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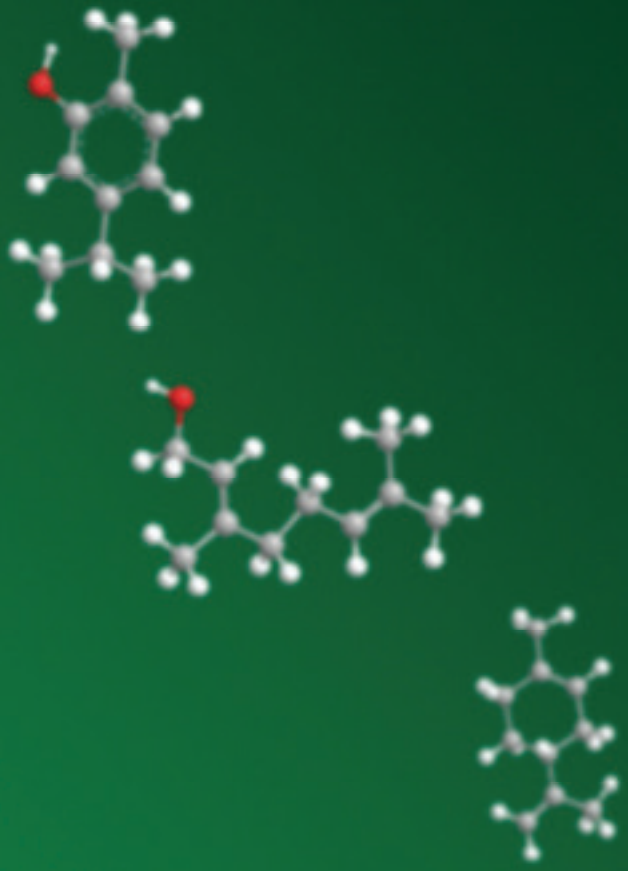
NATURAL VOLATILES & ESSENTIAL OILS

A Quarterly Open Access Scientific Journal



45th ISEO

7-10 September 2014, Istanbul

INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

ABSTRACTS

NVEO

Publisher BIO

NVEO

NATURAL VOLATILES & ESSENTIAL OILS

A Quarterly Open Access Scientific Journal

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Scope

NVEO is a major forum for the publication of new findings and research into natural volatiles and essential oils. It is created by the Permanent Scientific Committee of ISEO (International Symposium on Essential Oils). The journal is principally aimed at publishing proceedings of the ISEOs, but is also a peer reviewed journal for publishing original research articles and reviews in the field of natural volatiles and essential oils including wide ranging related issues on the analysis, chemistry, biological and pharmacological activities, applications and regulatory affairs, etc.

Published four times per year, NVEO provides articles on the aromatic principles of biological materials such as plants, animals, insects, microorganisms, etc. and is directed towards furthering readers' knowledge on advances in this field.

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Welcome

As president of the Organizing Committee of ISEO 2014, I am pleased to welcome you to the 45th International Symposium on Essential Oils (45thISEO).

This symposium series has been organized annually in Europe since 1969 in order to stimulate cooperation among scientists for the advancement of research and development into the science of essential oils, natural volatiles and aromachemicals.

This is the third time ISEO is organized in Turkey. Previously, the 28th ISEO was held in 1997 in Eskişehir and the 42nd ISEO in 2011 in Antalya. Both symposia were organized by us.

The venue of 45th ISEO is Istanbul, Turkey, which is one of the most attractive and historical cities of the world with a past reaching 12.000 B.C. It had been capital of the Byzantine Empire until 1453 and then the Ottoman Empire until 1923. Since then, it has been the most important city in Turkey in terms of history, culture, arts, industry, education and finance. Being situated along of the banks of Bosphorus, it is a town at the junction of two continents, Europe and Asia, and with shores on two seas Marmara and Black Sea.

The meeting has taken place in the convention center of Istanbul University with the participation of scientists and aroma therapists working in academia, trade, industry and regulatory affairs dealing with various aspects of essential oils, natural volatiles and aromachemicals.

Abstracts of the 42nd ISEO were published in a special issue of the Journal of Essential Oil Research (JEOR). We are proud to publish the abstracts in the special issue of new journal Natural Volatiles and Essential Oils (NVEO).

I wish you all a successful symposium and a very pleasant stay in Turkey.

Prof. Dr. K. Hüsnü Can Başer

President of the Organizing Committee of ISEO 2014

Editor-in-Chief of NVEO

Committees of ISEO 2014

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Topics of ISEO 2014

- Utilization and biological activities of essential oils
- Breeding, cultivation and post-harvest treatment of essential oil bearing plants
- Ecological importance of volatiles
- New trends in the production and analysis of essential oils
- Sourcing and trade of essential oils
- Biological variability of volatiles
- Sensorial aspects of natural volatiles
- Molecular and metabolomic trends in volatiles
- Biotransformation and biocatalysis of volatile compounds
- Regulatory aspects

Supporting Organizations

Anadolu University (http://www.anadolu.edu.tr/)

Istanbul University (http://www.istanbul.edu.tr/)

Bahçeşehir University (http://www.bahcesehir.edu.tr/)

International Council for Medicinal and Aromatic Plants (ICMAP) (http://www.icmap.org/)

The Scientific and Technological Research Council of Turkey (TÜBİTAK) (http://www.tubitak.gov.tr/)

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Sponsors

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Gülçiçek (http://www.gulcicek.com/)

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Aromsa (http://www.aromsa.com.tr/)

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International Federation of Essential Oils and Aroma Trades (IFEAT) (http://www.ifeat.org/)

Stand Sponsors

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LiKrom (http://www.likrom.com/en-US)

Milestone (http://www.milestonesrl.com/)

Talya (http://www.talyabitkisel.com/)

Association of All Pharmacist Cooperatives (TEKB) (http://www.tekb.org.tr/)

ISEO 2014 Organizing Committee is grateful to International Federation of Essential Oils and Aroma Trades (IFEAT) and EPS Fragrances (Erdoğmuş) for supporting the registration fees of 20 and 4 students, respectively. BadeBio supported the registration fees of 3 local students.

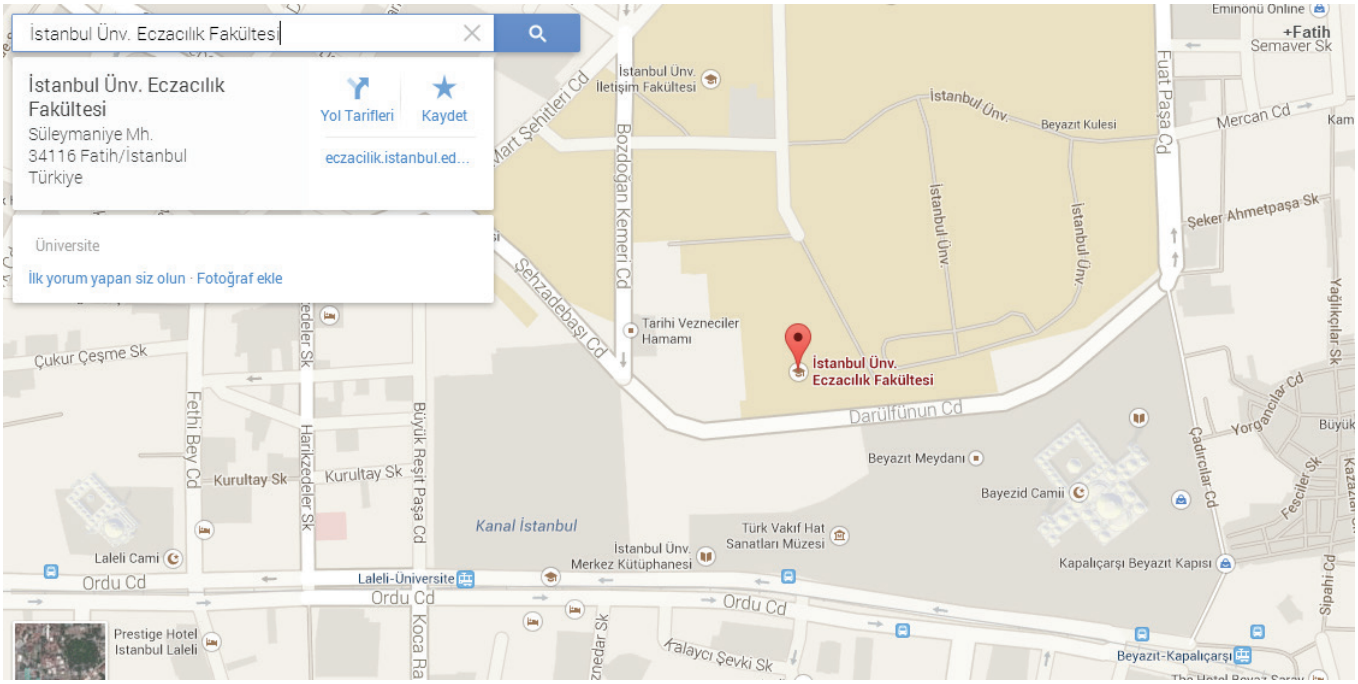
After the intense selection procedure, 27 students have been selected as Registration Fellowship Award Winners. We are grateful to IFEAT, Erdoğmuş and BadeBio for their kind gesture.

Registration Awardees

Aisheh Marwa Abu	University of Jordan, Jordan
Aijas Ahmad	Tshwane University of Technology, South Africa
Opeyemi N. Avoseh	University of Fort Hare, South Africa
Fatma Ayaz	Gazi University, Turkey
Emilie Belhassen	Université de Nice, France
Betül Büyükkılıç	Anadolu University, Turkey
Bhuwan Khatri Chhetri	University of Alabama in Huntsville, USA
Elif Dündar	Anadolu University, Turkey
Ines Ellouze	Institut National Agronomique de Tunis, Tunisia
Ceren Elmacı	Eskişehir Osmangazi University, Turkey
Daniela Gruľová	University of Presov, Slovakia
Yeşim Haliloğlu	Anadolu University, Turkey
Bilge Kara	Anadolu University, Turkey
Amber Khan	University of Witwatersrand, South Africa
Mahmoud Malekzadeh	Chamran University, Iran
Giovanni Mazzarrino	University of Teramo, Italy
Mohammed Mebruka	Addis Aaba University, Ethiopia
Rim Mecheri	Annaba University Hospital, Algeria
Özge Özşen	Eskişehir Osmangazi University, Turkey
Gezi Sander	Istanbul Technical University, Turkey
Elias Alves Da Silva	Universidade Federal de Lavras, Brazil
Nikola Stojanovic	University of Niš, Serbia
Daniel Strub	Wrocław University of Technology, Poland
Sylvain Sutour	Université de Corse, France
Görkem Şener	Anadolu University, Turkey
Dusan Vukicevic	University of Kragujevac, Serbia
Süleyman Yurt	Anadolu University, Turkey

Symposium Venue

Istanbul University Congress Centre,
Istanbul University Faculty of Pharmacy (İstanbul Üniversitesi Eczacılık Fakültesi)
Süleymaniye Mh. 34116, Beyazıt – İstanbul Türkiye
http://www.iseo2014.org/location.html



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General Information

Registration
The registration desk is located in the foyer of the Istanbul University Congress Centre

Resgistration Hours

Sunday, September 7.	14.00-19.00
Monday, September 8.	08.00-19.00
Tuesday, September 9.	08.00-18.30
Wednesday, September 10.	08.00-12.00

Symposium Language

Official language of ISEO 2014 is English. There will be no simultaneous translation.

Badges

Participants are requested to wear their badges at all times during the symposium, lunch and all social events.

Plenary Lectures and Oral Presentations

The Plenary Lectures (PL) are for 45 min., Oral Presentations (OP) for 20 min., and Young Scientists' Oral Presentations (YSL) for 10 min., including dissussions. Oral presenters are requested to download their presentations at least 1 h before the beginning of their respective sessions.

Posters

Posters will be exhibited according to their designated poster numbers. Odd (1,3,5,...) and even (2,4,6,..) numbers will be displayed Monday and Tuesday, respectively. The authors are requested to stand by their posters during the poster sessions.

Meals

Lunches on Monday, Tuesday and Wednesday are included in the registration fee. Lunches will be served in the Orangery of the Faculty of Pharmacy building nearby between 12.00-13.30 on Monday, Tuesday. At 13.00-14.30 on Wednesday.

Welcome Reception

Welcome Reception will be held in the Symposium Venue, the Istanbul University Congress Centre.

Symposium Dinner

Symposium Dinner will be served on board a cruise ship on the Bosphorus between 20.00-23.00 on Tuesday, September 9, 2014. Symposium Dinner tickets can be purchased at the Registration Desk. Dress code is smart casual.

Health and Insurance

The participants are requested to arrange their own insurance for health, travel and property. The Organising Committee will not accept any liability for personal injuries, loss or damage to property.

Scientific Programme

7th September 2014, Sunday		
14.00-18.00	Registration – (Istanbul University Congress Centre - near the Faculty of Pharmacy)	
19.00-21.00	Welcome Reception – Istanbul University Congress Centre	
8th September 2014, Monday		
08.00-09.30	Registration	
09.30-10.00	Opening Ceremony • Musical Performance • K. Hüsnü Can BAŞER, President • Patrizia RUBIOLO, Chairperson of the Permanent Committee	
10.00-10.30	Session Break – Tea & Coffee	
Session 1	**Chair persons: K. Hüsnü Can Başer & Éva Németh-Zámboriné**	
10.30-11.15	PL 1: Microencapsulation and sustained release formulations of volatiles – a revival of industrial interest and applications	Jan Karlsen
11.15-11.35	OP 1: Activity and activity enhancement of thymoquinone in cancer therapy: via loading in nanocarriers	Fadwa Odeh
11.35-11.55	OP 2: The aroma of turmeric depends on the combination of groups of several odor constituents	Toshio Hasegawa
12.00-12.30	Gülçiçek Workshop	
12.30-13.30	Lunch Break (Orangery of the Faculy of Pharmacy)	
Session 2	**Chair persons: Gerhard Buchbauer & Stanislaw Lochynski**	
13.30-14.15	PL 2: Chemistry and analysis of *Cannabis* and its essential oil	Mahmoud A. ElSohly
14.15-14.35	OP 3: Volatile components of stink bugs discolor finger and palm skin	Yoshinori Asakawa
14.35-14.55	OP 4: Chemical fingerprinting of essential oils derived from vegetative parts and flowers of wild *Anvillea garcinii* from the Central Region of Saudi Arabia	Merajuddin Khan
15.00-15.30	Session Break – Tea & Coffee	
Session 3	**Chair persons: Johannes Novak & Afife Mat**	
15.30-16.15	PL 3: The Investigation of three *Cinnamomum* essential oils from a pest management perspective	Nurhayat Tabanca
16.15-16.35	OP 5: Alkaloids from the essential oil of *Choisya ternata* attenuate diclofenac- and ethanol-induced gastric lesions in rats	Pavle J Randjelovic
16.35-16.55	OP 6: GC/MS fingerprinting of Yakushima's liverworts	Agnieszka Ludwiczuk
17.00-19.00	Poster Session I (odd numbers like 1,3,5,...) İstanbul University - Foyer of the Conference Centre	

9th September 2014, Tuesday		
Session 4	**Chair persons: Luigi Mondello & Jan Demyttenaere**	
09.00-09.45	PL 4 : Effects of essential oils and aroma chemicals on animal performance and health	Chlodwig Franz
09.45-10.05	OP 7: Variation of volatile oil composition of some Jordanian *Achillea* plants collected at different growth stages and different extraction methods	Hala Ihala Al Jaber
10.05-10.25	OP 8: Biocidal activity evaluation methods for essential oils: Searching new antiseptics and disinfectants in the golden age of antimicrobial resistance	Juan Bueno
10.30-11.00	Session Break – Tea & Coffee	
Session 5	**Chair persons: Yoshinori Asakawa & Neşe Kırımer**	
11.00-11.20	OP 9: Essential oils external use - internal use criteria of choice	Dominique Davenne
11.20-11.40	OP 10: Identification of volatile compounds, antimicrobial properties and antioxidant avtivity from leaves, cones and stems of *Cupressus sempervirens* from Algeria	Zoughi Nafila
11.40-12.00	OP 11: Geraniol restores antibiotics activities against multidrug-resistant isolates from *Enterobacter aerogenes*	Liliane Berti
12.00-13.30	Lunch Break (Orangery of the Faculty of Pharmacy)	
Session 6	**Chair persons: Jan Karlsen & Nurhayat Tabanca**	
13.30-14.15	PL 5: The European Flavouring regulation and how to deal with "Restricted Substances"	Jan Demyttenaere
14.15-14.35	OP 12: The regulatory framework for the use of essential oils, as herbal medicinal products, in the EC. An up-to-date scientific view	Ioanna Chinou
14.35-14.55	OP 13: Smoking desert plants: the highly diverse character of medicinal, chemosemiotic or psychoactive volatiles in Australian Aboriginal ethnopharmacology	Nicholas John Sadgrove
15.00-15.30	Session Break – Tea & Coffee	
Session 7	**Chair persons: Chlodwig Franz & Nicolas Baldovini**	
15.30-16.15	PL6: Advanced preparative techniques for the collection of pure components from essential oils	Luigi Mondello
16.15-16.35	OP 14: Conventional and enantioselective GC microfabricated columns versus FSOT columns in the analysis of essential oils	Cecilia Cagliero
16.35-16.55	OP 15: Introducing Supercritical Fluid Extraction - A Green Extraction Technique	Oleg Pokrovskiy
16.55-17.15	OP 16: Average-mass-scan-of-the-total-ion-chromatogram (AMS) profiling of essential oils – a useful tool for tracking storage-induced changes. The case of *Artemisia alba* Turra essential oils	Dragana D Stevanovic
17.15-17.35	OP 17: Antioxidant activities of essential oil components - An experimental and computational investigation	William N Setzer
17.40-18.30	Poster Session II (even numbers like 2,4,6) İstanbul University- Foyer of the Conference Centre	
20.00	Symposium Dinner – Bosphorus Cruise	

10th September 2014, Wednesday		
Session 8	**Chair persons: Patrizia Rubiolo & Agnieszka Ludwiczuk**	
Young Scientists Special Session		
09.00-09.40	KEYNOTE: Exploring the antimicrobial properties of South Africa's aromatic flora	Sandy van Vuuren
09.40-09.50	YSL-01: Inactivation dynamics of *Listeria monocytogenes* and *Salmonella enterica* during exposure to selected essential oils	Giovanni Mazzarrino
09.50-10.00	YSL-02: Key odorants of vetiver and atlas cedarwood: Analytical studies and structure-odor relationships	Emilie Belhassen
10.00-10.10	YSL-03: Toxicity of thujone and thujone-containing medicinal plants? The case of *Salvia officinalis, Artemisia absinthium*, *Thuja occidentalis* and *Tanacetum vulgare* essential oils	Nikola M Stojanovic
10.10-10.20	YSL-04: The elusive floral scent of *Daphne blagayana* Freyer (Thymelaeaceae)	Dusan R. Vukicevic
10.20-10.30	YSL-05: Utilization of constituents of thuja and fennel essential oils towards synthesis of novel fragrant compounds	Daniel Jan Strub
10.30-11.00	Session Break – Tea & Coffee	
Session 9	**Chair persons: Sandy van Vuuren & Temel Özek**	
Young Scientists Special Session (cont.)		
11.00-11.10	YSL-06: Chemical compositions, biological activities and molecular docking studies on some aromatic medicinal plants from Yemen	Bhuwan Khatri Chhetri
11.10-11.20	YSL-07: Apoptosis inducing abilities of *Ocimum sanctum* essential oil in *Candida albicans*	Amber Khan
11.20-11.30	YSL-08: In vitro antimicrobial activity of *Cymbopogon martini* essential oil and its synergystic interaction with silver ions	Aijaz Ahmad
11.30-11.40	YSL-09: Characterization of the aromatic quality of Tunisian orange blossom water essential oils: comparison between traditional and industrial extraction processes	Ines Ellouze
12.00-12.45	Closing Ceremony -Patrizia Rubiolo, K.Hüsnü Can Başer	
12.45-13.00	Introduction of the 46th ISEO	
13.00-14.30	Lunch Break (Orangery of the Faculty of Pharmacy)	
15.00-18.00	ISTANBUL City Tour – Registration on desk	

PLENARY LECTURES

PL-01. Chemistry and analysis of *Cannabis* and its essential oil

M. A. ElSohly

National Center for Natural Products Research, School of Pharmacy, University of Mississippi University, MS 38677, USA

The cannabis plant is one of the most studied and oldest medicinal plants that has recently gained more attention by the general public for therapeutic use. This is in spite of the fact that the drug is still considered illegal to possess and scheduled as a schedule I narcotic with no medical benefits, at the Federal level.

This presentation will provide an overview of the chemistry of cannabis and the cannabinoids as well as analytical data on illicit cannabis products and the increase in the Δ^9-THC content over the years. Furthermore, the presentation will provide information on the chemistry of the essential oil of cannabis and the contribution of the oil components to the pharmacological effects of the drug.

PL-02. The investigation of three *Cinnamomum* essential oils from a pest management perspective

Nurhayat Tabanca[1], Betul Demirci[2], Zulfiqar Ali[1], Ulrich R. Bernier[3], Abbas Ali[1], Aruna Weerasooriya[1], Sui Zhang[4], Jian Zhang[5], Peng Nan[6], K Husnu Can Baser[2,9], Ikhlas A. Khan[1,7,10], David E Wedge[8]

[1]National Center for Natural Products Research, The University of Mississippi, University, MS 38677 USA
[2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey
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[8]USDA-ARS, Natural Products Utilization Research Unit, University of Mississippi, University, MS 38677 USA
[9]King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia
[10]Department of Pharmacognosy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia

Plants produce chemicals for defense and communication, but can also generate their own form of chemical warfare targeting proliferation of pathogens. The chemical warfare between plants and their pathogens shows promise to offer natural products as new antimicrobial agents for human and animal health, as well as for agricultural pest control. Our current research efforts are directed on identification of natural product based fungicides and biopesticides. In this research program, we evaluated 20 essential oils using a direct bioautography method to detect antifungal activity against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides*. Of these essential oils, *Cinnamomum zeylanicum* bark essential oil followed by *C. cassia* bark oil showed the most promising antifungal activity. Leaf oils of *C. subavenium* and *C. cassia* oils demonstrated weak antifungal activity. Identification of active antifungal compounds from *C. zeylanicum* oil was confirmed on the overpressured layer chromatography (OPLC) plate in toluene-ethyl acetate (9:1, v/v) against *Colletotrichum* species. Antifungal activity was visualized directly on the OPLC plate as "clear zones" where no fungal mycelia, stroma or conidia grow. Two antifungal compounds with R_f values of 0.69 and 0.73 were easily demonstrated by direct bioautography assay and these two active antifungal compounds were isolated by High Performance Flash Chromatography. Their identification was accomplished by 1D- and 2D-NMR spectroscopy. Isolated pure compounds were subsequently evaluated using a dose-response format in a micro-dilution broth assays against *Colletotrichum, Botrytis*, *Fusarium,* and *Phomopsis* species. As a part of our effort to search for new insecticides to control mosquitoes, isolated active compounds were showed good repellent activity using the "cloth patch assay" with three human volunteers against adult female *Aedes aegypti* and larvicidal activity against 1-day old *A. aegypti*.

PL-03. Advanced preparative techniques for the collection of pure components from essential oils

Danilo Sciarrone[1], Sebastiano Pantò[1], Francesco Cacciola[1,3], Luigi Mondello[1,2,3]

[1] Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute (SCIFAR), University of Messina, Messina, Italy
[2] Centro Integrato di Ricerca (C.I.R.), University Campus Bio-Medico of Rome, Roma, Italy
[3] Chromaleont s.r.l. A start-up of the University of Messina, c/o Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute, University of Messina, Messina, Italy

The collection of pure components from complex samples can be considered a hard task, especially at milligram level in a reasonable time. Recently a heart-cut multidimensional GC-prep system based on the hyphenation of three chromatographic dimensions, equipped with stationary phases characterized by different selectivities, have proven to be suitable to collect in a very short time chemicals from real samples in a one-day work time characterized by a degree of purity higher than 95% [1]. The demands for the collection analytes at concentrations <10%, would consist in an increased sample injection volume, but this option could lead to exceed the GC liner capacity. To improve the capability of the system, an on-line 4D chromatographic system (prep LC-GC-GC-GC) instrument was developed. Such a system enabled the injection of higher sample volumes, the reduction of collection times, while maintaining high levels of purity.

The present research reports the use of the system in order to demonstrate the suitability of this approach for the collection of enantiomeric pure components from different samples. The system was thus equipped with a cyclodextrin based stationary phase in the third chromatographic dimension with the aim to separate the enantiomers of components purified on the first (apolar) and second (medium polarity) GC columns. The collection station connected to the third GC dimension allowed the collection of different enantiomers components in the same run due to the presence of a 10-position carousel.

Acknowledgments

The Project was funded by the “Italian Ministry for the University and Research (MIUR)” within the National Operative Project “Hi-Life Health Products from the industry of foods”. Project ID: PON01_01499.

Reference

1. Sciarrone, D., Pantò, S., Ragonese, C., Tranchida, P. Q., Dugo, P., Mondello, L. Anal. Chem. 2012, 84, 7092–7098.

PL-04. Microencapsulation and sustained release formulations of volatiles – A revival of industrial interest and applications

Jan Karlsen

Department of Pharmaceutics, Faculty of Pharmacy, University of Oslo, Norway

The application of volatiles and specifically essential oils are dependent upon biological/physiological activity such as flavour, aroma, perfumery, antibiotic, insecticidal and solvent properties. For some of these applications the volatility is a benefit but for most applications we would like to achieve a more lasting effect. To achieve this effect the volatility of the compounds must be reduced. If the volatility can be reduced new applications will be available and the industrial use of specific volatiles or volatile mixtures will increase. Only new and efficient applications of volatiles can ensure continuous financial support for basic research in the field of essential oil investigations. This was previously driven by the perfumery industry for the development of gas chromatography separations and instrumentation.

The use of microencapsulation and the incorporation of essential oils essential oil constituents and other volatiles in sustained release formulations has increased tremendously during the last five years and the number of patents has increased likewise. This talk will focus upon the importance of interdisciplinary collaboration to benefit from the research on essential oils, which has been reported through all the meetings of the ISEO. Some highlights of new essential oil products will be discussed. The new applications will lead to a development in instrumental techniques change in perfumery technology, increased use of volatiles as aroma chemicals and give rise to new industrial products. The importance of attracting industrial interest for the application of basic volatile components research will be described. The most important aspect of research and development in this field of essential oils and volatiles is an understanding of the industrial constraints and requirements which can make microencapsulation viable from laboratory scale experiments to full-scale industrial production.

PL-05. Effects of essential oils and aroma chemicals on animal performance and health

Chlodwig Franz

WG Functional Plant Products, Department of Farm Animals and Veterinary Public Health, University of Veterinary Medicine, A-1210 Vienna, Austria

Following the science-based development in human nutrition, health care and medicine, that functional plant products as e.g. essential oils show many beneficial effects, the interest in the use of essential oils and plant extracts as health care products has grown also in veterinary medicine. Especially in animal keeping and nutrition a rapid change could be observed due to the fact that since 2006 the use of antibiotic growth promoters is banned in the EU.

Although the understanding of their mode of action is a prerequisite for the optimal application of herbal products in terms of efficacy, a full understanding of these aspects in animals has not yet been achieved, as shown by the majority of papers being published as reports on production experiments ('feed and weight' type) only. But for instance, aromatic compounds and essential oils act along the entire animal digestive tract to improve appetite and modulate the bacterial flora, and are able to induce a number of other 'non-nutritive' benefits. The antimicrobial properties of essential oils and extracts can be dose dependently bacteriostatic or/and bactericidal. In addition, several investigations have shown their antioxidative effect, their effects on digestive physiology and digestion at weaning and on the microbiology of the gut. One advantage of essential oils in particular is that they occur in nature as complex mixtures rather than as single compounds, hence resistance is less likely to become a problem than with single synthetic compounds, and the mode of action is principally different. But the variability and biodiversity of essential oil makes also a clear botanical and phytochemical characterization of the plant material used absolutely necessary.

References

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PL-06. The European Flavouring Regulation and how to deal with "Restricted Substances"

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The European Flavouring Regulation (EC) No 1334/2008 [1] entered into force on 20 January 2009 and applies since 20 January 2011. According to this Regulation certain substances, most of which are common constituents of natural (food) ingredients, are restricted. These "restricted substances" (RS) as they are called, are substances that occur naturally in source materials for flavourings and food ingredients with flavour properties, but whose presence in certain foods is restricted and/or for which maximum levels are set [2].

Thus, two types of restrictions are foreseen: Annex III Part A of the Flavouring Regulation lists 15 substances "which shall not be added as such to food", whereas Part B of Annex III lists 11 substances which are naturally present in flavourings and food ingredients with flavouring properties and to which "maximum levels" apply in specific food categories.

Also elsewhere in the world flavour regulations contain such lists of so-called "Restricted substances". For example a list of substances to be controlled can be found in the Mercosur Technical Regulation Concerning Flavourings [3], the new Russian Federation Customs Union Technical Regulation on Food Additives [4], and many flavour regulations of South-East Asian countries, such as Malaysia, Indonesia, Singapore, *etc*.

Only a few publications refer to the determination of RS in compound flavourings or their raw materials, and the latter only concern the analysis of one or two individual RS in single essential oils. This presentation will discuss a method for the determination of various RS in flavourings and their raw materials by gas chromatography-mass spectrometry using selected-ion monitoring (GC-MS-SIM) and internal standards. An initial evaluation of a highly-complex surrogate flavouring containing the analytes under investigation, at concentrations that would be likely to produce levels in finished foods of around typical maximum limits, has been carried out by 9 laboratories. Results of this evaluation, using different column types and different flavour carriers, will be presented, demonstrating the robustness of the method. The method is intended for flavour-industry laboratories in order to enable them to inform their customers (*e.g.* food industry) of the amounts of these substances in commercial flavourings, but is not intended for their analysis in finished foods.

The presentation will further focus on Business-to-Business requirements when flavourings are sold to food producers (customers) and provide some elements from EFFA's Guidance Document [5].

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KEYNOTE

SL-01. Exploring the antimicrobial properties of South Africa's aromatic flora

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With over 24 000 species of flowering plants, South Africa is considered to be the third most biodiverse area in the world and 10% of the world's total flora is contained within its boundaries. Many of these species are aromatic and are extensively used in local traditional healing practices. The rich indigenous knowledge systems which have developed on local plant use acted as a catalyst to explore the medicinal aromatic flora of South Africa. We initiated a project in 1999 to explore the exceptional flora of South Africa with an acute focus on plant volatiles. This research culminated in the very first special issue of Journal of Essential Oil Research, which was devoted to discoveries on South Africa's aromatic plants. Our research is not only aimed at profiling the chemical composition of essential oils but also to unravel the biological properties of these oils and their constituents, with a special interest in synergy research and gaining a better understanding of structure activity relationships. We invite you on a journey where we present a reflection on the research achievements over the past 15 years. We will demonstrate some interesting time-kill efficacies on South African medicinal plants in comparison with commercial oils. The chemical composition is vitally important to the biological activity and this will be demonstrated by showing how chemical compounds within *Osmitopsis asteriscoides* contribute towards synergy. *Artemisia afra*, one of the most well-known medicinal plants in South Africa, will be used as a model to demonstrate how studies can progress from a screening process to interaction and then culminating into formulations. The traditional plant use by rural inhabitants of Northern Maputaland will validate how essential oil bearing plants play a role in the treatment of respiratory conditions. As antibiotics play a major part in current anti-infective treatments, their use in combination with popular South African medicinal plants will be presented.

ORAL PRESENTATIONS

OP-01. Activity and activity enhancement of thymoquinone in cancer therapy: via loading in nanocarrieirs

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In the context of this research, thymoquinone (TQ) was complexed with β-cyclodextrin (CD) to form nanosized aggregates. Various TQ:CD ratios were tested and it was found that the ratio of (1:0.25) TQ:CD formed distinguishable nanoparticles with minimum toxicity towards normal cells. These nanoparticles had an average size of 445±100 nm with a charge 21.8 mV using Zeta-sizer. Particle size measurement using scanning electron microscopy (SEM) showed an average size of 400 nm and it also revealed the presence of smaller structures, with an average size of 50 nm. The *in vitro* antiproliferative activity on MCF7 cells was determined using MTT assay and an IC_{50} of 4.70 ±0.60 µM for TQ-CD nanoparticles in comparison to 24.09 ±2.35 µM of free TQ solution after 72 hours of incubation. Simultaneously, TQ-CD nanoparticles showed lesser toxicity when was compared to TQ solution using human periodontal fibroblasts as a model for normal cells. It could be concluded from the results that TQ loaded cyclodextrin nanoparticles might serve as a potential nanocarrier to improve TQ solubility as well as its antiproliferative activity with little toxicity to normal tissues.

OP-02. The aroma of turmeric depends on the combination of groups of several odor constituents

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Turmeric plays an important role in the flavor and fragrance industries. Although many compounds have been reported as components of turmeric, the aroma profile of turmeric has not been determined. Recently, it has been reported that an odorant molecule is recognized by several odor receptors with different intensities [1]. Furthermore, an odor receptor responds to odorant molecules that have similar structures with different intensities [2]. Based on these studies of the mechanism of odor recognition, we have developed a new approach for evaluating the complex odors of materials [3], and we used this approach to study the aroma profile of turmeric. The hexane extract of turmeric had a turmeric-like odor, whereas turmeric oil obtained by steam distillation had a pungent, non-turmeric-like odor. Bulb-to-bulb distillation of the hexane extract produced a main fraction with a turmeric-like odor, which consisted of *ar*-turmerone and *β*-turmerone (group A) as the main components, and another fraction, which consisted of *α*-curcumene and *β*-sesquiphellandrene (group B) as the main components. In contrast, the bulb-to-bulb distillation of the turmeric oil obtained by steam distillation produced a fraction (group C) containing compounds that were not present in groups A and B. These results indicate that the turmeric-like odor of the group A fraction was altered by the addition of the group C fraction. The group C fraction caused the different odor of the turmeric oil obtained by steam distillation. The variation in the aroma of turmeric was therefore produced by the combination of these groups of odor constituents.

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OP-03. Volatile components of stink bugs discolor finger and palm skin

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Sting bugs emit *(E)-2-hexenal*, *(E)-2-octenal* and *(E)-2-decenal* and their related unsaturated aldehydes which are responsible for unpleasant odor of bugs. Graphosoma rubrolineatum is the harmful insect, which attacks *Foeniculum vulgare* L. (fennel). When man holds some stink bugs, which producing unpleasant odor, in his palm of hand for 1 to 3 min, the palm and fingers are immediately discolored to display yellowish-red color, which never disappears for 2 weeks even washing by any organic solvents or soaps. The purpose of the present study is to understand what type of biochemical reaction occurs on man's palm and fingers' skin during clasping stinkbugs in the palm and to identify the attractant component of F. vulgare against G. bubrolineatum.

Nezara antennata and *G. rubrolineatum* were collected and clasped in the palm for 1 to 5 min. On the other hand, each 1 ml of (*E*)-2-hexenal or (*E,E*)-2,4-hexadienal was put into the bottom of polyethylene sack and the hand was inserted in it, followed by closing the sack and heated by drier. In order to clarify the mechanism of discolor on the skin, several amino acids were reacted with (*E*)-2-hexenal or (*E,E*)-2,4-hexadienal in acidic, neutral and alkaline conditions.

In order to identify the stingbug's attractant component from *F. vulgare,* the flowers of this herb was extracted with ether and the crude extract analyzed by GC/MS and NMR.

G. rubrolineatum produced *n*-decane (4.0 %), (*E*)-2-decenal (24.4 %) and *n*-tridecane (70.3%) and *Nezara antennata* did *E*-2-decenal (80 %), tridecane and (*E*)-2-decenyl acetate, respectively. *N. antennata* showed the strongest dark-red color on the palm and finger skin. *Agriosphodrus dohrni* does not show discoloration on the skin because it biosynthesizes neither (*E*)-2-decenal nor its related a,b-unsaturated aldehydes. Thus, it is clear that discoloration phenomenon originates from the chemical reaction of gaseous unsaturated aldehydes with protein (amino acid). The chemical reaction of cysteine with (*E,E*)-2,4-hexadienal was carried out to afford reddish-yellow oil. This liquid was painted on the palm skin to show the similar dark-red color which is very similar to that produced by several stink bugs and this color did not disappear for a week. A reaction mixture contains unidentified thiazoline derivative.

(*E*)-2-Hexenal and (*E,E*)-2,4-hexadienal do not discolor on palm and finger skin when the hand was put into the sack which was occupied by those gases even more than 5 min. The biodiscoloration by stinkbugs is very fast, for example, for 30 seconds, at 36^{o}C, in case of *Nezara antennata.* Thus, it is suggested that some enzymes in insects might play an important role in this discoloration on man's palm and finger skin, because neither dead stinkbugs nor the direct exposure of gaseous (*E*)-2-hexenal or (*E,E*)-2,4-hexadienal on the hand skin discolor at all.

The GC/MS of the extract of the flower of *Foeniculum vulgare* showed the presence of limonene (9.6%) and anethole (77.7%). Limonene and anethole were isolated and they were separately painted on the leaves of *Polygonum hydropiper* belonging to the Polygonaceae, which contains a pungent sesquiterpene dialdehyde, polygodial and then the adult of *G. rubrolineatum* was introduced in the plastic cage in which *P. hydropiper* was kept into the Erlenmeyer flask. The stinkbug did not move to limonene-painted leaves, but all of the bugs willingly moved on anethole-painted leaves. Thus it is clear that the attractant to the bug was not limonene but anethole.

The production of the discolored components for some crime prevention spray is under progress.

OP-04. Chemical fingerprinting of essential oils derived from vegetative parts and flowers of wild *Anvillea garcinii* from the Central Region of Saudi Arabia

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The chemical components of essential oils derived from wild *Anvillea garcinii* in Saudi Arabia were studied here for the first time. The phytochemical analysis of the essential oils from the flowers and vegetative parts (leaves and stems) was performed using gas chromatography techniques (GC–MS, GC–FID, Co-GC, LRI determination, and database and literature searches) on polar and nonpolar columns. This analysis led to the identification of 141 compounds, among which 131 compounds were identified for the first time in the *Anvillea* genus. The results indicate that 127 compounds were identified in the oil obtained from the flowers of *A. garcinii*, whereas 120 compounds were identified in the oil obtained from the vegetative parts; these compounds account for 95.7% and 94.9% of the oil composition, respectively. The major components in the oil from flowers were bornyl acetate (33.7%), *cis*-nerolidol (7.3%) and camphene (6.1%). In contrast, the major compounds in the vegetative oil were *cis*-nerolidol (16.0%), terpinen-4-ol (10.4%) and cabreuva oxide B (6.4%). The two oils exhibited a qualitative similarity: 106 compounds were observed in oils derived from both parts of the plant. However, quantitatively, the two oils differed significantly from one another. In particular, 21 constituents were found only in the oil from flowers, whereas 14 constituents were found only in the oil from vegetative parts.

OP-05. Alkaloids from the essential oil of *Choisya ternata* attenuate diclofenac- and ethanol-induced gastric lesions in rats

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Methyl (M) and isopropyl (I) *N*-methylanthranilates, natural volatile alkaloids from the essential oil of *Choisya ternata* Kunth (Rutaceae) [1], were assayed for their possible overall effect on intact gastric mucosa and their protective properties towards the onset of gastric lesions induced by diclofenac (a non-steroidal anti-inflammatory drug, NSAID) or ethanol. The influence of I and M on gastric mucosa integrity was assessed by oral administration in doses of 200 mg/kg. The gastroprotective action of I and M in doses of 50, 100 and 200 mg/kg was analyzed in the diclofenac and ethanol-induced gastric lesion models in rats. After the treatment, the stomachs of the animals were analyzed. Ulcer scoring, morphometric and histopathological analyses of the stomachs were done.

The oral application of these compounds on their own, even in quite high doses (200 mg/kg) did not induce gastric lesions. Both alkaloids exerted a very strong antiulcer activity, even in low doses (50 mg/kg), by decreasing the number of lesions caused by the application of either diclofenac or ethanol, eliminating them completely or reducing them to a form of mucosal hyperemia. The mucosa of animals treated with the highest doses (200 mg/kg) of substances I and M was without any lesions.

Substances I and M were shown to possess very strong antinociceptive effect in several models [1]. Additionally, as the application of these compounds on their own, even in quite high doses (200 mg/kg) compared to those which were administrated in the antinociceptive assays (0.3 mg/kg), did not induce gastric lesions, I and M might represent new safer NSAIDs for pain management. The tested compounds, in a dose dependent manner, reduced the number, length and thickness of lesions caused by ET and DC administration, indicating very strong antiulcer activity, even in low doses.

The protective action of I and M against DC- and ET-induced ulceration could be the consequence of their possible anti-inflammatory mechanism of action partially evident from the results of the acetic acid-induced writhing test [1]. Acute and chronic application of high doses of benzodiazepines produced a significant reduction of stress-induced ulcerations (cold-restrain method) considered to be the consequence of a combination of sedative, anxiolytic and antisecretory actions [3]. Having this in mind, anxiolytic and antidepressant compounds I and M [2] may be considered for use as new possible medications for gastrointestinal ulcerations in patients with/without psychiatric disorders.

Due to their many positive properties including anxiolytic, antidepressant, antinociceptive, anti-inflammatory and gastroprotective activities, as well as a cheap and simple synthetic route for their preparation, methyl and isopropyl *N*-methylanthranilates, both alike, might represent a cost effective alternative sought for in the treatment of peptic ulcers and/or new safer NSAIDs for pain management.

Acknowledgments

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OP-06. GC/MS fingerprinting of Yakushima's liverworts

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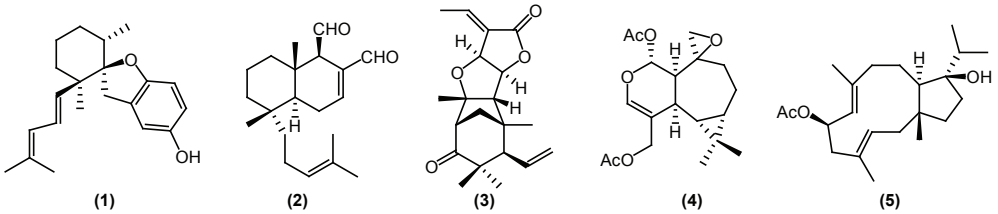
Chemical fingerprints obtained by chromatographic techniques are fantastic tool which can be used for identification and authentication of herbal samples, as well as in purpose of chemosystematic studies. Chromatographic fingerprinting by use of hyphenated methods, e.g. GC/MS, can successfully demonstrate both sameness and differences between various samples (1).

Liverworts are highly diverse group of spore-forming plants that can grow in almost every available habitats. Because of the small size, morphological simplicity, and enormous diversity, the identification of these plants is especially challenging (2). Species identification is fundamentally important within the fields of biology, ecology, and conservation, but also crucial in phytochemical studies, especially when searching for new pharmaceuticals. Our previous studies showed the possibility of using the liverworts chemistry to resolve the taxonomic problems at species (3), genus (4), and family level (5).

The major purpose of this study was to show whether the chromatographic fingerprinting of liverworts collected in Yakushima island (Japan) can be useful in identification and taxonomic differentiation of this group of plants. GC/MS analysis of the 38 liverworts specimens was carried out. All analyzed liverworts species were characterized by high diversity of volatile terpenoids, especially sesqui- and diterpenoids. We was able to find chemotaxonomic markers of the liverwort species and genera. Merosesquiterpene, riccardiphenol A (**1**) was the major volatile of Yakushima's *Riccardia crassa*. Sacculatane type diterpenoids (e.g. **2**) were the most characteristic components for *Pellia endiviifolia*. All analyzed *Pallavicinia* specimens produced labdane-type diterpenoid, pallavicinin (**3**), which is chemical marker of this genus. 2,3-Seco-aromadendrane-type sesquiterpenoids (e.g. **4**) were found in two analyzed *Plagiochila* species. The most characteristic chemical feature of *Odontoshisma denudatum* is the presence of dollabellane type diterpenoids. Acetoxyodontoschismenol (**5**) were detected in Yakushima's specimen.



(1) (2) (3) (4) (5)

To extract a maximum of chemical information from experimental data, the multivariate statistical methods were employed. Based on CA analysis (**fig.1**) three major cluster were recognized. The first and third one are made of simple thalloid and complex thalloid liverworts, respectively. Cluster II contains leafy liverworts. Employment of the statistical analysis showed also some interrelationships between some liverworts species. CA analysis point out on the chemical similarity between *Jubula japonica*, *Frullania tamarisci* subsp. *obscura*, and *Porella densifolia*, All of mentioned liverworts are classified in order Porellales. Statistical analysis also showed, that *Scapania* and *Odontoshisma* species are chemically very closely related, which is in agreement with phylogenetical data (2).

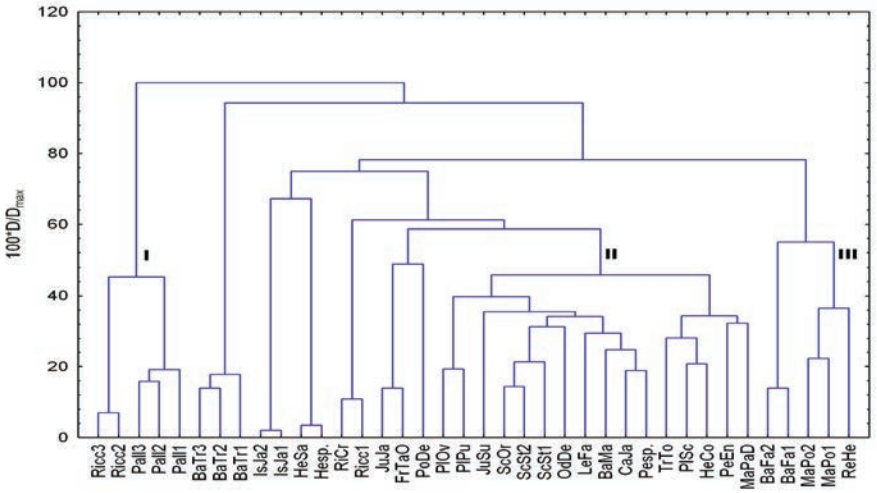


Fig.1. Hierarchical dendrogram representing chemical composition similarity relationships among Yakushima's liverworts.

In conclusion, a wide chemical variability of volatile components has been demonstrated for Yakushim's liverworts. Studies using non-morphological characters, such as chemical features, to discriminate species seem to be very important from evolutionary and ecological point of view. Results from the chemical investigations can help to understand real relationships among the taxa, together with the other biological or genetic information.

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OP-07. Variation of volatile oil composition of some Jordanian *Achillea* plants collected at different growth stages and different extraction methods

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Different *Achillea species* (Asteraceae) are commonly used in traditional medicine for healing wounds and in the treatment of many ailments including abdominal pain and stomach ache. There are 5achillea species that are reported to grow wildly in Jordan.

In a continuous effort conducted for the investigation of the chemical composition of the volatile and nonvolatile constituents of Jordanian medicinal plants, were report here a summary of our recent results concerning the variation of the volatile oil composition of some *Achillea* plants collected at different flowering stages. Moreover, variation of the essential oil composition using different extraction methods, namely the hydro-distillation, Solid Phase Micro-Extraction (SPME) and Super-Critical Fluid (SCF), will also be discussed.

OP-08. Biocidal activity methods for evaluation of essential oils: searching new antiseptics and disinfectants in the golden age of antimicrobial resistance

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Infectious disease is an important global public health issue, and the increase of development of microbial resistance to the most powerful antibiotics have raised the alarm. In the U.S. hospital system around 2 million people have contracted infections each year, and 90,000 die for this cause. Biocides as antiseptics and disinfectants play an essential role in limiting the spread of infectious disease. These agents are used broadly in healthcare, home and food industry environments. The efforts in biocide development has focused on products that avoid bacteria colonization and biofilm formation on surfaces, looking for to prevent growth and dissemination of infectious organisms [1].

Making it necessary to develop new biocide agents with broad-spectrum activity, which includes efficacy against bacterial endospores as well as biofilms, and lack of environmental toxicity.

In this way, essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, their antimicrobial activity is due to inhibition or interaction with multiple targets in the microbial cell as well as to non-specific antimicrobial effects due to the hydrophobic properties of the mixtures and components. Such as, microbial alteration of the structure, leakage of cell contents, and death cell [2]. Equally, posses antimutagenic properties [3], which makes them ideal candidates for the development of new biocides alone or in combination for to provide an alternative approach to combat emerging drug resistance.

In this order of ideas, implement *in vitro* techniques to evaluate the biocidal activity of essential oils and their formulations robust, reproducible and automatable, controlling the different variables offered by the complex chemical mixture that contain is an important step for obtain new antiseptics and disinfectants for medical and industrial use with less toxicity and broad activity. Also, screening labs interested should be focused in develop biocide suspension test as primary screening, evaluate the ability of surface decontamination, antibiofilm activity both static as flow, *in vitro* wound dressing activity for determinate the utility of essential oil in wound healing and whole animal infectious model that have the ability of to evaluate activity and toxicity in the same screening platform. Likewise to determine the synergism between essential oils and existing biocides [4].

Finally, the aim of this lecture is to give tools to begin the search and development of new biocides from essential oils that can be useful in medical healthcare and industry for the control dissemination of infectious disease as well as resistance emergence.

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OP-09. Essential oils external use - internal use - criteria of choice

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This presentation is connected to medical and pharmaceutical use of essential oils.

Efficiency and safety will be developed.

Essential oils can be used according different routes:

A Oral route

B Cutaneous application

C Inhalations

A Oral route:

This approach allows a wide flexibility regarding doses of essential oils. Traditionally vegetable oils, honey, sugar have been used and are steel used. Gastric anti-acid gels allows the use, even at high level, of essential oils that can be irritating for the stomach. Capsules and stomach acidity resistant Capsules can be easily prepared by pharmacists. Soft capsules produced industrially are also available. Some pharmaceutical syrups contains essential oils, it is easy to add EO to saccharose syrup. Alcoholic solutions can be prepared with EO.

B Skin application:

First choice way of application in the fields of dermatology and cosmetics. Many essential oils have total diffusion through epidermis, they are suitable to treat pulmonary diseases.Vegetable oils, gels, creams, pulps, are the more common supports.

D Respiratory route:

Upper respiratory tract: inhalations and nasal sticks. Pulmonary diseases: inhalations and aerosols. According essential oils families one of the routes will be favorite. Sometimes association of two routes can be useful.

OP-10. Identification of volatile compounds, antimicrobial proprieties and antioxidant activity from leaves, cones and stems of *Cupressus sempervirens* from Algeria

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Cupressus sempervirens L. (Cupressaceae) leaves, cones and young branches have been used in traditional medicine and aromatherapy. The composition of the isolates obtained by water distillation from the areal parts (plant material collected at Bainem forest, in northwest of Alger, Algiers), were analyzed by GC and GC-MS. The leaves isolate (yield 0.22 % w/w), was mainly composed of monoterpene hydrocarbons (60.8 %), α-pinene (38.4 %), δ-3-carene (13.9%), α-cedrol (10.6 %), α-terpinyl acetate (3.5 %), (*E*)-totarol (3.0 %). The cones isolate (yield 0.34 % w/w) was predominantly composed of monoterpene hydrocarbons (33.I8 %), with α-pinene (20.3 %), δ-3-carene (6.0 %), terpinen-4-ol (9.0 %), α-terpineol (9.0 %), α-terpinyl acetate (5.9 %), α-cedrol (9.1 %), (*E*)-totarol (4.4 %). The major components of steams isolates (yield 0.03 % w/w) are rich in diterpenoids (51.9 %), namely: α-pinene (5.9 %), α-cedrol (14.4 %), manool (5.6 %), (*E*)-Totarol (34.7 %), ferrugenol (6.0 %).

Isolates were also tested against four bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*), and two fungi (*Saccharomyces cerevisiae* and *Candida albicans*), using the Kirby Bauer disk-diffusion method. All bacteria were susceptible to the *C. sempervirens* volatiles isolates.

Antioxidant activity of the isolate was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method together with two antioxidant standards, butylated hydroxytoluene (BHA) and tert-butyl-4-hydroxy toluene (BHT). The results showed antioxidant effect of all isolates less significant as BHA and BHT.

OP-11. Geraniol restores antibiotics activities against multidrug-resistant isolates from *Enterobacter aerogenes*

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The constant use of antibiotics in the hospital environment has selected bacterial populations that are resistant to many antibiotics. In particular, *Enterobacter aerogenes*, a commensal Gram-negative bacterium of human intestinal flora, has been rapidly emerging as an important nosocomial pathogen with an increasing frequency of isolates resistant to many antibiotics and antiseptics. Efflux systems provide efficient extrusion of antibiotics that contribute to multidrug resistance (MDR) phenotypes of bacteria. An important medical challenge is to identify compounds capable of circumventing MDR by the inhibition of efflux systems.

The aim of this study was to screen essential oils obtained from Corsican plants that modulate antibiotic resistance of Gram-negative bacteria. We demonstrated that fractions isolated from the essential oil of *Helichrysum italicum* significantly reduce MDR of *Enterobacter aerogenes*. Geranoil was identified as an active compound of the essential oil and significantly increased the efficacy of various antibiotic classes, including ß-lactams and quinolones in addition to chloramphenicol. To understand the relationship between the structure of geraniol and its inhibitory activity, we have extended our study to examine compounds with similar structure to geraniol. We also compared the antibacterial activity of geraniol and derivatives like geranylamine on two strains of Enterobacter, a clinical MDR isolate and its *acrAB* deletion mutant. Geranylamine represents a new type of efflux pump inhibitors that are not substrates but that are able to block antibiotic transport.

Such knowledge may facilitate the development of new synthetic drugs that will be able to decrease the efficiency of the antibiotic resistance mechanisms in human and animal pathogens.

OP-12. The regulatory framework for the use of essential oils, as herbal medicinal products, in the EC. An up-to-date scientific view

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All over the world medicinal plants and their essential oils have been used therapeutically for centuries while many scientific studies are conducted around the world, describing their remarkable healing properties. It is well known, that the chemistry of essential oils is influenced by the local geography and weather conditions, as well as the season and time of day when the plants are harvested, how they are processed, and how they are packaged and stored.

The essential oils can be used mainly to be applied to the skin (the oil is diluted in a liquid carrier and applied to a dressing or directly to the affected area); to be inhaled; to be gargled and ingested as well as to be used as bath additives (drops of the oils are added to bath water). These methods of administration result in absorption through the skin and oromucosa, as well as inhalation of the volatilized oil.

The European Union has recently considered medicinal use of essential oils as herbal products through mainly the Traditional Herbal Medicinal Products Directive (Directive 2004/27EC amending Directive 2001/83/EC as regards THMPs). The Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA, London) has drafted and adopted a couple of guidelines which are intended to support assessment of (traditional) herbal medicinal products considering their particular characteristics. One of the major tasks of the HMPC is to establish community monographs and list entries of herbal substances. Currently, about 12 monographs on essential oils (fennel, anise, peppermint, thyme, rosemary, lavender, juniperus, cinnamon, clove, eucalyptus. tea-tree and chamomile), have been finalised and are available at EMA's website. In these monographs, the accepted quality, as well as the finally adopted indications among EU countries, together with potential risks, adverse drug reactions and contraindications in their use are presented; based in their longstanding medicinal uses and European experience (1,2). A viewpoint of the regulatory Authorities Experience in EU will be discussed, in details, through several examples.

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OP-13. Smoking desert plants: the highly diverse character of medicinal, chemosemiotic or psychoactive volatiles in Australian Aboriginal ethnopharmacology

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Lacking the technology to produce hydrodistilled essential oils in the conventional sense, the Australian Aboriginal people practiced fumigation or inhalation modalities to mobilise and employ the therapeutic effects of bioactive volatiles. Although the Aboriginal people in prehistoric times viewed disease and medicine in an entirely different way to that informed by contemporary scientific theory, there is now an emerging recognition that specific compounds present in the smoke contribute to therapeutic benefits. Smoke fumigation or inhalation rituals were used to achieve effects consistent with chemosemiosis in women related to fertility and childbirth, psychoactive or antinociceptive activity, decongestion in chest infections and antimicrobial, antiviral and anti-inflammatory activity. In many such applications the chemical character of volatiles is almost always the same as the essential oils, but since it has been shown that the delivery of essential oils quantitatively influences measures of biological activity, fumigation rituals are expected to produce therapeutic outcomes that are not the same as those produced using the unaltered hydrodistilled essential oils. One reason for this is that volatiles have increased antimicrobial activity if delivered in warm air. Another reason is that hydrophilic compounds, which are dissolved in the hydrosols and hence lost in hydrodistillation, can be present in smoke during fumigation rituals. Furthermore, both volatile and fixed components may be altered chemically during partial pyrolysis, thus profoundly affecting their bioactivity and therapeutic potential.

In our laboratory we have utilised various new and unusual methods to attempt to measure the significance of these factors, using many desert plants in the Aboriginal *materia medica* of Australia, which were used in smoke fumigation or inhalation rituals for various therapeutic effects. We have employed a thermocycler to investigate the influence of temperature gradients on bacterial cultures treated with condensates derived from laboratory controlled smoke trials aimed at simulating custom use. The chemical and biological character of smoke condensates was investigated, revealing a number of novel derivatives with significant bioactivities. In addition, larger molecular mass molecules, not normally present in essential oils, were also detected in smoke condensates. In summary, heat derived artefacts of partial pyolysis and larger molecular mass volatiles not normally seen in orthodox hydrodistilled oils may complement and/or synergistically enhance essential oil components in therapeutic effects achieved in smoke fumigation or inhalation rituals.

OP-14. Conventional and enantioselective GC microfabricated columns versus FSOT columns in the analysis of essential oils

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Microfabricated columns are highly attractive for gas chromatography because of the number of possible applications in particular for on-site environmental monitoring and in-field analysis. Microfabricated columns are therefore ideal for mobile micro GC instruments because of their small size and low thermal mass affording rapid temperature programming with relatively low power. Silicon micro-electromechanical system (MEMS) technologies are often adopted for microfabricated column preparation.

This study adopts etched silicon columns (equivalent, for both pneumatic impedance and chromatographic performance, to a 100 µm dc conventional capillary column) fabricated in silicon substrates that are statically coated with a 0.1-0.2-µm-thick film of different stationary phases microfabricated columns 1.68 and 3.60 m long coated with conventional polysiloxane, polyethylene glycol and 30% 2,3 diethyl – 6 *t*-butyldimethylsilyl-β-cyclodextrin in PS-086. All columns showed an efficiency comparable to that of corresponding conventional columns, i.e. 10000 theoretical plates per meter for SE 52 and 7000 for CD columns respectively using hydrogen as carrier gas.

The performance of the resulting microfabricated columns were tested with a conventional GC system and their performance compared to those of conventional columns of the same length and phase ratio and under the same analysis conditions. Comparison was carried out by analyzing a set of essential oils from different plants and evaluating the enantiomer separation of racemate standards.

OP-15. Introducing supercritical fluid extraction - A green extraction technique

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Supercritical Fluid Extraction (SFE) Systems extract chemical compounds using CO_2 in its supercritical state in place of organic solvents. The result is an extract with little or no residual solvent, superior purity and yield, and lower operating costs compared to traditional hydrocarbon-based solvent extraction systems. SFE provides a faster, safer and cleaner technology for analysts from the food, natural product, flavor, fragrance, pharmaceutical, nutraceutical, polymer, and chemical industries.

The supercritical fluid state occurs when a fluid is above its critical temperature (Tc) and critical pressure (Pc), when it is between the typical gas and liquid state. This supercritical state allows CO_2 to take the properties of a gas (high diffusivity, low surface tension), as well as maintaining the solvent power of a liquid. Manipulating the temperature and pressure of the fluid influence its density which can solubilize the material of interest and selectively extract it. The sample is placed in an extraction vessel and pressurized with CO_2 to dissolve the sample. Transferred to a fraction collector, the contents are depressurized and the CO_2 loses its solvating power causing the desired material to precipitate. The condensed CO_2 can be recycled.

SFE has been around for many years. Although the selectivity benefits and the cost-effectiveness of the technique have become widely recognized, one of the most tedious steps of developing a SFE method, is the screening of conditions (P, T, flow composition...), because of the lack of small scale automated instrumentation.

Recently, Waters introduced the MV10-ASFE instrument, the SFE system offering accelerated, multi-vessel sample extraction, resulting in a faster, greener, more selective alternative for analyte extractions from a wide variety of sample matrixes. The MV-10 ASFE can rapidly screen for conditions that can then be scaled-up for larger process or simply process small quantities of different samples.

During this oral presentation, you will have the opportunity to understand the basics of Supercritical Fluid Extraction, its range of application and the associated benefits.

OP-16. Average-mass-scan-of-the-total-ion-chromatogram (AMS) profiling of essential oils – a useful tool for tracking storage-induced changes. The case of *Artemisia alba* Turra essential oils

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Evaporation and chemical transformations of the native plant volatile constituents are known to occur during the storage of plant material. The duration of plant material storage can have a profound effect on the chemical composition of the essential oils isolated from that material [1]. Having in mind the economical, commercial and pharmacological importance of essential oils, the development of a method that would enable fast, easy and reliable tracking of these storage-induced changes is of high importance. We have recently shown that AMS (average mass scans of the total ion chromatogram) profiles-these are readily available from standard GC-MS instruments-of essential oils may be used as their "fingerprints" [1] and, in combination with multivariate statistical analyses (MVA), could be used to explore similarities/dissimilarities between essential oil samples. The aim of this work was to investigate whether AMS profiles are suitable for tracking storage-induced changes in the plant volatile profile (essential-oil chemical composition). To assess this, we have collected *Artemisia alba* Turra (Asteraceae) plant material and divided it to several independent batches. These were kept under controlled conditions during different storage periods (0 – 270 days) and then subjected to hydrodistillation. The essential oils obtained in this way (S1-S6) were analyzed by GC and GC-MS analysis; for every oil sample, we have generated an AMS profile [1]. The dominant constituents of S1-S6 oils were as follows: yomogi alcohol (3.3-11.1%), 1,8-cineol (1.5-7.0%), artemisia alcohol (2.6-18.3%), artemisia ketone (8.0-24.0%), camphor (7.8-27.2%), silfiperfol-5-en-3-ol A (tr-9.2%), unidentified I (1.9-17.0%), unidentified II (0.6-5.3%).

Both GC profiles (essential-oil compositional data) and AMS profiles of S1-S6 oils were mutually compared by means of MVA analyses (variables: relative abundances of individual oil components ("traditional" variables), i.e. relative abundances of the appropriate AMS *m/z* values). Both traditional and AMS MVA approaches gave comparable results and pointed out that during the storage of the (already dried) plant material, volatile plant metabolites could undergo chemical transformations to a substantial extent (e.g. pronounced differences in the relative abundances of artemisia alcohol, artemisia ketone, etc.; presence/absence and changes in the relative abundances of selected *m/z* values). The same *A. alba* oils were re-analyzed (GC, GC-MS) after storage of the oils for 27–36 months (S1'-S6'). The results of the mentioned analyses showed that although there were some noticeable compositional differences between the corresponding "pairs of oils" (S1/S1', etc.), these were much less pronounced than the changes in the volatile profiles that occurred during the storage of plant material.

Coherence in the GC-MVA and AMS-MVA results proves that the AMS approach is a promising tool for fast and reliable tracking of storage-induced changes of plant volatiles. It takes only several minutes to generate an essential-oil AMS profile, while the identification of every component of complex essential oils is much more time consuming.

Acknowledgments

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OP-17. Antioxidant activities of essential oil components - An experimental and computational investigation

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The antioxidant activities of eighteen different essential oil components have been determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation assay, and the ferric reducing antioxidant power (FRAP) assay. The phenolic compounds, carvacrol, thymol, and eugenol, showed the best antioxidant activities, while camphor, menthol, and menthone were the least active. The structural and electronic properties of the essential oil components were assessed using density functional theory (DFT) at the B3LYP/6-311++G** level. Correlations between calculated electronic properties and antioxidant activities were generally poor, but bond-dissociation energies (BDEs) seem to correlate with DPPH radical-scavenging activities, and the ferric reducing antioxidant power (FRAP) assay correlated with vertical ionization potentials calculated at the Hartree-Fock/6-311++G** level.

YOUNG SCIENTIST LECTURES

YSL-01. Inactivation dynamics of *Listeria monocytogenes* and *Salmonella enterica* during exposure to selected essential oils

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The antimicrobial activity of essential oils (EOs) is well established, however few studies are focused on the inactivation kinetics, which are instead helpful to optimize antimicrobial treatments.

Twenty-one commercial EOs were screened for antimicrobial activity against 10 *Listeria monocytogenes* and 10 *Salmonella enterica* strains. The inactivation kinetics of the most resistant strains in presence of the most effective EOs was investigated.

According to disk diffusion and Minimal Inhibitory Concentration (MIC), *Origanum vulgare*, *Caryophillus aromaticus* (clove), *Cinnamomum zeylanicum* (cinnamon), *Thymus vulgaris* (red thyme) and *Melaleuca alternifolia* (Tea tree) EOs were the most effective against both microorganisms, although different sensitivities among strains were highlighted. In detail, oregano EO was particularly effective, with MIC values between 0.6 and 1.2 µl/ml, while tea tree showed MIC up to 20.0 µl/ml for *Salmonella enterica*.

Inactivation kinetics of *L. monocytogenes* ATCC 7644 and *S.* Derby S1 in presence of MIC/4, MIC/2, MIC, MIC x 2, MIC x 4 EOs concentrations were determined by means of automated turbidimetry. The data obtained were modelled by means of Baranyi, Roberts and McClure (1993) equation. Red thyme and oregano at MIC concentration exerted a bacteriostatic activity on *L. monocytogenes*, causing lag phase extension and growth rate decrease, if compared to control, while for concentrations above the MIC value, cells death was observed, as characterized by negative μ_{max} value (death rate). After treatment with cinnamon, tea tree and clove EOs, a bactericidal effect already at the MIC concentration was observed. Regarding *Salmonella,* red thyme, cinnamon, tea tree and clove at MIC concentration achieved a bactericidal effect, while oregano had only a bacteriostatic effect, with a lag phase extension and a decrease of growth rate and maximum growth value.

Among the twenty-one tested, five EOs were particularly effective against *L. monocytogenes* and *S. enterica*. These EOs exerted bactericidal or bacteriostatic effect at very low concentrations and modified growth dynamics.

Inactivation dynamics provide highlights on the specific effect of EOs on growth parameters, such as lag phase and maximum growth rate and values. Results could be useful to develop control strategies based on natural antimicrobial agents that exert antimicrobial activity at low concentrations.

YSL-02. Key odorants of vetiver and atlas Cedarwood: Analytical studies and structure-odor relationships

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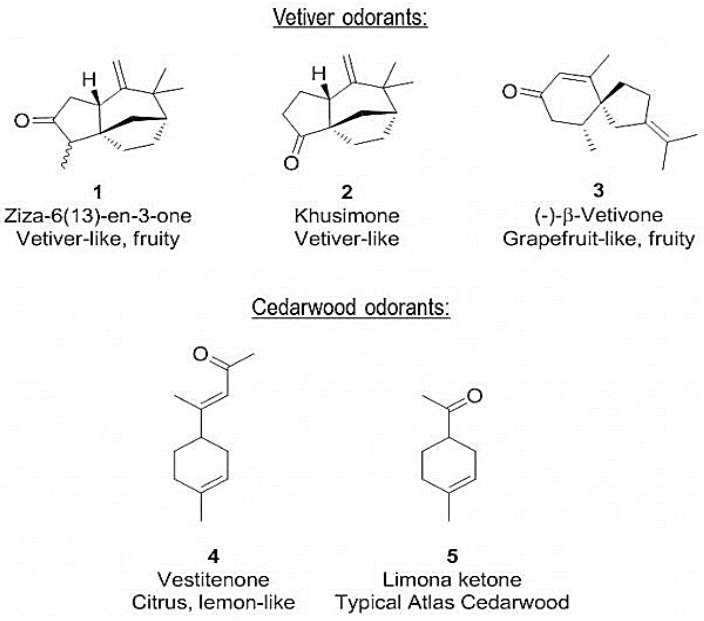
The identification of key odorants in natural extracts is often a first step for the discovery of new fragrances. Indeed, they constitute a rich pool of odorant ingredients and are also a source of inspiration for the synthesis of new compounds for perfume formulations. These syntheses are usually based on the evaluation of the olfactory properties of structural analogues, to better understand their Structure-Odor Relationships (SOR).

The woody natural raw materials are extremely important perfumery ingredients, and vetiver essential oil is commonly incorporated to contribute to base notes. Atlas Cedarwood is another interesting natural extract, with a characteristic sweet odor bringing original facets in some perfume formulations. GC-O studies have shown that the main odorant constituents of Vetiver and Atlas Cedarwood essential oils are respectively sesquiterpenic derivatives such as **1**-**3**[1] and the norterpenic ketones **4**-**5[**2]. In the course of our SOR studies, we synthesized several analogues of the zizaanes **1-2** by taking advantage of our new methodology for the large scale preparation of khusimone **2**[3]. The olfactory character and relative potencies of these analogues were characterized by means of GC-O.



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YSL-03. Toxicity of thujone and thujone-containing medicinal plants? The case of *Salvia officinalis, Artemisia absinthium, Thuja occidentalis* and *Tanacetum vulgare* essential oils

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For decades thujone diastereomers have been regarded as key toxic ingredients of many essential oils, mainly on the basis of past notoriety initiated by the phenomenon of "absinthism". It is still uncertain whether thujones truly cause the symptoms of so-called "absinthism", but it is shown that neurotoxicity is the principal toxic outcome in several animal models with convulsions as the most prominent symptom. In this study, we wanted to test whether this widespread conviction is justified. Thus, we evaluated the toxic effect of α- and β-thujone and the essential oils of four medicinal plants (*Salvia officinalis* L., *Artemisia absinthium* L., *Thuja occidentalis* L. and *Tanacetum vulgare* L.), that have these isomeric monoterpenones in differing quantities, on both prokaryotic and eukaryotic cells in order to possibly correlate the toxicity of the oils with the amount of thujones present in the oils. Additionally, to probe the effect of thujone and thujone-containing essential oils on the central nervous system, thujones and the oils were evaluated in several *in vivo* rodent models (light/dark, open filed, and diazepam-induced sleep tests) that include activation of the central nervous system.

According to GC-MS analyses the relative percentages of α- and β-thujone were as follows: 28.2 and 5.1% in *Salvia officinalis*, 0.8 and 3.4% in *Artemisia absinthium*, 51.8 and 5.3% in *Thuja occidentalis*, and 0.9 and 66.6% in *Tanacetum vulgare* essential oil. A panel of bacterial laboratory control strains and isolates were used as model organisms for testing toxicity in prokaryotes and the results revealed that there is no clear link between the amount of thujones and the observed toxicity. As with prokaryotes, the toxicity of the tested oils in two eukaryote organisms, the fungus *Candida albicans* and brine shrimp *Artemia salina*, could not be positively related to the amount of thujones in the oils. The results of the light/dark tests suggested that almost all tested oils, as well as the pure thujones, possessed an anxiolytic effect. However, when the results from this test were compared with those obtained from the open-field test, we could conclude that the mentioned observed anxiolytic activity arises from the hypnotic/muscle relaxing/paralaysing activity of the oils (especially in higher doses) and the thujones. The only essential oil that can be regarded as a putative anxiolytic is *T. vulgare* oil.

Hence, it appeared that other constituents except thujones contribute to the overall toxicity of the tested oils and that title of "the main toxic principle" is often too straightforwardly unreasonably assigned to thujone. Both the oils and the thujone isomers have demonstrated their significant impact on the central nervous system and these results at least partially justly the notion that thujones are among the psychoactive constituents of these oils.

Acknowledgments

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YSL-04. The elusive floral scent of *Daphne blagayana* Freyer (Thymelaeaceae)

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Daphne blagayana Freyer (Thymelaeaceae) is an evergreen low-growing shrub native to the mountains of south-eastern Europe and adjacent regions. This very rare and endangered plant species, also known as "king's flower" or "Balkan Daphne" ("Jeremičak" in Serbian), usually grows on the sunny woody slopes of pine forests. It is regarded as the first harbinger of spring as it blooms very early, even when the snow is not completely withdrawn, and its ivory-white blossoms are a source of a characteristic intoxicating scent.

Although *D. blagayana* is a highly appreciated plant species because of heady fragrance of its flowers, characterized as sweet and clove-like, there are no reports of its chemical composition in the literature. In this study we performed the first GC and GC-MS analysis of headspace constituents of intact *D. blagayana* flowers in order to detect and identify its odoriferous components. Capturing the floral volatiles of this *Daphne* species turned out to be more challenging than expected. Several attempts at bringing intact plants in full anthesis to our lab failed as the individuals that were removed from its natural surroundings demonstrated a tendency to lose their floral smell. Finally, direct field sampling (at a location near Leposavić, Kosovo, Serbia) and careful transportation of the used SPME fiber resulted in a myriad of detected constituents.

Over 100 compounds were successfully identified, many of which were easily recognized as having odorous properties. The floral volatiles were made up largely of oxygenated monoterpenoids (48.9%) and benzenoid compounds (37%), with smaller amounts of fatty-acid-derived compounds (4.3%). The major components were found to be benzyl benzoate (7.6%) and linalool (5.5%). A number of linalool-related compounds were detected: *trans*-hotrienol, *cis*- and *trans*-rose oxides, diastereomers of furanoid and pyranoid linalool oxides, lilac alcohols and aldehydes, and several hydroxylinalool isomers. Along with benzyl benzoate, the following benzenoid compounds were present in appreciate amounts: *o*-guaiacol, veratrol, eugenol and its isomers, cinnamic alcohol, benzoic acid, methyl benzoate, methyl salicylate, benzyl salicylate etc. Methyl nicotinate was the only nitrogen-containing compound detected. Benzyl alcohol and the linalool oxides might well be important contributors to the heavy "note" or "sweet character" of the fragrance. Interestingly enough, species producing benzenoid esters, benzenoid alcohols, linalool and/or oxygenated monoterpenes are known to be moth-pollinated flowers [6]. This fact suggests to a possible reproductive way of *D. blagayana*.

Acknowledgments

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YSL-05. Utilization of constituents of thuja and fennel essential oils towards synthesis of novel fragrant compounds.

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A few oximes are known to possess pleasant scents and only seven of them are used in fragrances. From this group only citral and citronellal oxime belong to the terpenoid class [1]. Studies regarding Structure-Odour Relationship (SOR) of terpenoid oxime ethers are even scarcer than parental oximes. Literature data is limited to three patents [2-4], where authors describe mostly *O*-methyl ethers with various scents ranging from floral, fruity to amber and spicy. From the described compounds only citral *O*-methyl oxime belong to the terpenoid class.

We have prepared some novel fragrant oxime ethers with preserved fenchane system. Starting materials were (+)-fenchone – constituent of fennel essential oil and (–)-fenchone – from thuja essential oil. Using (+)- and (–)-fenchone as substrates, let us compare olfactory properties of both isomers of oxime ethers from the same series. Synthetic details and olfactory evaluation of all novel oxime ethers will be presented. Significant fragrance diversity is observed between homologous series of fenchone oxime ethers. More agreeable are scents of ethers derived from (–)-fenchone oxime. Their odours range from turpentine like and resinous to vegetable, floral or woody whereas scents of (+)-ethers range from turpentine, resinous to onion like and slightly floral.

Acknowledgments

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YSL-06. Chemical compositions, biological activities and molecular docking studies on some aromatic medicinal plants from Yemen

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The current scenario of drugs used in the treatment of diseases owes most of its part to medicinal plants, where ethnomedicine and *in-vitro* biological screening has always been a path to direct scientists for the search for new lead compounds. Essential oil and their components are becoming increasingly popular as naturally occurring bioactive agents. They are known to show a variety of effects as antibacterial and antifungal agents, chemo-preventive and cancer suppressing agents, as antioxidants, as anti-diabetic agents and many more. Keeping in mind the ethnomedicinal value, we undertook the analysis and biological study of sixteen different essential oil samples from Yemen. The volatile oils were analyzed using GC-MS and further tested for their *in-vitro* biological activity, including cytotoxic activity against MCF-7 and MDA-MB-231 human tumor cell lines, acetylcholinesterase (AChE) inhibitory activity, antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and antifungal activity against *Aspergillus niger*, *Botrytis cinerea*, and *Candida albicans.*

Lavandula pubescens, *Tecrium yemensis*, *Stachys yemensis*, *Otostegia fruticosa*, and *Plecranthus barbatus* showed very good activity against both the cancer cell lines. Although most of the oil samples showed some AChE activity, two samples of *Lavandula pubescens* and *Plecranthus barbatus* showed promising AChE inhibitory activity. This can be attributed to the presence of high concentration of carvacrol in these oils, which has been shown to be a good AChE inhibitor in other studies as well. Other major compounds present in these oil samples were also studied for potential AChE activity through molecular docking studies.

In conclusion the ethnomedicinal importance of some of the tested plants could be correlated to their *in-vitro* biological activities. This study has paved a way for the present and future studies that we will undertake with these oil samples. We are currently studying the bacterial bio-film inhibition as well as potential synergistic activities of the major components.

YSL-07. Apoptosis inducing abilities of *Ocimum sanctum* essential oil in *Candida albicans*

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Manipulation of endogenous responses during programmed cell death (PCD) in fungi can lead to the development of effective therapeutic strategies. In the present study we evaluate the physiology of cell death in *Candida albicans* in response to *Ocimum sanctum* essential oil (OSEO) at varying inhibitory concentrations. Apoptotic cell death was studied on the basis of externalization of membrane phosphatidylserine (PS) revealed by annexin-V-FITC labeling; morphological alterations revealed by transmission electron microscopy; and DNA fragmentation by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assay. Exposure of fungal cells to MIC/4 of OSEO resulted in morphological features characteristic of apoptosis while necrosis was observed at higher concentrations. Necrotic cells displayed reduced TUNEL staining and an inability to exclude propidium iodide. Besides, they lacked a defined nucleus and an intact external morphology. Exposed cells were TUNEL positive, showed chromatin condensation and margination, nuclear envelope separation, nuclear fragmentation, cytoplasmic shrinkage and plasma membrane blebbing. A dose dependent decrease in cytochrome *c* oxidase activity was observed with the essential oil but the decrease was not comparable to that elicited by H_2O_2 eliminating the primary involvement of cytochrome *c* release in the apoptosis so induced. Previously reported data clarifies induction of apoptosis at low concentrations as a result of oxidative insult. Studies aimed to identify other mitochondrial factors activated in this course to mediate apoptosis will further elaborate mechanism of vast antifungal action of this essential oil.

YSL-08. I*n vitro* antimicrobial activity of *Cymbopogon martini* essential oil and its synergystic interaction with silver ions

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Cymbopogon martini (Palmarosa) essential oil is known to exhibit antimicrobial activity. Metals have been described to be effective antimicrobial agents, and the efficacy of silver ions as a disinfectant has been also known for centuries. In the present study, the *in vitro* antimicrobial activity of *Cymbopogon martini* essential oil (CmEO) alone and in combination with silver ions (Ag^+) against two Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*), two Gram-negative (*Escherichia coli* and *Morexella cattarhalis*) and two yeast species (*Candida albicans* and *C. tropicalis*) is presented. The nature of the interaction was studied by determining fractional inhibitory concentration indices (FICIs) for CmEO and Ag^+ calculated from microdilution assays. Minimum inhibitory concentration (MIC) results depicted that all the tested pathogens are susceptible to both CmEO as well as Ag^+ to variable degrees as shown in the Table below. The MIC of CmEO and Ag^+ against *E. coli* was 0.125 mg/ml and 0.008 mg/ml respectively, whereas when assayed in combination the FICI value was 0.266 indicating synergy. Similarly, all the other tested pathogens except *S. aureus* ($\sum$FIC=0.52) showed synergism when exposed to combinations of CmEO and Ag^+. The most prominent interaction was observed against *C. albicans* with $\sum$FIC=0.141. Results suggested that CmEO and Ag^+ when used in combination reduce the effective concentration required to control important infectious pathogens and can be implicated in the formulation of EO based antimicrobials.

YSL-09. Characterization of the aromatic quality of Tunisian orange blossom water essential oils: comparison between traditional and industrial extraction processes

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Neroli essential oil extracted from sour orange flowers through hydrodistillation is extensively used in fragrance, flavour and pharmaceutical industries and in aromatherapy. During hydrodistillation, a part of the essential oil is dissolved in condensate or distillation water and is lost as this water is discarded. The aim of this work was to characterize the chemical profile of recovered oils from distillation water obtained by either traditional or industrial processes. Essential oils from sour orange *Citrus aurantium* L. flowers were extracted by both artisanal and industrial distillation processes. In order to get a complete extraction of essential oils from flowers, the isolation of dissolved essential oil (recovered) from the distillation water was performed using a method employing hexane as an extractant, and analyzed using GC and GC–MS.

Twenty-one components were detected and identified in Neroli primary essential oil, and were predominated by linalool (39.4%), and terpenes (38.6%). Eighteen compounds, comprising 98.5% of the total recovered oil obtained by the industrial process, and a total of 13 volatile compounds in oil (91.7%) obtained by the traditional process were identified. The major components in industrial and traditional orange blossom water recovered oils, of linalool (respectively 78% and 68%), linalyl acetate (respectively 11.7% and 12%), geraniol (respectively 0% and 6.8%), α- terpineol (respectively 2.4% and 0.1%) and nerol (respectively 0.8% and 2.1%) were found. No terpenes were found in recovered oils.

These results may indicate that recovered oils are richer in organoleptically important oxygenated compounds such as alcohols, esters, aldehydes, than the Neroli and recovered essential oil of differing qualities can be obtained according to the hydrodistillation process.

POSTER PRESENTATIONS

PP-001. Assessing *Nigella sativa* (Black Seed) variability using NMR-based metabolomics

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The medicinal plant *Nigella sativa* L. (NS) is used traditionally for different diseases, including diabetes, hypertension and allergy. Its metabolites may vary depending on the origin of NS, production and processing methods. NMR-based Metabolomics offers a novel technology platform for plant extract analysis that has not been applied for NS.

Using H^1-NMR spectroscopy combined with Principal Component Analysis (PCA) to establish a robust and reproducible method for the metabolomic analysis of NS products, investigate potential variations between same and different species of Nigella metabolites, and identify chemical quality markers.

Twenty three seed and six oil samples were collected from thirteen different global locations. Four different NMR solvents were evaluated to determine suitable extraction technique. H^1-NMR combined PCA using SIMCA statistical software was utilized for exploring significant variability among the samples.

Methanol-D4 was found to be a suitable solvent for seed samples, while chloroform-d for NS oil samples. Linoleic acid was selected as a key phytochemical marker. PC-1 vs PC-2 PCA score plot (Pareto scaling) of seed samples showed significant deviation (outlining the 95% confidence level) of two samples. However, PC-2 vs PC-3 score plot (UVN scaling) was able to distinguish between different species. PC-1 vs PC-2 plot (Pareto scaling) for oil samples had two slightly deviated samples.

An innovative economical technique for NMR-based metabolomic analysis of NS products has been established. This methodology was able to identify some variations from the others in five samples of seeds and two samples of oil, discriminating between different species and identifying fraudulent products. In the future, these metabolomic fingerprints may be used as a base for developing a validated PCA model for the quality evaluation of NS products. More sample size and different NS product forms are needed in further studies as well as a comparison with validated methods.

PP-002. Biosynthesis sites and chemical composition of essential oils of *Aldama kunthiana* and *A. tenuifolia* (Asteraceae)

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This study aimed to analyze the chemical composition and to indicate the sites of biosynthesis of *Aldama tenuifolia (Gardner)* E.E.Schill. & Panero (=*Viguiera tenuifolia*) and *A. kunthiana* (Gardner) E.E.Schill. & Panero, (=*Viguiera kunthiana*). They are morphologically similar and occur in open physiognomies of the Cerrado domain, which are characterised by intense solar irradiation, frequent fires and water scarcity during the autumn and winter. The samples were collected and separated into roots, xylopodia, stems and leaves. Anatomical analyses were performed using usual histological techniques. The extraction was carried out by hydrodistillation in a Clevenger-type apparatus, analysed by gas chromatography coupled with mass spectrometry and the compounds were identified by comparing the mass spectra with library data. The biosynthesis sites of the essential oils comprised the glandular trichomes in the leaves, the accumulation of lipid droplets in the chlorophyllous parenchyma cells of *A. tenuifolia* leaves, and internal secretory spaces, in all analyzed organs. The secretory spaces consisted of simple or multiseriate epithelium and variable diameter. In the leaves, they are associated with the midrib and the veins, but in the stems they occur in the cortical parenchyma region and medullary parenchyma. In the xylopodia occur in the cortex and secondary phloem and medullary parenchyma, and in the roots they were observed in the cortical region, in the secondary phloem and in the expanded pith of the tuberized roots. The EO's yield (% w/w) in *A. tenuifolia* and *A. kunthiana* was 0.01 and 0.04 in leaves, 0.04 and 0.01 in aerial stems, 0.10 and 0.04 in roots, and 0.02 and 0.12 in xylopodia. The EO's from the both species are characterized by the presence of mono, sesqui and diterpenes. Monoterpenes represent the largest component of EO's obtained from stems and roots of *A. tenuifolia* (53.52% and 49.72%) and from roots of *A. kunthiana* (60.51%). EO's from the leaves of both species and from the stems of *A. kunthiana* primarily consisted of sesquiterpenes (64.49% to the *A. tenuifolia* leaves and 86.53% and 34.41% to the leaves and stem of *A. kunthiana*). Diterpenes were the major compounds in the EO's from the xylopodia in both species, representing 70.21% to *A. tenuifolia* and 72.51% to *A. kunthiana*. The most representative monoterpenos in the EO's from *A. tenuifolia* were β-phellandrene, β-myrcene, α- and β-pinene, δ-3-carene and β-t-ocymene. For A. kunthiana, α-pinene represents 76.38% of the total of the monoterpenes in the EO's from the roots. The major sesquiterpenes compounds found in the EO's of A. tenuifolia and A. kunthi*ana* were germacrene D and t-caryophilene for both species, α-isocomene exclusively to *A. tenuifolia* and cyperene only in the EO from *A. kunthiana*. Some compounds were unique for each species: β-phellandrene, β-patchoulene, α-isocomene and longifolene-(V4) for *A. tenuifolia* and only the ciperene for *A. kunthiana*. The range of compounds indentified provides a characteristic chemical profile for each one of these species and can thereby assist with the differentiation of them. Besides that, several compounds identified in the essential oils of the studied species appear to possess various biological activities in phytochemical studies.

Acknowledgments

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PP-003. Characteristics of SFE CO_2 extracts manufactured from some plants cultivated in Poland

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Humulus lupulus L. (common hop), *Fragaria ananassa* L. (strawberry) and *Ribes nigrum* L. (blackcurrant) are plants often cultivated in Poland. These plants provides valuable ingredients for food and cosmetic industry. *H. lupulus* is a flowering plant from *Cannabaceae* family. Common hop cone (inflorescence) contains essential oil, resins, tannins and bitter substance hens there are used for the beer production.

F. ananassa is a species represented by many varieties, which belong to *Rosaceae* family. Strawberry fruits became one of the most popular food component during the summer months in Poland. *R. nigrum* is a shrub plant, which belongs to *Grossulariaceae* family. Whole plant of *R. nigrum* delivers many products, such as tasty, vitamin C rich fruits, buds essential oil as well as cold pressed seed oil. Strawberry and blackcurrant seed extracts as well as common hop cone extract are considered as precious ingredient for cosmetic and food industry.

Common hop cone extract, strawberry and blackcurrant seed extracts were produced by means of Supercritical Fluid Extraction (SFE) with carbon dioxide as a solvent. Production process was provided by Fertilizer Research Institute in Pulawy in Poland. In these three products the composition of fatty acids, volatile compounds, tocopherols and phytosterols as well as antioxidant properties were determined.

Strawberry and blackcurrant seed extracts were greasy liquids characterized by low viscosity whereas the common hop cane extract were thick substance with high viscosity. The main group of components in strawberry and blackcurrant extracts were fatty acids. The content of these compounds were 950 mg/g and 800 mg/g respectively. Among fatty acid the highest amounts of constituents classified as EFA e.g. α-linoleinic acid and linoleic acid were determined.

There was determined that analyzed extracts contains phytosterols. The total content of sterols amounted to 4.5 mg/g, 5.5 mg/g and 2.4 mg/g, in *F. ananassa*, *R. nigrum* and *H. lupulus* extract, respectively.

Common hop extract reveal very high content of volatile oil about 1840 mg/100g. In this product the presence of 87 volatile organic compounds (VOCs) were confirmed. The total content of volatiles in strawberry extract was 27 mg/100g and 127 VOCs were identified. Pleasant strawberry scent of SFE extract could be explained by the presence of large quantity of γ-decalactone. In blackcurrant extract, 99 volatile constituents were determined and the content of volatiles was 13 mg/100g.

According to our results, strawberry and blackcurrant seed extracts produced by SFE with CO_2 as a solvent are very valuable mixtures, which contain many biologically active compounds. Therefore, they can be used as precious ingredients in cosmetic production, in particular for ecological and organic products.

Acknowledgments

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PP-004. Homologues of citronellol, grapefruit odorants and their bioactivity

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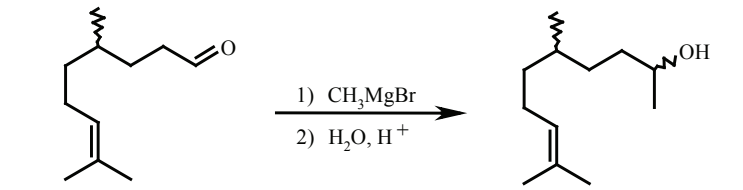
Nootkatone is olfactory one of the most important grapefruit essential oil component. Its scent is described as grapefruit, citrus, sweet with a woody nuance [1]. Because of its organoleptic properties,it is a desirable component of flavour and fragrance. On the other hand, isolation of natural and synthesis of nature-identical nootkatone is expensive. Compounds having similar to nootkatone odour characteristics are successfully used, for example: 4,7-dimethyloct-6-en-3-one, 2,2-dimethyl-4-phenylpentanenitrile (grapefruit nitrile), isobutyl *N*methylantranilate, 2,4,8-trimethylnon-7-en-2-ol or the (*E*,*E*)dodeca-2,4-dienal.

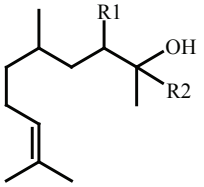
During our research, as one of the intermediate product to the synthesis of acyclic sesquiterpene analogues we have obtained alcohol **1**, as it turned out to have a pleasant citrus-grapefruit scent similar to nootkatone. Due to its attractive organoleptic properties and uncomplicated method of obtaining, we have decided to synthesize and describe all diastereoisomers of alcohol **1**. Obtained diastereoisomeric mixtures were separated via enzyme-catalysed resolution.



4,8-dimethylnon-7-enal — 5,9-dimethyldec-8-en-2-ol

1

We have alsoobtained a series of aliphatic alcohols with the general formula:



R1 = H, Me
R2 = H, Me, Et

Summarizing, six new compounds were obtained. All of them could be characterised as an odour mimic of nootkatone. Synthesised alcohols were subjected to study of an antimicrobial activity.

Acknowledgments

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PP-005. Composition of Scots Pine hydrolate in the relation to the time of distillation

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Scots pine (*Pinus sylvestris* L.) is the most common pine species in Europe, northern Asia and northern America. Scots pine cetin (twigs with needles) is valuable raw material for desired Pine sylvestris oil production. This oil according to European Pharmacopoeia is suggested as a treatment especially for respiratory system diseases. Pine oil and pine hydrolate can be used as ingredients in pharmaceutical, cosmetic, and household applications. Pine oil was produced in Poland for many years but have not been any longer. However, it is worth to reconsider the production of both pine oil and hydrolate that is characterized by pleasant woody scent. While composition and properties of pine oil are well documented, no information on composition and properties of pine hydrolate is available.

The aim of this study was to estimate the most appropriate volume (measured by distillation time) of Scots pine hydrolate produced as a by-product by industrial hydrodistillation of pine oil.

Plant material collected in 2013 in Poland (19.7 kg) was subjected to hydrodistillation and seven fractions of hydrolate (1.5 L each) were produced. Along with pine hydrolate essential oil was received that was separated from hydrolate after distillation. Representative sample of the whole hydrolate was also prepared by mixing equal volume of each fraction. The volatiles from fractions and representative sample of hydrolate were isolated by liquid-liquid extraction with diethyl ether. The essential oil and hydrolate volatiles were analyzed by GC-FID-MS and confirmed GC x GCTOF-MS.

The total content of volatiles in representative sample of pine hydrolate amounted to 230 mg/L whereas in hydrolate fractions the value was varied and changed irregularly from 140 mg/L to 280 mg/L.

Nonpolar volatiles such as mono- and sesquiterpene hydrocarbons were the main groups of constituents of Scots pine oil. Polar monoterpene alcohols were identified as dominant compounds in hydrolate. Although qualitative similarities in volatile composition of every fraction of pine hydrolate were visible, , significant quantitative differences were observed. In representative hydrolate sample and every hydrolate fraction monoterpene alcohols such as *p*-cymen-8-ol, *p*-cymen-9-ol, terpinen-4-ol and γterpineol were dominant. The first fraction was richer in more volatile compounds such as (*E*)- and (*Z*)-hex-3-en-1-ol, hexan-1-ol then other fractions. According to the obtained results distillation time significantly affects the quality of pine hydrolate.

PP-006. Chemical composition of essential oil and biological activities of *Marrubium deserti* growing in Algeria

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The genus *Marrubium* (L.) belongs to the Lamiaceae family. It comprises about 30 species indigenous to Europe, the Mediterranean area and Asia (1). In the flora of Algeria, this genus includes six species and one hybrid [2], and shows various activities such as: antigenotoxic [3] antioxidant [2-3-4] and antimicrobial [2].

The essential oil was obtained from aerial parts of *Marrubium deserti* de Noé., collected from the North fringe of the Algerian Sahara, by hydrodistillation by using Clevenger apparatus according to European Pharmacopeia (5). Then the oil was analyzed by GC-EI-MS coupled with DB-1 column and ion trap analyzer. The antioxidant potential of the essential oil of *Marrubium deserti* is evaluated using different antioxidant tests; namely, CUPRAC, DPPH, and β-carotene bleaching assays. The anticholinesterase activity was measured by the spectrophotometric method developed by Ellman with slightly modifying (6-7) against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are the chief enzymes of Alzheimer disease. The essential oil was also tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* by the agar disc diffusion and broth dilution assay.

Forty-one compounds were identified, representing 95.5% of the total oils. The major constituents of the essential oil were tetracosane (31.11%), Germacrene D (7.91%), Δ-Cadinene (6.52%), α-cadinol (6.26%) and *t*-cadinol (5.81%), and respectively. In β-carotene bleaching assay essential oil (IC_{50}: 22.14 mg/L) demonstrated good activity. The essential oil also indicated antibacterial activity, particularly against *Bacillus subtilis* (14 mm). However, the essential oil showed no anticholinesterase activity.

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PP-007. Essential oils composition, antioxidant and antibacterial activity of *Satureja baborensis* (Batt.) Briq.

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The genus *Satureja* (Lamiaceae) comprises more than 200 species of aromatic herbs and shrubs, widely distributed which includes Europe, Asia, tropical Africa, and the Americas [1]. *Satureja* oils represent sources of natural larvicidal substances [2]. The antiviral activity of savory's essential oils against HIV has been documented [3]. Essential oils of *Satureja* are used as a food ingredient in the Mediterranean region as an aromatic and flavoring agent in Algeria; the leaves of *Satureja* Algerian herbs are used to purify water and to increase the time of conservation of dry figs and sun-dried tomatoes. This study reports chemical compositions and *in vitro* antioxidant and antibacterial potentials of hydrodistilled essential oils of the fresh (FA) and dry (DA) arerial parts of the endemic species *Satureja baborensis* (Batt.) Briq. (Lamiaceae) [4]. GC and GC/MS analyses of the essential oils led to the identification of 34 components representing 99.3 % for FA and 32 components representing 98.43% for DA. The main constituents of the essential oils were limonene (FA 1.2%, DA 4.7%), piperitenone oxide (FA 7.8%, DA 18.2%), menthone (FA 9.3%, DA 1.2%), pulegone (FA 53.9%, DA 25.9%), *cis*-piperitone oxide (FA 3.0%, DA 15.1%). β-Carotene/linoleic acid assay of the essential oils showed moderate inhibition percentages (FA 58.4% and DA 61.5 %).

By the use of the disk diffusion method, the essential oils showed the best antibacterial activity against *E. coli ATCC* (FA 24 mm, DA 20 mm), *P. aeruginosa* ATCC (FA 21 mm, DA 15 mm), *S. aureus* ATCC (FA 15 mm, DA 19 mm).

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PP-008. Essential oil composition of *Centaurea stenolepis* Kerner

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Essential oil composition of *Centaurea stenolepis* Kerner flowers and stems were investigated with GC, GC/MS. Flowers and stems of *C. stenolepis* had very low essential oil yield <0.01% (v/w). Twenty eight compounds were identified in the flower oils which represent 53.5% of the oil. Main components of the flower oils include caryophyllene oxide 12.6%, hexadecanoic acid 10.6% and β-Eudesmol 7.2%. Forty compounds were identified in the stem oils which represent 74.2% of the oil. Main components of the stem oils include hexadecanoic acid 38.4% and phytol 12.9%. Both flower and stem oils were rich on oxygenated sesquiterpene hydrocarbons and fatty acids. The present study shows that flower oil of *C. stenolepis* from Turkey is rich in sesquiterpene hydrocarbons, hexadecanoic acid and stem oil is rich in higher alkanes, fatty acids unlike the previous reports. These differences observed in both studies could be related to differences in collection time, ecological factors, climatic conditions, soil conditions, methods employed in the analysis and genetical differences. However differences in the biosynthetic origins of the main compounds in both studies strongly suggest chemotype variation in this species exist. In order to determine the exact situation in this species essential oil, non-volatile secondary metabolite comparisons on samples of *C. stenolepis* from different locations should be done together with DNA comparisons and seasonal variation studies.

PP-009. Composition of the essential oil from the leaves of *Plectranthus amboinicus* (Lour.) Spreng. from the Seychelles

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Plectranthus amboinicus (syn. *Coleus amboinicus*) is an aromatic perennial plant of the Lamiaceae (Labiatae) family which grows wild in tropical Africa and Asia and is also found flourishing in the Seychelles. The leaves are strongly scented and are used for flavourings as well as for medicinal purposes. Studies on the chemical composition of the essential oil indicated major differences that may result from geographical and seasonal variations as well as from genetic, environmental or developmental causes.

In this study, the fresh leaves and twigs of *P. amboinicus* were subjected to hydrodistillation, yielding 0.18% (v/w) of essential oil. The plant material was collected in Mahé, the largest of the Seychelles islands, from a location close to the capital Victoria, during September 2011.

The essential oil has been studied by GC–MS and GC–FID, leading to the determination of more than 40 components, representing more than 95% of the total. The enantiomeric distribution of some relevant constituents has been assessed by enantio-GC through the use of a chiral cyclodextrin-based stationary phase.

The essential oil predominantly comprised oxygenated compounds, of which fenchone (30.3%) and carvacrol (18.8%) where the major constituents. Other mono- and sesquiterpenes identified included *p*-cymene (2.9%), α-terpinene (2.8%), limonene (2.6%), germacrene-D (7.5%), (E)-β-caryophyllene (6.7%), δ-cadinene (2.6%), α-humulene (2.0%), bicyclogermacrene (1.6%) and β-selinene (1.2%).

Furthermore, chiral analysis revealed that the enantiomeric distribution of fenchone in *P. amboinicus* essential oil is (+)-fenchone (100%): (–)-fenchone (0%). As a result, this essential oil would be an excellent source from which to isolate pure (+)-fenchone.

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PP-010. Essential oil composition and secretory structures of the aerial organs of four Brazilian *Aldama* species (Asteraceae)

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Aldama La Llave (formerly *Viguiera* Kunth) genus, which comprises the species of this study, belong Asteraceae family. To the plants of this family, it is related the occurrence of secretory structures, which are responsible for the production of secondary metabolites. In the *Aldama* genus representatives, the secretory structures usually found are glandular trichomes and internal spaces. These structures produce lipophilic substances, especially essential oils (EOs). The identification of chemical compounds could assist in survey of bioactive compounds and studies from our research group have demonstrated the medicinal potential of the EOs from *Aldama* species. The species of this study are *Aldama bakeriana*, *A. discolor*, *A. grandiflora* and *A. squalida*, which were chosen due to their resiniferous potential. The essential oils were obtained from the vegetative organs, from different populations for each species, by hydrodistillation in a Clevenger-type apparatus, analysed by gas chromatography coupled with mass spectrometry and by gas chromatography coupled with flame ionization detector. The compounds were identified by comparing the mass spectra with library data. For the anatomical analysis, samples of leaves and stems from each species were processed according to usual techniques. The EOs yield (% w/w) varied among the species, with the highest essential oil yield observed in *A. grandiflora* leaves (0.18%) and the minor values in the leaves and stems from *A. bakeriana* (0.01% in both). The EOs from the leaves and stems of the four analyzed species were characterized primarily by sesquiterpenes that represented, respectively, 89.4 and 74.0% in *A. bakeriana*, 69.4 and 41.9% in *A. discolor*, 77.7 and 69.8% in *A. grandiflora* and 87.7 and 44.4% in *A. squalida*. It was identified 30 compounds to *A. bakeriana* EOs and the major compounds were *trans*-caryophyllene, germacrene D and bicyclogermacrene. To *A. discolor*, 38 compounds were identified and the major to this species were spathulenol, viridiflorol, intermedeol<neo> and intermedeol. *A. grandiflora* also had 30 identified compounds, of which β-phellandrene, germacrene D, bicyclogermacrene and α-cadinol were the most representatives. Finally, 31 compounds were related to the EOs of *A. squalida* and stood out as major α-pinene, germacrene D, 10s,11s-himachala-3(12),4-diene, bicyclogermacrene, spathulenol and pimaral. The secretory structures found in the four analysed species comprised two types of trichomes and the canals, as well. The capitate glandular trichome (CGT) was observed only on the abaxial surface of the leaves, whereas the linear glandular trichome was distributed on the two leaves surfaces. To the stems, these trichomes were observed only on the younger parts. Secretory canals in the leaves are always associated to the midrib and to the sheath extensions of the lateral veins. In the stems they could be observed in the cortical region, in the phloem and in the perimedullary parenchyma. Several compounds indentified to these species have proven biological or pharmacological activity, which confirms the potential of them.

PP-011. Volatiles of Algerian *Daucus reboudii* Coss. rich in *(E)*-anethole and their antioxidant activity.

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The essential oils obtained from the aerial parts of *Daucus reboudii* Coss. (Apiaceae) which is endemic to north Africa collected from Algeria were analyzed by GC/MS. 28 compounds were identified accounting for 97.8% of the total oil, the result shows the dominance of the phenylpropanoids derivatives. *(E)*-anethol (59.4%) was the main constituents identified in the essential oil, followed by estragol (21.2%) and dodecanal (4.4%). In vitro antioxidant antioxidant activity of the Essential oils were assayed using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, ß- carotene and $ABTS^{+}$ assay, the results indicated that *D.reboudii* oil recorded a moderate capacity.

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PP-012. Essential oil composition of leaves of *Piper aduncum* under different radiation conditions

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Piper aduncum L, known as long-pepper, has a great potential for economic exploration because of the proven use of its essential oil in the agriculture and in the human health, however, the essential oil production may vary depending on several factors. Light, both in term of quality and quantity, is a factor which stand out because it alters directly or indirectly these compounds synthesis. Thus, this work aimed was to assess the production and composition of the essential oil of *P. aduncum* under shade (50%, 70% of irradiance) and sunny conditions. Moreover, the effects of red shading net (RN) and blue shading net (BN) on essential oil of *P. aduncum* were verified. The young plants of *P. aduncum* were cultivated for four months in those conditions of radiation. The samples (leaves) were dried (30 ^{o}C) and hydrodistilled in a Clevenger type apparatus. The obtained oil was diluted in dichloromethane and analysed by GC and GC/ MS using an apolar column. The compounds were identified according to their mass spectra and retention indices [1]. In the leaves were verified the predominance of sesquiterpenic compounds , with (E).- nerolidol (14,28- 16,65%) as the major components and Linalool (9,33 – 13,44%); α-humulene (8,45 – 10, 62%). All the major compounds from leaves had their level increased when plants were cultivated in sunny conditions and under blue net as well as in sunny conditions plants of *P. aduncum* exhibits greater biomass. These results reforce the importance of the studies of plants grown under artificial ambient conditions considering that *P. aduncum* in natural ambient conditions survive better than in shade conditions.

Acknowledgments

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PP-013. Effect of Chitosan on the essential oil contents and composition of *Piper molicommum*

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Piper molicommum L. has a great potential for economic exploration because of the proven use of its essential oil in the agriculture and in the human health. However, the essential oil production may vary depending on several factors, such as, attack of pathogenic organisms. The elicitors, as chitosan (CH), are substances used to simulate pathogens action in plants. Thus, the aim of this work was to evaluate the effectiveness of CH in the increase of biomass production, content and composition of the essential oil of *P. molicommum*. The young plants of *P. molicommum* were cultivated and chitosan (CH) solved in HCl 0.05 N 0.25; 0.5; 1.0 % solved in were sprayed in all leaves of the plant after seven months of cropping. The samples (roots and leaves) were dried (30 ^{o}C) and hydrodistilled in a Clevenger type apparatus. The obtained oil were diluted in dichloromethane and analysed by GC and GC/ MS using an apolar column. The compounds were identified according to their mass spectra and retention indices [1]. The amounts of essential oil in both organs, leaves and roots, of *P. molicommum* were 1,06% and 1,74 %, respectively. Linalool (15,5%) and *cis*-nerolidol (12,8%) are majority component of essential oil of leaves of *P. molicommum*. The samples obtained from roots showed 86% of phenylpropanoid compounds, such as dillapiol (21.14%) and the myristicin (17.76%). Chitosan 0.5% was effective to increase the dillapiol and the biomass of roots. Several variations were verified in essential oil composition. The main target of our group are in order to check the main physiological mechanism involved in action of this elicitor.

Acknowledgements

The research was supported by FAPEMIG, CAPES and CNPq

Reference

1. R. P. Adams, Identification of essential oil components by Gas Chromatography / Mass Spectrometry, 4th ed. Allured Pub. Corp. Carol Stream, IL (2007).

PP-014. Essential oil composition of roots of *Piper aduncum* under different radiation conditions

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Piper aduncum L, known as long-pepper, has a great potential for economic exploration because of the proven use of its essential oil in the agriculture and in the human health, however, the essential oil production may vary depending on several factors. Light, both in term of quality and quantity, is a factor which stand out because it alters directly or indirectly these compounds synthesis. Thus, this work aimed was to assess the production and composition of the essential oil of *P. aduncum* under shade (50%, 70% of irradiance) and sunny conditions. Moreover, the effect of red shading net (RN) and blue shading net (BN) on essential oil of *P. aduncum* were verified. The young plants of *P. aduncum* were cultivated for four months in those conditions of radiation. The samples (roots) were dried (30 °C) and hydrodistilled in a Clevenger type apparatus. The obtained oil were diluted in dichloromethane and analysed by GC and GC/ MS using an apolar column. The compounds were identified according to their mass spectra and retention indices. These results showed that in roots of *P. aduncum* predominate compounds of phenylpropanoic pathway with apiol (18,36 – 29,51%) as the main component and dilapiol (13 – 18,36%), an essential oil with larger use in agriculture industry. Moreover, were detected in considerable amounts β- selinene and butanoate-2-methyl-geranyl. The apiol in roots exhibited increase in blue net conditions, however, the dilapiol increased in sunny conditions. *Piper aduncum* has great plasticity considering radiation factors, although, this plant is classified as shade species.

Acknowledgments

The research was supported by FAPEMIG, CNPq and CAPES.

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PP-015. Effect of methyl jasmonate on the essential oil contents and composition of *Piper molicommum*

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Piper molicommum L has a great potential for economic exploration because of the proven use of its essential oil in the agriculture and in the human health, however, the essential oil production may vary depending on several factors, such as, attack of pathogenic organisms. The elicitors, as methyl jasmonate (MJ), are substances used to simulate pathogens action in plants. Thus, the aim of this work was to evaluate the effectiveness of MJ in the increase of biomass production, content and composition of the essential oil of *P. molicommum*. The young plants of *P. aduncum* were cultivated and methyl jasmonate (MJ) 0.5; 2 and 8 mM were sprayed in all leaves of the plant after seven months of cropping. The samples (roots and leaves) were dried (30 ^{o}C) and hydrodistilled in a Clevenger type apparatus. The obtained oil were diluted in dichloromethane and analysed by GC and GC/MS using an apolar column. The compounds were identified according to their mass spectra and retention indices [1]. The amounts of essential oil in both organs, leaves and roots, of *P. molicommum* were 1,06% and 1,74 %, respectively. The essential oil of leaves predominate compounds of terpenic pathway. Linalool (15,5%) and *cis*-nerolidol (12,8%) are majority component of essential oil of leaves of *P. molicommum*. The samples obtained from roots showed 86% of phenylpropanoid compounds such as dillapiol (21.14%) and the myristicin (17.76%). Methyl jasmonate (2 mM) application results in increase of essential oil contents and in radicular biomass, the main site of essential oil accumulation. The experiment described, the first time, the characterization of the essential oil from this species. Moreover, the detection of dillapiol as major constituent shows the relevance of these studies.

Acknowledgements

The research was supported by FAPEMIG, CAPES and CNPq

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1. R. P. Adams, Identification of essential oil components by Gas Chromatography / Mass Spectrometry, 4 th ed. Allured Pub. Corp. , Carol Stream , IL (2007).

PP-016. Essential oils and essential oil fractions hatching inhibition activity against *Meloidogyne chitwoodi* nematode

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The Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi*, is an EPPO A2 type quarantine pest since 1998 [1]. This nematode causes severe damage in economically important crops such as potato and tomato, making agricultural products unacceptable for the fresh market and food processing. Commonly used nematicidal synthetic chemicals are often environmentally unsafe. Essential oils (EOs) may constitute safer alternatives against RKN. EOs, isolated, and analysed as detailed [2], from 44 plant samples, were tested against CRKN hatching, in direct contact bioassays. Some of the most successful EOs were fractionated. The hydrocarbon molecules (HM) and oxygen-containing molecules (OCM) fractions tested separately. Sixteen EOs displayed hatching inhibitions ≥90% at 2 µL/mL and were further tested at lower concentrations. *Ruta graveolens*, *Satureja montana* and *Thymbra capitata* EOs revealed the lowest EC_{50} values (< 0.15 µL/mL). *S. montana* and *T. capitata* OCM fractions showed hatching inhibitions higher than HM fractions. The comparison of EO and corresponding fractions EC_{50} values suggests interactions between OCM and HM fractions against CRKN hatching. *R. graveolens*, *S. montana* and *T. capitata* EOs showed to be potential environmentally friendly CRKN nematotoxics. These species EOs bioactivity should, nonetheless, be considered globally, since its HM and OCM fractions may contribute, diversely, to the full nematotoxic activity.

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PP-017. Glucosinolate of Croatian wild-growing *Brassicaceae* plants

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Glucosinolates, group of phytochemicals particularly abundant in plants belonging to *Brassicaceae* family, are interesting as a source of volatile degradation products possessing various biological activities. Besides commercial plants, such as vegetables and oilseeds crops, numerous wild-growing *Brassicaceae* plants could serve as a source of these compounds.

As a part of an investigation of glucosinolate degradation products and, indirectly, glucosinolates from Croatian wild-growing *Brassicaceae* plants, five plants, *Aurinia leucadea* (Guss.) K. Koch, *Cardaria draba* (L.) Desv., *Capsella rubella* Reut., *Calepina irregularis* (Asso) Thell. and *Bunias erucago* L., were investigated, respectively..

In this present study, indirect method consisting of thermal degradation of glucosinolates followed by GC-MS analysis of liberated volatile degradation products was used. Hydrodistillation was chosen as suitable isolation method because it insures simultaneous thermal degradation of glucosinolates and isolation of the liberated volatile degradation products.

The identification of volatile products isolated from *A. leucadea* revealed the presence of nine glucosinolates. The main glucosinolates were glucobrassicanapin and gluconapin, followed by glucoberteroin, glucotropaeolin, glucolepidiin and glucojiabutin. The dominating glucosinolate found in *C. draba* was glucoerucin, while *iso*-butyl glucosinolate and glucoiberverin were present in much smaller amounts. Among eight glucosinolates identified in *C. rubella*, the main one was sinigrin. Other interesting glucosinolates identified in this plant were 9-(methylthio)nonyl glucosinolate and 10-(methylthio)decyl glucosinolate. *C. irregularis* contained four glucosinolates, namely glucoiberverin, glucoerucin, sinigrin and glucotropaeolin. The dominating glucosinolate in this plant was glucoiberverin. *B. erucago* was found to contain glucodehidroerucin and glucoerucin as the most abundant compounds, plus gluconapin, hexyl glucosinolate, glucojiabutin, glucotropaeolin, glucolepidiin and glucosinalbin.

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PP-018. Phytochemical analysis of essential oil produced by intact plant and *in vitro* cultures of *Rhododendron tomentosum*

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Rhododendron tomentosum Harmaja (formerly *Ledum palustre*) is an evergreen shrub, grown in peaty soils in northern Europe, Asia and North America. Its biological properties have been used for ages in folk medicine to treat respiratory and joint diseases as well as various pains and infections [1]. Moreover, even now it is considered not only to be an effective repellent, but also a refreshing beverage, known as Labrador tea [2]. Modern research confirms the validity of the traditional use of this plant, indicating its anti-inflammatory, analgesic and anti-microbial activity, conditioned by the essential oil content [3].

Difficulties in obtaining the ground plant material from *R. tomentosum*, which is a protected species in Poland, resulted in establishing for the first time *in vitro* shoot cultures on Schenk-Hildebrandt (SH) medium supplemented with 2 mg/l 2-isopentenyladenine and 0.22 mg/l thidiazuron, as the alternative, continuous source of the valuable volatile fraction. In addition, the micropropagation protocol of the examined species was specified, including multiplication, elongation, rooting and *ex vitro* adaptation.

Parallel, phytochemical studies were carried out, aimed at developing a methodology for all stages of the analysis of the *R. tomentosum* essential oil's fraction. Firstly, the optimal drying method for the ground material was selected, taking into account a thermolability of volatile compounds. Subsequently, different methods of volatile oil extraction were examined (hydrodistillation, microwave and ultrasound assisted extraction), which allowed to determine the amount of essential oil in the intact plant (0,44%) and microshoots (0,25%).

Finally, two chromatographic techniques were employed to analyze the obtained volatile fractions. GC-FID and GC-MS was used to identify qualitatively and quantitatively the essential oil composition and to recognize the metabolic profile of the plant obtained by micropropagation process, while HPTLC was focused on the selected volatile compounds, especially on sesquiterpenoid ledol, in order to compile the effective screening scheme. Both methods were validated and the results were compared to each other.

In conclusion, as a result of our research, *in vitro* shoot cultures of *R. tomentosum*, a continuously available source of its essential oil, were established for the first time. Moreover, the optimization of the all stages of phytochemical analysis was performed, resulting in the possibility of the quick determination of the volatile fraction in the examined plant matrices. It is important to emphasize that the complete biotechnological protocol for up- and downstream procedures of *R. tomentosum* volatile oil's production was developed.

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PP-019. Characterization of secretion sites and chemical composition of essential oils of aerial organs of Brazilian species of *Aldama* La Llave (Asteraceae)

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The secretory structures are widely recognized in the Asteraceae and are associated with the secondary metabolites production. Phytochemical analyses demonstrate their pharmacological potential and their application in the phylogeny and chemotaxonomy of species, including members of genus *Aldama*. The secretory cavities and ducts, as well as glandular trichomes, present in *Aldama* are associated with essential oils production, characterized by the presence of low molecular weight terpenes. However, only one analysis evaluates the yield and chemical profile of essential oils of three Brazilian species. Therefore, the present study aimed to characterize the secretion sites of essential oils, analyze their chemical composition and detect possible chemical markers on leaves and stems of *Aldama anchusifolia* (DC.) E.E.Schill. & Panero, *A. megapotamica* (Malme) Magenta & Pirani, *A. nudibasilaris* (S.F.Blake) E.E.Schill. & Panero, and *A. pilosa* (Baker) E.E.Schill. & Panero. Samples were collected in the flowering period and from three different populations located in the states of Rio Grande do Sul and Minas Gerais, Brazil. The characterization of the secretion sites was performed on fully expanded leaves and stems of different diameters according to usual techniques in plant anatomy. The essential oils were obtained by hydrodistillation in a Clevenger-type apparatus. They were analysed by gas chromatography coupled with mass spectrometry and flame ionization detection. In the leaves, the synthesis of essential oil takes place in the capitate glandular trichomes and secretory ducts. Only *A. anchusifolia* and *A. megapotamica* have oil drops in the mesophyll cells. The trichomes are found on the abaxial surface of the leaves, and the ducts occur in association with vascular bundles embedded in the mesophyll and in the midrib parenchyma. *A. nudibasilaris* and *A. pilosa* have ducts in the phloem of the midrib. The same ducts also are found in the stem cortex, in the medullary parenchyma and associated with the secondary phloem. Only *A. anchusifolia* and *A. nudibasilaris* have secretory ducts in the primary phloem. The essential oil yield from the aerial vegetative organs was different among species and among organs of the same species. The leaves of *A. pilosa* demonstrated a highest average yield (0.26 % w/w), that is five times higher than the lowest average yield of the stem of *A. nudibasilaris* (0.05 % w/w). The essential oils of vegetative organs of all four species are characterized by predominance of sesquiterpenes. However, differences in chemical composition occurred between populations. We identified 57 compounds in the leaves and 52 in the stems, of which 35 occur in both organs. The sesquiterpenes β-Caryophyllene, Germacrene-D, Bicyclogermacrene and Spathulenol are common to the leaves and to the stems of four species. The compounds which are found in at least two populations of the same species are considered as potential chemical markers. Some of them are remarkable in the leaves as Carotol in *A. anchusifolia* and butyl citrate in *A. megapotamica*, and also in the stems as β-selinene in *A. nudibasilaris* and globulol in *A. pilosa*. The characterization of the secretion sites and the identification of chemical markers improve the differentiation of the species and provide useful information for the phylogeny of *Aldama*.

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PP-020. Chemical composition and allelopathic potential of essential oils from *Tipuana tipu* (Benth.) Kuntze cultivated in Tunisia

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The present work describes the chemical composition and evaluates the allelopathic effect of the hydrodistilled essential oil from roots, stems, leaves, flowers and samaras of *Tipuana tipu* (Benth.) Kuntze gathered in the area of Mogran (Tunisia). The chemical composition of the different parts of the species, determined by GC-FID and GC-MS, is reported for the first time. Remarkable differences were found between the constituent percentages in different studied organs. The most important compounds detected in roots were *β*-caryphyllene (24.1%), germacrene D (20%) and isopropylmyristate (11.7%). Whereas, in stems oil the main compounds found were 4-terpineol (14.6%) and elemicin (12.6%). Hexahydrofarnesyl acetone was the major oxygenated sesquiterpene constituent of leaves and samaras (10.2% and 19.9%, respectively). Essential from *Tipuana tipu* flowers was mainly rich in α-longipinene (8.3%), 1.7-dimethylnaphthalene (15.6%) and (*E*)-*β*-inone (33.8%). Allelopathic effects of the essential oils tested at different concentrations were evaluated on the seed germination and the early growth of *Lactuca sativa* seedlings. The highest inhibition of 100% was detected for the roots oil at the concentration of 1 mg/mL.

PP-021. Compositions of essential oils of *Salvia adenophylla*, *Salvia pilifera* and *Salvia viscosa* in Turkey

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In this study, water-distilled essential oil of three species of *Salvia* (Lamiaceae) were analyzed. The analysis was performed by using a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. The major components were found as α-pinene (16.2 %) and β-pinene (14.4 %) for *Salvia adenophylla* (endemic)*;* β-pinene (24.9 %), myrcene (9.0 %), α-humulene (7.9 %) for *Salvia pilifera* (endemic)*;* α-copaene (13. 0 %), β-caryophyllene (10.8 %), γ-muurolene (9.8 %), δ-cadinene and caryophyllene oxide (8.0 %) for *Salvia viscosa*.

PP-022. The composition of the essential oil of *Helianthemum canum* (L.) Baumg growing in Turkey

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The genus *Helianthemum* Miller (Cistaceae) are evergreen or semi-evergreen subshrubs which are widely distributed in the Mediterranean region. The leaves and flowers of different *Helianthemum* taxa are traditionally used as anti-inflammatory, antiulcerogenic, wound healing, antiparasit, antimicrobial, analgesic, cytotoxic and vasodilator remedies, especially locals in Spain and The Americas (1). In Turkey, this genus is represented by 16 species (2). Some of them have been used in constipation and as a styptic in folk medicine (3). In this present study, it was aim to determine the composition of essential oil of *Helianthemum canum*. Water-distilled essential oil from the aerial part of *H. canum* was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The identified major components were myristicin (29.4%), T-cadinol (6.5%), hexadecanoic acid (5.2%), spathulenol (4.0%), decanoic acid (3.6%), respectively.

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PP-023. Essential oil composition of *Scaligeria napiformis* (Sprengel) Grande native to Turkey

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The genus *Scaligeria* DC. belongs to the Apiaceae family. The species of this family are rich sources of essential oils. They may contain essential oils originating in any organ including fruits, leaves, roots or whole plant (1). *Scaligeria* DC. is represented in Turkey by seven species of which two are endemic (2). A few studies on essential oils of some *Scaligeria* species has been carried out (3, 4). Antimicrobial and antifungal activities of several *Scaligeria* species have been reported (3). In this study, the aerial parts of *S. napiformis* were hydrodistilled for 3 h using a Clevenger apparatus to obtain essential oil (5). The essential oil was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Water-distilled essential oil from the aerial part of *S. napiformis* was separated contained spathulenol (7.4%), salvial-4(14)-en-1-one (6.3%), eudesma-4(15),7-dien-1β-ol (6.2%), torilenol (5.0%), (*Z*)-β-farnesene (4.5%) as the major components.

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PP-024. Improving the strong scent of *Citrus junos* Tanaka (yuzu) juice with an underwater shock wave pretreatment

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Citrus fruits have been widely cultivated in regions between the tropical and temperate zones, and are some of the most important commercial crops. *Citrus junos* Tanaka (*yuzu)*, a sour fruit, has been cultivated mainly in Japan and Korea. In Japan, the annual production amounted to approximately 25,000 tons in 2009. Yuzu has a strongly characteristic aroma in comparison with other citrus fruits, and yuzu juice is popularly used in Japanese foods. Yuzu produced in Rikuzentakata (Iwate prefecture) is called “Northern Limit Yuzu” and has good aroma. Additionally, it is expensive because it is produced in small quantities. Rikuzentakata sustained great damage during the Great Tohoku Earthquake and subsequent tsunami in 2011. Currently, Northern Limit Yuzu is being promoted as part of support reconstruction. The authors have reported that an underwater shock wave treatment of the leaves of *Alpinia zerumbet* is expected to result in more effective extraction of essential oil by subsequent steam distillation[1,2]. These results show that essential oil can be extracted more effectively applying an underwater shock wave preprocessing treatment. As a preprocessing step, the underwater shock wave treatment of yuzu is expected to result in a stronger scent after subsequent squeezing. In the present study, we evaluated the content of volatile and functional compounds to determine whether the pretreatment affects the scent and functionality of yuzu juice.

Yuzu was grown in Rikuzentakata, and Kochi for comparison. The materials were subjected to shock wave pretreatment or left untreated before squeezing, and juice was then obtained by squeezing with a juice press for exclusive use. The volatile compounds of the juice were analyzed by head-space gas-chromatography-mass spectrometry and components were identified from the peak area and mass spectrometry libraries. We identified 20 compounds in the juice of Rikuzentakata, which represented 97.2% of the total components detected. The major compounds detected were limonene (72.5%), γ-terpinen (14.7%), β-phellandrene (4.1%), myrcene (1.9%), α-pinene (1.8%), β-pinene (1.1%) and linalool (1.1%). The juice of Kochi produce contained 21 identified compounds, which represented 96.7% of the total components detected. The predominant components were limonene (73.9%), γ-terpinen (11.5%), β-phellandrene (4.8%), myrcene (2.2%), α-pinene (2.0%), β-pinene (1.1%) and linalool (1.2%). These results indicate that the composition of the volatile component hardly changes between Rikuzentakata and Kochi juices, but the content of the volatile component in the Rikuzentakata juice was 1.2 times that in the Kochi juice.

With shock wave pretreatment at 3.5 kV and 4.9 kJ, there was an increase in the content of the most abundant compounds in the juice of Rikuzentakata, compared with that obtained from untreated yuzu, by a factor of 10.6. Moreover, the amount of functional compounds such as flavanones in the juice with shock wave treatment was 1.3 times that without treatment. These results suggest that the scent of juice can be extracted more effectively through underwater shock wave preprocessing treatment. This treatment can also improve the functionality of the juice, as determined by the analysis of functional compounds.

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PP-025. The insecticidal activity of selected aromatic compounds against larvae of *Alphitobius diaperinus* Panzer.

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Plants have evolved plenty of secondary chemicals to protect themselves against herbivores and pathogens. Essential oils (EOs) produced by higher plants are complex mixtures containing a few dozen components, especially monoterpenes, sesquiterpenes, and phenols in varying concentrations. Phenolic compounds as components of essential oils, have confirmed harmful impact on many insect species. These chemicals are characterized by hydroxyl group bound directly with the benzene ring, which is responsible for their toxic character. Carvacrol, eugenol or anethole contain this group and they are main components of oregano (*Origanum vulgare* subsp. *hirtum*), clove (*Syzygium aromaticum*) and anise (*Illicium verum*) EOs, respectively. The identification of the compounds was performed using gas chromatography (GC) coupled with mass spectrometry (MS) performed on a Saturn 2000 MS Varian Chrompack.

The insecticidal activity of carvacrol, anethole and eugenol was evaluated in laboratory against different larval stages of lesser mealworm (*Alphitobius diaperinus* Panzer, Coleoptera, Tenebrionidae), major insect pest inhabiting poultry houses in vast number. The earlier (10-day old) and later larval stages (20-day old) were reared on diet containing 1 % acetone solutions of tested compounds. Body weight gain, mortality, number of pupae and adults, and their body mass were recorded. Insecticidal activity of the applied compounds depended on the age of larvae. The highest toxicity against younger larvae showed carvacrol, followed by eugenol and anethole. The total mortality caused by these compounds was 97.50, 65.00 and 45.00%, respectively in comparison with control trials only 5.00%. The older larvae have shown lower sensitivity to carvacrol than the younger, however mortality was quite high for the older stage (77.5%). The mortality of both larvae stages treated with eugenol and anethole was similar. The studied phenols incorporated into the diet of larvae disturbed the growth, extended their developmental cycle in comparison to the control conditions as well as resulted in an increase of cannibalism among them.

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PP-026. Chemical composition of essential oils from two Aldama species (Asteraceae) in different phenological stages of development

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Aldama arenaria (=*Viguiera arenaria*) and *A. robusta* (=*Viguiera robusta*) are subshrubs endemic from Cerrado domain (Brazilian savannah) with seasonal development. During favourable periods of development, these species emit aerial stems from the thickened underground system (xylopodia and roots) and produce flowers. In cold and dry periods, these species lose their aerial stems and survive only via underground organs, which characterise the dormant period. Both species are strongly aromatic due to the production of essential oils (EOs) mainly in secretory structures (trichomes and internal spaces) and phytochemical studies have demonstrated the biological activity of their root compounds. The essential oils were obtained from leaves, stems, xylopodia and roots (separately) by hydrodistillation in a Clevenger-type apparatus, analysed by gas chromatography coupled with mass spectrometry and the compounds were identified by comparing the mass spectra with library data. The present study compared the yield and chemical composition of EOs from the vegetative organs of *Aldama arenaria* and *A. robusta* during flowering and dormant stages. The highest yield of EOs in *Aldama arenaria* in the flowering stage was obtained from the leaves (0.23%), followed by roots (0.19%) and xylopodia (0.07%). In the dormant stage, the level of EOs from the xylopodia increased by 100% relative to the flowering stage, whereas EOs from the roots increased by 73%. In *A. robusta*, in the flowering stage, the highest yield of EOs was obtained from the roots (0.32%), followed by the leaves (0.21%), and xylopodia (0.08%). In the dormant stage, the level of EOs from the xylopodium increased by 62% compared to the flowering stage, while the level of EOs from the roots did not change. Monoterpenes represented the largest component of EOs obtained from the roots and xylopodia in both developmental stages. In the dormant stage, the monoterpene content increased in both organs, while the sesquiterpene and diterpene contents decreased. Since sesquiterpenes are metabolically active during plant growth, it would be expected that these species invest more in the biosynthesis of sesquiterpenes during the flowering stage, when the aerial structures are forming. In turn, the increase in monoterpenes in EOs during the dormant stage may be interpreted as a chemical defence mechanism in a plant that not only survives in conditions unfavourable to growth, but also has only an underground system to maintain itself until the next growth stage. Limonene was the major compound in the EOs in the leaves (5.18%) and aerial stems (15.3%) of *A. arenaria*, whereas α-pinene (9.02% and 19.64%), β-pinene (9.82% and 11.59%), limonene (11.16% and 15.84%), and germacrene D (11.98%, 7.56%) were detected in the EOs in the leaves and aerial stems, respectively, of *A. robusta*. In the EOs of the underground systems, the major compounds in *A. arenaria* were α-pinene, β-pinene, and carotol, whereas the major compounds in *A. robusta* were α-pinene, cyperene, and germacrene D. Sesquiterpene carotol, which exhibits antifungal activity, was only detected in *A. arenaria,* whereas germacrene D, which exhibits antimicrobial activity, was exclusively detected in *A. robusta*.

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PP-027. Variations in Essential Oil of Selected *Origanum dubium* Boiss Genotypes

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Origanum species are economically important medicinal and aromatic plants in Turkey. They are commonly used pharmaceutical and industrial products in many areas. Number of researches have been conducted on *Origanum* species and their essential oils. These studies have generally focused on essential oil yield, components and its biological activities. *Origanum dubium* Boiss is one of the economically important wild oregano species in Turkey and it is collected from the natural flora of Antalya. This species is used mainly for essential oil production due to its high essential oil and carvacrol yield. In the experiment, one hundred genotypes of *Origanum dubium* originated from the wild flora of Gazipasa town of Antalya were selected according to their agronomic and chemical features. Also, essential oil colours were observed in all genotypes since it could be important for marketing choices. This study was conducted in Antalya located in Mediterranean Region of Turkey and this location was characterized by Mediterranean climate. Essential oils of different genotypes were obtained by hydrodistillation of the aerial parts of plants and they were analysed by GC-MS. Among the selected genotypes carvacrol was the major component and followed by *p*-cymene, γ -terpinene , α-thujene and myrcene. According to the results, essential oil yields were varied between 6% to 14%; carvacrol rates were varied between 73.26% to 88.21%. Also, different colours of essential oils ranging from light yellow to mustard colour were observed in the selected genotypes under cultivated condition.

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PP-028. Essentail oils composition from leaves and flowers of *Lonas annua* L. from Algeria

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Essential oils were obtained by separate hydrodistillation from leaves and flowers of *Lonas annua* L. (Compositae) . were analyzed by means of gas chromatography – mass spectrometry (GC-MS) , the main constituents of the essential oil from the leaves were (*E,E*)-farnesol (23.63%), caryophyllene oxide (18.42%), limonene (12.01%), and 1,10-di-*epi*-cubenol (3.15%), and those from the flowers caryophyllene oxide (27.30%), β-cedrene (15.10%), sabinene (8.84%), cedrol (6.48%), spathulenol (5.73%), and 1,10-di-*epi*-cubenol (4.74%). This analyzed is the first study of composition of the essential oil obtained from the leaves and flowers of *Lonas annua* L. growing in Algeria .

PP-029. Essential oil composition of the aerial parts of *Ferula orientalis* L. and determination of its antimicrobial properties by bioautography

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Apiaceae is a big family with 400-450 genera and 3500-3700 taxa throughout the world.[1] The genus *Ferula* L. has 180-185 species and highest biodiversity is known to be present in the Central and Southeast Asia[2]. *Ferula orientalis* L. grows naturally in Turkey and is recorded in Bern Convention App. I[3], and within our knowledge, this is the first study indicating the composition of the aerial parts of the plant growing in Turkey. Volatile oil from the aerial parts of the species was obtained by hydrodistillation and analyzed by GC and GC-MS and main component was found to be α-pinene (75.9%). Results of antimicrobial activity study *via* bioautography method showed that aerial parts were active against *Staphylococcus aureus* ATCC 6558 and *Candida albicans* ATCC 90028 strains. However, the oil was not active against *Escherichia coli* NRRL B-3008 strain at the tested concentration.

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PP-030. Chemical diversity of essential oil and lipids of *Galatella punctata* (Waldst. & Kit.) Nees

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Galatella punctata (*syn*. *Aster punctatus)* (Waldst. & Kit.) Nees (Compositae) was collected during flowering stage in the vicinity of Karaganda city (Kazakhstan). Essential oil from herb of *G. punctata* was obtained by hydrodistillation in a Clevenger apparatus while lipids were extracted by Folch method. The essential oil yield was 0.1% (calculated on a moisture free basis). Fraction of fatty acids was methylated with Boron trifluoride-methanol reagent. The essential oil and fatty acid methyl esters were analyzed by GC/FID and GC/MS techniques.

Seventy nine compounds were identified in the essential oil, while 17 compounds were detected in the fatty acid fraction. The essential oil was characterized with a high content of oxygenated monoterpenes (34.1%) with *trans*-verbenol (7.6%) and *trans*-pinocarveol (6.4%) as main constituents. Monoterpene hydrocarbons (13.0%) were presented by α-pinene (6.9%), β-pinene (2.5%) and limonene (1.4%). Oxygenated sesquiterpenes constituted 20% of the essential oil with caryophyllene oxide (4.5%) and spathulenol (3.4%) as the major constituents. Fatty acids (16.0%) were mostly presented by hexadecanoic acid (12.3%) and tetradecanoic acid (2.1%). The essential oil was subjected to preliminary test for antioxidant activity using DPPH reagent and HP-TLC technique.

Lipids fraction consisted of C_{10}-C_{24} fatty acids with predomination of unsaturated forms. Methyl linoleate (25.9%), methyl linolenate (19.9%) and (Z)-9-methyl octadecenoate (8.1%) were found to be major constituents. Among saturated fatty acids methyl hexadecanoate (24.4%) was detected in high abundance. The present work is the first detailed contribution into the chemistry of the essential oil and lipids of *Galatella punctata*.

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PP-031. Antimicrobial effects of the thyme (*Thymus vulgaris* L., Lamiaceae) and coriander (*Coriandrum sativum* L., Apiaceae) essential oils – source for alternative treatment of vaginal infections

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Although antimicrobial properties of essential oils were acknowledged long time ago, there are still many investigations that deal with their antimicrobial properties pointing to them as potential new sources of novel antimicrobial compounds. Due to the growing resistance of bacteria and fungi to common antibiotics and antimicotics, there is an increasing interest in medicinal plants and their products as an alternative to synthetic preservatives and antibiotics. Furthermore, essential oils have demonstrated clinical efficacy for the treatment of oral and vaginal candidiasis in animals. With respect to that, commercial essential oils of thyme (*Thymus vulgaris* L.) and coriander (*Coriandrum sativum* L., Apiaceae), together with commercial antibiotics and antimicotics were analyzed for their antimicrobial potential against twelve vaginal clinical strains of bacteria: *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Enterococcus* sp., and including three ATCC strains: *Staphylococcus aureus* 25923, 6538 and *E. coli* 25922, as well as two clinical isolates of *Candida albicans* strains and one ATTC strain (10231). Their chemical composition was analyzed by GC-MS. Antimicrobial activities were assesed using disc, well and broth dilution method in 96 well microplates. Minimal inhibitory (MIC) and minimal bactericidan/fungicidal (MBC/MFC) concentrations were evaluated for both essential oils against all tested strains. The main constituents of the corinadrum essential oil were lynalyl acetate (43.1%) and limonene (24.9%), and those present in the essential oil of thyme were thymol (56,6%), *p*-cymene (12,3%) and carvacrol (8.7%). Recorded antimicrobial activity was higher for coriander essential oil (MICs 0.4-4.54 µL/mL) against almost all tested bacteria except multiple resistant strains of *Enterococcus* sp. and *Proteus* sp. However, thyme essential oil exhibited slightly better fungicidal activity reaching MIC at 0.11 mg/ml for all tested *Candida* strains. The obtained results provide *in-vitro* support for the possible use of these essential oils against *E. coli, Staphylococcus aureus* and *Candida albicans* vaginal infections in alternative gynecological treatment.

PP-032. Chemical characterization of different *Ocimum basilicum* L. gene bank accessions during the vegetation period

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In our study eight different *Ocimum basilicum* L. accessions stored in gene bank were evaluated. Essential oil composition in three phenological phase (early-, full flowering and seed ripening period) was analysed. Plant materials were dried in natural way protected from sunlight at room temperature. The dried samples were hydrodistilled for 2h in a Clevenger type apparatus (PhHg VII.). Essential oil components from leaves and inflorescences were analyzed by gas chromatography/mass spectrometry (GC-MS). Main components were mainly oxygenated monoterpenes and phenylpropane derivates. From chemical aspect two major essential oil profiles were distinguished among the eight basil accessions. The first group contains the accessions belonging to the linalool chemotype ('Arvada', 'Dark Opal', 'Genovese', 'Lengyel', 'Rit-Sat') where the percentage of linalool reaches 60.76-79.59 % of the oil. The second group consists of populations of linalool and methyl chavicol chemotype ('A-1', 'Mittelgroβblättriger Grünes', 'Piros'). Here the ratios of the two main components are 18.53-55.07 % and 15.74-62.62 %, respectively. In our experiment we observed that the concentration of monoterpenes (linalool, 1,8-cineole) generally decreased during the generative stage but in case of accession 'Piros' (Hungarian cultivated population) it drastically increased. At the same time the amount of sesquiterpene compounds (τ-cadinol, trans-α-bergamotene, germacrene-D, δ-guaiene, cis-γ-cadinene) in most cases increased. In contrary, the quantity of phenylpropane derivatives showed different patterns for different genotypes; in the samples belonging to the group of linalool and methyl chavicol chemotype it decreased. Our investigation showed that the maximum essential oil content is present during the flowering period (from early to full flowering) and its amount decreases afterward in each accession. Although essential oil composition seems to have taxon-specificities, the accumulation rate and dynamics of compositional changes during ontogenesis are characteristic for the species.

Acknowledgments

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PP-033. Chemical composition and anticandidal effect of *Thymus leptobotrys* essential oil from southwest of Morocco against the emerging nosocomial Fluconazole-resistant strains

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The purpose of this present work is to study the essential oils chemical composition of Thymus leptobotrys Murb. an endemic and aromatic Thymus species from southwest of Morocco. Additionally, we intend to determine the antifungal activity of the essential oil of these species towards nineteen strains of emerging nosocomial Fluconazole-resistant Candida species. The chemical composition of the essential oils was identified by chromatographic analysis (GC and GC/MS). Hydrodistillation yielded 2.00 ± 0.01% of essential oil. Carvacrol (63.38%) was the major constituents T. leptobotrys essential oil. Other minor compounds identified were *ρ*-cymene (8.34%), borneol (5.85%), linalool (3.12%), camphene (2.70%), α-pinene (2.69%), g-terpinene (2.52%) and β-caryophyllene (2.46%). This oil was dominated by the monoterpene fraction that amounted 91.13%. The results obtained for the anticandidal disc-diffusion assay shows that the 19 strains of Candida species tested were inhibited by the Moroccan Thymus essential oil to a varying degree, with the diameters of the inhibition zone ranging from 49 ±1.00 to 85±1.15 mm. The essential oil of T. leptobotrys showed larger inhibition zones than the positive control Fluconazole and Amphotericine B. Candida albicans showed a high sensitivity to essential oil of Thymus leptobotrys compared to controls. While non albicans Candida species showed less sensitivity to essential oil of T. leptobotrys. Interestingly C. krusei, C. dubliniensis and C. glabrata were found to be resistant to conventional antifungals (fluconazole and Amphotericine B), while our essential oil tested was able to inhibit the growth of Candida strains resistant to antifungal agents. The value of Minimal inhibitory concentration (MIC) ranged from 5.21-0.0814 mg/ml and Minimal Fungicidal Concentration (MFC) of the studied oil ranges from 0.33mg/mL to 0.90625 mg/mL. The oil possessed higher antifungal potential than classical fungicide.

PP-034. Chemical and antifungal properties of essential oils of three Moroccan endemic plants and study of the antifungal activity of 44 pure compounds

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Fruits and vegetables are subject to many postharvest phytopathogenic fungi that cause severe economic losses. The treatment of these fungi uses a lot of chemicals that negatively affect environment and human health. Our study aims to test the antifungal activity of essential oils extracted from three endemic medicinal plants against two phytopathogenic fungi. The composition of the essential oils isolated from the aerial parts of *Thymus satureioides*, *Thymus pallidus* belonging to *Lamiaceae* family and *Bubonium imbricatum* family of *Asteraceae*, endemic plants from Morocco, were investigated by GC and GC/MS. The antifungal activity against two post-harvest pathogens of the essential oils and 44 pure compounds of the essential oils were also studied *in vitro*. The oil of *T. satureioides* was found to be rich in monoterpenes hydrocarbons, with borneol (36,6%) being the major component. The most abundant compound in *T. pallidus* was also borneol (36,2), while the oil of *B. imbricatum* contained dimethoxy-*p*-cymene (17,2) as a major component. The oils were tested for their *in vitro* antifungal activity against two post-harvest pathogens: *Geotricum citri-aurantii* and *Phytophthora citrophthora*. Examination of the antifungal activity revealed that only *B. imbricatum* exhibited a definite activity against both fungi tested. Among the 44 essential oils pure compounds tested the phenols: carvacrol and thymol were the most effective with 100% of mycelial growth inhibition for the two fungi at 150 ppm. The alcohols mainly borneol exhibited a very low antifungal activity. While a moderate activity was found using (-)-carveol, citral and camphene.

PP-035. Chemical composition of essential oils and study the antimicrobial activity of two medicinal plants, *Myrtus communis* L. and *Myrtus nivellei* Batt et Trab. from Algeria

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This study focuses on the characterization of the volatile fraction of the species *Myrtus communis* L Mediterranean (Rihan) and Sahara- endemic *Myrtus nivellei* Batt and Trab species and evaluation of their antimicrobial effects. Analysis by GC / MS identified the major compounds of essential oils of both plants. For *M. communis*, essential oil belongs to chemotype: Limonene (16.22 %) / alpha pinene (15.93 %) with a remarkable presence of octadienol (15.04 %) and cineole (9.12 %). The chemotype δ-elemene (15.69 %) / α-terpineol (11.66 %) , was found in *M. nivellei* . Batt and Trab.

Regarding the study of the antimicrobial activity of pure HE , has shown that , on the one hand, the HE *M communis* has a strong effect on *E. coli* (ZI = 38mm), a significant effect on *Alternaria sp* (ZI = 20mm), and a moderate effect on *F.oxysporium fsp albidinis , F . oxysporium fsp lycopersici* and *Penicillium sp.* On the other hand *M. nivellei* , showed activity on *P. aerogenosa* with ZI = 15mm, a null action on *F. oxysporium fsp lycopersici and Penicillium sp.* Overall, *E. faecalis strains, S aureus, S epidermidis, S typhi, S flesineris and Candida albicans* were found susceptible to essential oil tested against by his last had an average share of phytopathogenic fungi.

PP-036. Mycorrhizae as promised biotechnological approach for essential oil production of *Thymus vulgaris* L.

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Mycorrhizal plants are better able to obtain their nourishment from the soil and resist environmental stresses thanks to the role that the fungal symbionts have in the biofertilization and crop protection.

The increase uptake of soil minerals by colonized plants can be considered a sustainable crop management strategy, reducing applications of fertilizers and pesticides.

Previous studies investigated the symbiosis between host plant and arbuscular mycorrhiza (AM) and its effect on the increase of essential oil (EO) production in several medicinal and aromatic plant.

Since there is no studies being done on AM fungi and EO increased production in *Thymus vulgaris* L., a perennial Mediterranean species with a long list of pharmacological and aromatic properties, and commonly used in the herbalist sector.

The aim of this work was to evaluate the effect of inoculation with AM fungi, as substitute of the phosphorus fertilization, on the thyme's essential oil yield.

We examined the influence of two AM species, *Glomus mosseae* Nicol. & Gerd. and *Glomus viscosum* H.T. Nicolson, maintained in the purity in our laboratories, one commercial formulate (Symbivit® product of MYBATEC Ltd), and two amounts of phosphorus nutrition (P) on yield and chemical composition of essential oils. In this context, pot experiment started from seeds was conducted in a greenhouse under controlled conditions. Leaves from 4 months old plants were collected; their essential oil was obtained using a Clevenger type- apparatus and analyzed by GC/MS. The data revealed that within the AM treatments the concentration of essential oil, based on the dry weight of the leaves, was higher than control in *Symbivit* inoculated plants, while no differences were recorded with the P supply.

Thymol was the major component among all the identified compounds regardless of the treatments. In term of essential oil composition*, G. mosseae* and *Symbivit* leaded to high content of thymol (66.66, 67.38%) respectively. While its precursor *ρ*-cymene increased with *G. viscosum (13.01%)*, γ-terpinene concentration was found more in *Symbivit* mycorrhizal plants. About the application of P fertiliser, neither quantity nor quality of oil was affected with more P supply. Mycorrhizal symbiosis appeared to have a role in accumulation of phenolic monoterpenoid (71.80%) by mycorrhization of *Symbivit* and also monoterpene hydrocarbons (19.46%) through *G. viscosum* over the control. The oxygenated compounds either monoterpenes or sesquiterpenes showed no such trend which increased slightly by non mycorrhizal or P-fertilised control. The current work confirmed the efficiency of mycorrhizal fungi as biofertilizers tool in order to improve quantities of the major constituents. For the first time, this study demonstrates the comparison of essential oils obtained from mycorrhizal and non mycorrhizal thyme plants and the positive effect of thyme-mycorrhizal interaction not only in oil content but also in improvement the quality of mycorrhizal plants through increasing the content and enhancing the chemical profile of active compounds of *T. vulgaris*. However, different inoculations might have dissimilar effect. Specific inocula could be necessary to give positive effects of yields or composition of EOs.

PP-037. Effectiveness of arbuscular mycorrhizal fungi on essential oil production in oregano cultivar Carva

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In the natural environment the arbuscular mycorrhizal (AM) symbiosis is a widespread association between the roots of plants and fungi. The principal benefit for the plants is the increased uptake of relatively immobile phosphate ions, while the plant supplies the fungus with carbohydrates.

Other than P, the improved uptake of macronutrients as well as micronutrients has also been recorded. Additional effects include drought tolerance, soil structure, resistance to pathogens, and ecosystem functionality. Therefore mycorrhiza is best known for remarkable growth responses.

Arbuscular mycorrhizal (AM) symbiosis is usual in aromatic plants and several studies showed the effects on the secondary metabolites such as flavonoids, carotenoids, phytoalexins, phenols, triterpenoids. Among these, only few research has been carried out on *Origanum* spp., an important Mediterranean aromatic plant with new applications in food and feed for its antimicrobial and antioxidant properties. In particular, one of the studies shows the positive effect of AM fungus in improving the productivity of essential oil is highly dependent on the genotype of the plants.

The objective of this work was to evaluate the effect of different inoculants of AM fungi on biomass production, development of secretory structures, qualitative and quantitative synthesis of essential oils (EO) of *Origanum vulgare* L. spp. *viridulum* (Martrin-Donos) Nyman x *O. vulgare* L. spp. *hirtum* (Link) Iestwaart, cv Carva (mediSeeds sàrl).

A complete randomized design including three mycorrrhizal treatments (*Glomus mosseae* Nicol. & Gerd, *Glomus viscosum* H.T. Nicolson or a commercial mix of 6 AM fungi) and non mycorrhizal control were replicated 4 times. Oregano seedlings were grown for 4 months under a controlled greenhouse. Before the harvest, ten leaves for each treatment at the same age and position were randomly excised and examined by stereomicroscopy. However, peltate trichome density was evaluated. Then the dried biomass of shoots was weighted and the dry leaves submitted to water distillation in a Clevenger type-apparatus. Chemical analyses were done using GC-MS.

Mycorrhizal symbiosis increased the shoot biomass of oregano, in particular *G. viscosum* inoculums led to more than 2-fold higher dry weight production compared with non-mycorrhizal plants. Leaves from *G. viscosum* and *G. mosseae* treated plants had a larger number of glands per square-centimeter than the others (respectively 4,8 and 4,7 vs. 3,7 (control) and 3,8 (commercial mix), while the higher EO content was obtained from *G. viscosum* and the commercial treatment (1,5 and 1,4 ml $100g^{-1}$ DW).

The EO constituents determined by GC/MS on 4 month-old plants were 63. Carvacrol was the most abundant component (69,40-77,73%), followed by thymol (5,80-13,84%) γ- terpinene (3,86-5,45%), *ρ*-cymene (2,43-4,67%) and trans-caryophyllene (3,02-3,40%).

Even if the percentages of carvacrol and thymol were different depending on mycorrhizal treatments and in comparison to the control, the total amount of phenolic monoterpenoids and their precursors (γ- terpinene and *ρ*-cymene) was quite similar.

The results of this study demonstrated the different effectiveness of mycorrhizal fungi on biomass production, gland density and EOs of oregano cv Carva showing the importance in the symbiosis not only of the genotype but also the AM inoculums.

PP-038. Chemical composition and bioactivity of the essential oils of Algerian medicinal plant: Artemisia herba alba asso.

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The aim of this present study was to examine the antimicrobial and antioxidant activities of three essential oils types obtained by hydrodistillation from the aerial parts of Artemisia herba-alba cultivated in three different locations from southern Algeria (Boussaada, Medjedel and Romana). The chemical composition was investigated by capillary GC and GC/MS techniques in combination with retention indices. Among the 114 identified constituents (accounting for 94.4 - 98.3% of the oils), the main components were camphor (49.3 – 14.5%), α-thujone (27.1-1.0%), chrysanthenone (23.3–3.2%), 1,8-cineole (13.5 – 6.5%), β-thujone (9.7 – 2.1%), borneol (7.3 – 4.7%), and cis-chrysanthenyl acetate (8.2 – 1.0%).. Variable antibacterial activities with the essential oils appeared on four Gram-positive (*Bacillus subtilis*, *Bacillus coagulans*, *Micrococcus luteus* and *Staphylococcus aureus*) and three Gram-negative (*Agrobacterium tumefaciens*, *Escherichia coli* and *Pseudomonas aeruginosa*) and five fungi (*Mucor ramannianus*, *Aspergillus ochraceus*, *Fusarium oxysporum* f. sp. *albedinis*, *Penicillium expansum* and *Fusarium oxysporum* f. sp. *lini*) and two yeasts (*Candida albicans* and *Saccharomyces cerevisiae*). The agar dilution method was used. The Minimum inhibitory concentration (MIC) of various essential oils shows a power inhibitor not exceeding 10 µg/mL against all microbial strains. The three chemotypes were slightly active and the weak DPPH radical scavenging activity (13.23 - 5.65%).

PP-039. Variability of the peppermint bio tea from different origin at the food market

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Biofood products are more required in past decade. Plants still make an important contribution to health care. Peppermint tea plays important role in daily use in many countries. With a refreshing mint flavour, this tea is one of the most popular infusions throughout the world, largely appreciated by both adults and children. It helps digestion and relieve flatulence. The peppermint tea can also help to control nausea and decongest the airways in case of colds and flu.

Insufficient data exist for most plants to guarantee their quality, efficacy and safety. The aim of our investigation was to compare the differences of peppermint raw material from different origin, used for the tea production. Five samples of peppermint from Austria, Egypt, Germany, Kosovo and Turkey were obtained from the german company Herbacut. The analyses were done in 2012.

The chemical composition of the essential oil was determined by using GC-MS and indicated the differences in content of the main components menthol and menthon. Free radical scavenging capacity of herbal extracts was evaluated by measuring the scavenging activity with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. All examined samples were able to reduce DPPH radicals into the neutral DPPH-H form, and this activity was dose-dependent. The phenolic content of each extract were estimated using spectrophotometric methods based upon the Folin-Ciocalteu reagent. The total phenols and flavonoids were quantified and compared between these extracts.

Environmental factors, growing and harvest conditions and genetics predisposition are responsible for the plant variability. Our examination presents the variability of the peppermint tea as a product of one brand at the market. This findings could be responsible for the different colour and taste of the final product – peppermint infusion. Its effect on human body require more specific analyses.

The trend in the domestication, production and biotechnological studies of medicinal plants will offer great advantages to obtain uniform and high quality raw materials which are fundamental to the efficacy and safety of herbal products.

PP-040. Trypanocidal and cytotoxic activity of the essential oil from *Lippia origanoides* Kunth.

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Natural products have been used since ancient times in many areas. Currently, many plant species are being studied with the aim of isolating their active principles, which serve as models for new chemicals. Although less extensively investigated, the use of essential oils for the treatment of parasitic diseases has acquired paramount importance. Essential oils can be effective in the treatment or prevention of parasitic diseases because properties such as the low density and rapid diffusion through cellular membranes that result from their lipid solubility can enhance the intracellular inclusion of the active components into parasites.

Despite the widespread use of essential oils, some of the constituents have been described as having cytotoxic, hepatotoxic (pulegone), genotoxic and carcinogenic (safrole) properties. This fact makes it important to broadly characterize the oils so as to better understand their actions in different biological systems and to discover secure applications both in health and in human consumption [1]. Lippia origanoides Kunth. (Verbenaceae) is a species native to northeastern Brazil. An ethnobotanical survey of some Brazilian medicinal plants showed that this plant, popularly known as alecrim-d'Angola, is used in the treatment of various diseases such as gastrointestinal and respiratory [2] infections. This study evaluated the potential activity of the essential oil from *Lippia origanoides* Kunth. against epimastigotes of *Trypanosoma cruzi* and its cytotoxic effect on VERO cells.

The plant material was collected in the Medicinal Plants Garden of the Federal University of Lavras (Lavras, Minas Gerais, Brazil), and the essential oil was obtained by hydrodistillation of the leaves in the Organic Chemistry-Essential Oils Laboratory of the university [3]. The cytotoxic and trypanocide activities of the oil were determined at the Institute Carlos Chagas (FIOCRUZ), Curitiba, Parana, according to the methods described by Mosmann (1983) [4]. For trypanocidal activity against epimastigotes of *Trypanosoma cruzi* culture and the cytotoxic effect against the VERO cell line (African green monkey kidney), tests were performed using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

A weak trypanocidal activity [IC_{50} (concentration causing lysis or death of 50% of the epimastigote forms of *T. cruzi*) = 198.4 mg L^{-1}], a high cytotoxic effect [CC50 (concentration that causes lysis or death of 50% of Vero cells) = 36.9 mg L^{-1}] and, consequently, a low selectivity index (0.19) were observed for the essential oil from *L. origanoides.* Thus, the oil probably does not represent a potent drug use for in the fight against *T. cruzi*. The oil has a low selectivity against parasites and offers no possibility for safe use in therapy.

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PP-41. Hemolytic activities of essential oils from *Citrus* fruits and of the limonene and citral standards

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The use of natural products for medicinal purposes dates back to the beginning of civilization, and the interest in medicinal plants has increased the number of investigations of the biological effects in humans and animals during the last decades. There are many examples of drugs that were produced by from natural sources, especially plants, such as morphine, vinblastine, vincristine, and others. Currently, one of the challenges for pharmaceutical chemistry, biochemistry and pharmacology is the elucidation of the active components in plants, as well as their mechanisms of action, since plants contain numerous constituents and may exhibit synergistic or antagonistic effects, when tested. The essential oils frequently present these characteristics. They are complex mixtures of various compounds, containing about 20 to 60 components in very different concentrations, with two or three components present at higher concentrations (20-70%) [1]. The hemolytic activities of the essential oils extracted from lime (*Citrus aurantifolia*), lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels were evaluated, together with limonene and citral standards and their mixture (limonene and citral).

The limes, lemons and oranges were collected in a rural area near the city of Lavras in the morning, and the essential oils were obtained by hydrodistillation of the shredded peels in the Organic Chemistry-Essential Oils Laboratory of the University [2].

The evaluation of the hemolytic activity of the essential oils, the citral and limonene standards and their mixture (limonene with citral) was performed at the Laboratory of Biochemistry, Federal University of Lavras. For the experiments, healthy blood from the author was collected in the UFLA Clinical Laboratory in Vacutainer tubes with a capacity of 10 mL and containing sodium heparin. The determination of the resistance to hemolysis was followed by incubation of suspensions of erythrocytes (Ht-0.15%) in isotonic medium with different concentrations of the essential oils (0.6, 1.0, 2.0, 4.0, 5.0 and 10.0 µL mL^{-1}). In addition to the hemolytic test, scans were performed at wavelengths from 500 to 700 nm to evaluate whether the oxidation of hemoglobin occurred [3].

The standards (citral and limonene) and their mixture (limonene with citral) possessed a greater effect at low concentrations in the hemolytic study (release of hemoglobin) than the three essential oils. The hemolytic effect of three essential oils was inversely proportional to the concentration of the essential oil used. The oxidizing effect of six treatments on hemoglobin was observed to be gradual and proportional to the concentrations of the oils.

Acknowledgments

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PP-042. Evaluation of the antibacterial and antifungal activities of *Mentha suaveolens* ssp. *insularis* (Req.) Greuter essential oil from Corsica.

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The *Lamiaceae* family represents an important source of essential oils. The genus *Mentha* includes many species sometimes not clearly distinct because hybridizations between some of the species occurs naturally. *Mentha suaveolens* ssp. *insularis* (Req.) Greuter is endemic from Corsica and Sardinia .

A detailed analysis of essential oil (EO) and hydrolate extract (HE) was carried out by combination of GC–RI, GC–MS and ^{13}C-NMR spectroscopy.

After fractionation by column chromatography, 51 components of the EO and 27 components of the HE, accounting for 96.1% and 98.3%, respectively, were identified.

The main components were pulegone (44.4% and 14.8%) and *cis*-*cis*-*p*-menthenolide (27.3% and 67.3%). This *a*-methylen-*g*-butyrolactone was isolated and its structure elucidated by 1D and 2D NMR spectroscopy.

Essential oil , fractions containing mainly lactone or pulegone and hydrolate extract were evaluated for antimicrobial activity using the disc diffusion method and the microdilution minimum inhibitory concentration (MIC) assay. Several common bacterial and yeast isolates were tested for their susceptibility . Both exhibited a fair antibacterial activity against Gram+ bacteria : Large prevailing effective zones of inhibition and low MIC were observed for *Staphylococcus aureus* and *Bacillus cereus*. But the more important activity was against the yeast *Candida albicans*.

PP-043. Effects of the essential oil of *Foeniculum vulgare* on melanogenesis and antioxidation

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Foeniculum vulgare Mill. (Umbelliferae) is widely distributed in East Asia. Its fruits have been used as antiemetics, ameliorating stomach conditions, and an analgesic. A variety of constituents have been isolated from the fruits of this plant, mainly many kinds of essential oils and some coumarins.

Melanin is an ubiquitous natural pigment for skin, eyes and hair in most organisms, its production in human skin is called melanogenesis. Melanin is synthesized from tyrosine by the action of tyrosinase in the melanocytes. Therefore tyrosinase inhibitors are important in cosmetic skin-whitening. Oxidative stress contributes to skin aging and can adversely affect skin health, which means antioxidants may protect skin cells from radiation and any other harmful factors.

The present study was carried out to investigate the melanin inhibition and antioxidant effect of the fruits of *Foeniculum vulgare*, which would contribute further development of the natural cosmeceuticals. For this purpose, anti-melanogenesis and antioxidant activities of their extracts, fractions, and isolated compounds (8 monoterpenes and two phenyl propanoids) were evaluated.

As a result, *n*-hexane fraction inhibited the mushroom tyrosinase activity (37.2±2.25%), the melanin biosynthesis without cytotoxicity (<38 μg/ml) against B16 murine melanoma cell, and showed proper antioxidant activities against DPPH radical, lipid peroxidation and superoxide anion. Among 10 isolated constituents, three monoterpenes inhibited markedly the tyrosinase activity at 0.01 ug/ml and suppressed the melanin formation in the melanoma cell. In addition, antioxidant activities of all isolated compounds are demonstrated.

PP-044. Chemical composition of *Cornus mas* L. essential oil: influence of ecological/ geographical factors

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Cornus mas L. (Cornaceae Dumort), Cornelian cherry or dogwood ("dren" in Serbian), is a deciduous shrub, native to central, southern, and eastern Europe, Caucasus and Asia Minor. Despite its ethnopharmacological significance (there is even a saying in Serbian-"Zdrav ko dren."-to be healthy as a cornelian cherry), phytochemical data on this plant taxon are rather scarce. There is only one previous study that reports the chemical composition of an essential oil sample obtained from *C. mas* [1]. According to therein presented results, the dominant flower volatiles of two geographically/climatically/ecologically distinct Ukrainian populations of *C. mas* (Simferopol and Kharkiv districts; distance around 500 km) were camphor, verbenone, borneol and carvacrol (monoterpenoids constituted 43-49% of the total oils); the second most abundant compound class in both oil samples were the alkanes (*c.a.* 20-30%; mainly medium-chain-length *n*-alkanes) [1]. In order to further explore the variability of *C. mas* volatile profile, more precisely its change with geographical/climate/ecological factors, we decided to perform detailed GC and GC/MS analyses of an essential oil sample obtained by hydrodistillation from fresh flowers of a Serbian population of this plant taxon (flowers of dogwood were collected in the vicinity of the village Kajkovo, near Leposavić, Kosovo, Serbia).

Analyses by GC and GC/MS of the *C. mas* essential-oil sample (oil yield was 0.05%, based on the mass of fresh plant material) allowed the identification of more than 100 different components, comprising *c.a*. 90% of the total oil composition. The major identified volatile compounds were long-chain *n*-alkanes: heneicosane (11.0%), tricosane (11.2%), pentacosane (7.3%), heptacosane (7.0%), and nonacosane (6.4%) (*n*-alkanes comprised some 50% of the total oil). A significant amount of isomeric alka(poly)enes (C_{16}-C_{30}; 1-4 double bonds) were also identified (*c.a.* 20%). Alongside with these classes of constituents, (a small amount of) sesquiterpenoids, diterpenoids, fatty acids and other fatty acid related compounds (fatty acid esters, long-chain *n*-aldehydes, 1-alkyl acetates, alkanones, *etc*.) were also detected in the herein studied oil sample.

Pronounced differences in the chemical composition of the oils obtained from Serbian and Ukrainian *C. mas* populations (presence/absence and/or abundance of monoterpenes, alka(poly)enes *etc*.) may be explained by the influence of external (geographical/climate/ecological) factors; the latter might have caused the development of different *C. mas* chemotypes in Serbia and Ukraine (distance between the locations of Serbian and Ukrainian *C. mas* populations was around 1000-1500 km). Nevertheless, these differences might not be as pronounced as it may seem at first glance, as a quite large percent (from 25-38%) of oil components remained unidentified in the course of the previous study [1].

Acknowledgments

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PP-045. Essential oil of *Cephalaria ambroisioides* (Sibth. & Sm.) Roem. & Schult. (Caprifoliaceae): chemical composition and chemotaxonomic significance

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The genus *Cephalaria* (Caprifoliaceae) includes 93 species of annual or perennial plants scattered over the Eastern Mediterranean, the Balkans and the Middle East with an extension in Africa [1]. Both the genus and the family to which it belongs to are considered taxonomically difficult. The status of Dipsacaceae has recently been changed from the family level to a subfamily within Caprifoliaceae [2]. *Cephalaria ambrosioides* (Sibth. & Sm.) Roem. & Schult. is a robust perennial plant species endemic to the Balkan Peninsula. This taxon has been phytochemically neglected and there is one study reporting the isolation of triterpene saponins from the aerial parts of this plant species [3]. The composition of the essential oil has not been published thus far.

The unavailability of data on the essential oil composition prompted us to perform the first investigation of this species volatiles. The second goal of this study was to assess the usefulness of the chemical compositional data in resolving some uncertainties concerning the taxonomy of the *Cephalaria* genus and its infrageneric relations. Multivariate statistical analyses (particularly principal component analysis (PCA) and agglomerative hierarchical cluster analysis (AHC)) proved to be useful and often necessary for the processing of large data sets corresponding to volatile secondary metabolites. Herein, we presented the results of MVA comparison of essential-oil composition of 17 different Dipsacaceae species (25 samples including our own) with an intention to juxtapose them with morphologically implemented classification.

For this purpose, plant material was collected on the mountain Suva Planina, near the city of Niš. The essential oils hydrodistilled separately from air-dried flowers (fw) and leaves (lf) of *C. ambrosioides* were obtained in low yields (0.008% and 0.004%, w/w, respectively). GC and GC-MS analyses revealed a complex mixture of compounds constituting both essential oils. Among them 284 components, accounting for 88.6% and 96.1% of the total peak areas detected, were successfully identified. The main components were found to be palmitic acid (24.3% (lf); 32.5% (fw)), hexahydrofarnesyl acetone (1.4% (lf); 10.8% (fw)), (3*Z*)-hexen-1-ol (7.0% (lf); <0.1% (fw)) and linoleic acid (1.9% (lf); 6.5% (fw). Carotenoid-derived compounds represented one of the most diverse and abundant compound classes in the leaves and roots' oils, representing 15.9% (lf) and 12.5% (fw) of the essential oils. The remaining parts of the oils were comprised of "green leaf" volatiles and fatty acid derived compounds, 10.7% (lf), 1.2% (fw) and 38.3% (lf), 48.7% (fw) respectively. MVA demonstrated that the evolution of the volatiles' metabolism of Dipsacaceae taxa was both genera-specific and is in agreement with their morphological evolution. These analyses showed a close relationship between the genera *Cephalaria* and *Scabiosa*, and provided evidence for the exclusion of *Morina* spp. from this plant subfamily.

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PP-046. Evaluation of antifungal activity of essential oil from leaves of *Pimenta pseudocaryophyllus* against the fungus *Aspergillus flavus*

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A common problem in grain storage is fungal growth that may cause the occurrence of damaged and moldy grains. These types of grain present quality loss, with nutritional reduction, lower commercial value and the possibility of mycotoxin presence. Mycotoxins are toxic compounds that can be produced by fungi, and are dangerous to both human and animal health. Synthetic fungicides are currently used to control fungal growth, but natural alternatives with a lower risk of environmental damage and food contamination have been sought. Essential oils obtained from some plant species have been studied as natural antifungals. *Pimenta pseudocaryophyllus* (Gomes) Landrum is a native species from Brazil, where it is found in the Cerrado and Atlantic Forest biomes. The essential oil (EO) from leaves of *Pimenta pseudocaryophyllus* has presented, in other studies, some antimicrobial effect. In this study we evaluated its antifungal activity on the mycelial growth and production of aflatoxin B1 (AFB1) by *Aspergillus flavus*, a commonly found fungus in grain storage. Leaves of *P. pseudocaryophyllus* were collected in Ilha Comprida, SP , Brazil, in areas of dense forest, and were hydrodistilled by using a Clevenger-type apparatus for 2 h to obtain the EO. The antifungal activity was measured by the determination of Minimum Inhibitory Concentration (MIC). The methodology for MIC evaluation was the dissolution in Yeast Extract Sucrose (YES) culture medium. Doses of oil at 0, 250, 500, 750 and 1000 ppm were evaluated. Acetone was used as an emulsifier of oil in culture medium, even at the 0 ppm dose (control). Each treatment dose consisted of 5 replications of Petri dish with a diameter of 9 cm, where 20 mL of culture medium containing the oil dose were added. The fungus used was an isolate of *A. flavus* (CCT 7638). The inoculum was prepared from a colony of the fungus that was grown on Potato Dextrose Agar (PDA) for 15 days at 28°C. The density of conidia in the inoculum was 1.2 x 10^5 conidia/mL, counted with the aid of a Neubauer chamber. Inoculated plates were maintained without exposure to light for a period of 10 days at 28°C. The mean diameter of colonies was assessed after 2, 4, 6, 8 and 10 days by measuring it in perpendicular directions. The production of AFB1 was monitored at the end of the experiment in the culture medium using High Performance Liquid Chromatography (HPLC). It was observed at the end of the experiment (10 days) that fungal growth was visible only up to the dose of 500 ppm, hence the dose of 750 ppm was considered as the MIC (lowest dose evaluated where no growth was observed). The AFB1 contamination was 9023.1, 1465.4, and 0.7 ppb at 0, 250, and 500 ppm oil doses, respectively. The composition of the oil was evaluated by GC/MS, and eugenol, methyl-eugenol, and 1,8-cineole were detected as major compounds.

PP-047. Properties of the essential oil extracted from *Alpinia zerumbet* flowers

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Alpinia zerumbet (Pers.) Burtt & Smith is an aromatic perennial plant that is widely distributed throughout the tropical and sub-tropical regions of the world, and which grows freely from southern Kyushu to the Okinawa Prefecture in Japan. *A. zerumbet* produces a pink flower that blooms from April to May. The essential oil of this flower has been reported to show antioxidant activity [1]. less attention has been given to its flowers and seeds. In our study, essential oil, total phenolics and antioxidant capacities assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH], but it is very expensive because large quantities of the flower are required to obtain relatively small amounts of the oil. In our previous research, we have reported that underwater shock-wave treatment can be used to enhance the amount of essential oil that can be extracted from *A. zerumbet* leaves by steam distillation. Underwater shock wave treatment can also be used to improve the functionality of the extracted oil, as demonstrated by its antioxidant activity and its ability to suppress melanogenesis [2,3]. In this study, we extracted the essential oils from dried and fresh *A. zerumbet* flowers and evaluated their antioxidant activities to determine whether the picking season affected the composition of the volatile components present in these essential oils, as well as their overall functionality.

A. zerumbet flowers were collected in Henoko (Nago City, Okinawa) in April and May, 2013. The essential oil, aqueous extract and residual water, which remained in the container following the distillation process, were all obtained by the hydrodistillation of the flowers. The aqueous extract was analyzed by headspace gas chromatography-mass spectrometry (HS-GC-MS), and the volatile components present in the extract were identified by comparison with MS libraries. The antioxidant activity of the residual water was measured using the 1,1-diphenyl-2-picrylhydrazyl method[4]. The aqueous extract obtained from the flowers collected in April (AE-A) was analyzed by HS-GC-MS, which resulted in the identification of 55 compounds. The main compounds detected in the extract were 1,8-cineole (44.7%), terpinen-4-ol (35.8%), linalool (7.2%), α-terpineol (2.3%), camphor (1.0%), and borneol (0.2%), which represented 91.2% of the total components detected. The aqueous extract from the flowers collected in May (AE-M) contained 41 volatile compounds and AE-A contained more minor components than AE-M. Furthermore, AE-M contained higher levels of 1,8-cineole and camphor than AE-A, although the amount of terpinen-4-ol decreased sharply in AE-M compared with AE-A. AE-A also had a much sweeter scent than AE-M.

The aqueous extract and residual water sample were evaluated to determine their antioxidant activity. The water extract did not show any discernible antioxidant activity, whereas the residual water sample showed significant free radical-scavenging ability, and its trolox equivalent value was determined to be 5.4 mmol/gDW. These findings therefore indicated that *A. zerumbet* flowers contain nonvolatile and hydrophilic compounds with significant free radical-scavenging properties. The hydrophilic nature of these compounds explains why they dissolved in the residual water during the hydrodistillation process. The results of this study also showed that the antioxidant activity disappeared when the extracts were dried at a low temperature, and was recovered when the flowers were subjected to underwater shock wave treatment.

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PP-048. Essential oil content of cultivated *Origanum vulgare* ssp. *hirtum*

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Two different populations of *Origanum vulgare* ssp. *hirtum* were cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece). After one year of cultivation, the essential oils of their aerial parts were analyzed. Although both samples belonged to the same subspecies, the origin plants were collected from different places: Orvh1 from Thermi (N. Greece); Orvh2: from Mont Taygetos (S. Greece). The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their chemical analyses were performed by GC and GC-MS. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries and those described by Adams, as well as on comparison of their retention indices and with literature values. Their main constituents were found to be for Orvh1: thymol (24.6%), trans-sabinene hydrate (24.2%), γ-terpinene (8.6%) and terpinen-4-ol (7.1%); Orvh2: *p*-cymene (34.7%), carvacrol (29.6%) and γ-terpinene (14.9%). It is the first time that trans-sabinene hydrate is found in high amounts in *O. vulgare* subsp. *hirtum*, while it is abundant in *O. microphyllum*, *O. majorana*, *O. rotundifolium*, *O. micranthum*, O*. husnucan-baserii*, *O. syriacum* var. *sinaicum*, *O. dayi* and *O. ramonense*. In addition, there is one more study where terpinen-4-ol was abundant in *O. vulgare* subsp. *hirtum*; this evidence was attributed to a certain chemotype.

PP-049. Essential oil content of *Origanum cordifolium*, cultivated at the laboratory of conservation and evaluation of the native and floricultural species (North Greece), originated from Cyprus.

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O. cordifolium (Montbret & Aucher ex Benth.) Vogel was collected from Nicosia, Cyprus and cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece). After one year of cultivation, the aerial parts were analyzed on the basis of their essential oil, obtained by hydrodistillation in a modified Clevenger-type apparatus. The chemical analyses were performed by GC and GC-MS. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries and those described by Adams, as well as on comparison of their retention indices and with literature values. It is noteworthy the total absence of monoterpene hydrocarbons, while oxygenated monoterpenes (79.6%) and sesquiterpene hydrocarbons (18.6%) constitute the total composition of the essential oil. The results are in accordance with two previous studies concerning native and cultivated populations in France. Therefore, it can be assumed that although the plant was cultivated far away from the locality where it grows wild, the cultivation does not affect the yield and the composition of its essential oil.

PP-050. Effect of chitosan on the essential oil contents and biomass of aerial part of *Mentha arvensis*

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Menthol, the majority component of essential oil in *Mentha arvensis* is widely used in pharmaceutical, cosmetic and food industry. The essential oil production may vary depending on several factors, such as, attack of pathogenic organisms. The elicitors, as chitosan (CH), are substances used to simulate pathogens actions in plants[1]. Thus, the aim of this work was to evaluate the effectiveness of CH in increase of biomass production and contents of essential oil of *M. arvensis*. The plants were cultivated for 72 days and chitosan 0 (only acetic acid); 0.25; 0.5 and 0.75% solved in acetic acid (1%) were sprayed in plants of *M. arvensis*. The samples (aerial parts and roots) collected after 7 days of chitosan application when the plants were harvested and subjected to the hydrodistillation using the Clevenger type apparatus. The content of essential of all treatments and the control (intact plants) were measured. The essential oil yielded (about 0.4%) [2] were very similar, although were verified the tendency of increase of essential oil in the plants treated with chitosan 0.5%. The biomass of aerial part reduced significantly (about 40%) with the application of acetic acid and CH doses, therefore, especially to *M. arvensis* more study must be performed. In order to determine the minor dosage of acetic acid in chitosan solution others experiments are been proposed for our research group and the possible translocation of the essential oil of aerial part to roots will be considered.

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PP-051. Production of essential oil in young plants of *Mentha rotundifolia* L. in function of different types of cutting and Embiotic Line application

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In the last decade, the growth of commercial demands of essential oils, especially of *Mentha* species, widely used in industries, is relevant the development de agriculture techniques including propagation of plants. In this study, were used different propagation structures of *Mentha rotundifolia* L., apical and medium cuttings (from aerial parts) and tip cutting (from rhizome part) (1) and a products "Embiotic Line" (EM) mixed in substrate Plantmax® (Eucatex, São Paulo, SP) in five different doses (0; 0,25 ml/Kg; 0,75 mL/ Kg; 1,25 mL/Kg; e 1,75 mL/Kg). The experimental design was completely randomized in 3x5 factorial schemes (three propagation structures and five dose of EM). The cutting had 4-5 cm long. After 42 days were determined height, number of leaves, aerial and roots biomass, mortality of plants. The results show that apical cutting from aerial part had higher rooting, aerial part development without any mortality. Medium cuttings from aerial part and tip cutting from rhizome part were verified 20 and 15 % of mortality and low indices of rooting. The interaction among cuttings and mixed EM in substrate was not significant for the growth parameters. Related to EM doses mixed in substrate is not verified significant different among the treatments, however were observed a tendency for the better development in plants treated with EM. Only plants obtained from apical cuttings from aerial parts were used to extraction of essential oil, although in minimal quantities. Another cutting produces dry matter insufficiently for extraction. Were verified that, the use of EM in dose 1,25 mL/Kg of substrate yielded 0,45% of essential oil. Considering that the plants were harvested in the first stages of development this preliminary data is relevant, however, some experiments must be performed in order to determine the main period for harvest the essential oil composition, as well as, the influence of the use of EM during the life cycle of *Mentha rotundifolia*. Essential oils from *Mentha rotunfolia* were a complex mixture. Concerning to the essential oil composition of this species were reported two chemotypes according to seasonal variations. Pulegone or β-Caryophyllene as majority constituent (2). In this study the composition of essential oil are been performed. These questions are been target of investigations of our group.

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PP-052. Flower extract and chemical analysis of flower scent of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae)

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Volatile organic compounds (VOCs) have protective and signaling effects, including defensive, attractive and alelopathic functions. *Zeyheria tuberculosa* is a large tree found in tropical region, occurring in Brazilian seasonal dry forests. Its flowers are tubular, yellow with red nectar guides, nectariferous and pollinated mainly by medium-sized bees. Once this is an endangered species, it is important to know, as much as possible, how is going the reproduction of the remaining individuals of this species in the vegetation patches. This way, we studied pollinating-attractive features with the goal of providing information that can help to guarantee its permanence in these ecosystems throughout the time. We performed the characterization of the flower extracts by qualitative phytochemical analysis and thin-layer chromatography, as well as the floral scent characterization by means of GC/MS-HS analysis to identify chemical compounds potentially related to plant-pollinators interaction. Flower extracts (hexane, chloroform and methanol) were evaluated by several chemical reactions and thin-layer chromatography. Recently-opened flower samples were placed in a 20ml borosilicate glass vial and analyzed on a Thermo Scientific Focus system, equipped with an ISQ 230ST mass spectrometer and a Triplus automatic sampler. Separations were achieved by using an OV5-MS column under optimized conditions and identification of volatiles was based on the comparison of their mass spectral data and retention index with those from NIST data base and literature. Our study revealed the presence of antraquinones in methanol extract, and terpenes (ursolic acid, β-sitosterol and β-amyrin) in hexane, chloroform and methanol extracts. Floral scent of *Zeyheria tuberculosa* comprises a complex mixture of four aldehydes, six alcohols, two non-terpene hydrocarbons, two phenol compounds, one monoterpene (β-ocimene) and six sesquiterpenes. Further studies are being carried out aiming at elucidating the role of these compounds on *Zeyheria tuberculosa* reproductive biology.

PP-053. Floral scent of *Jacaranda oxyphylla* Cham. (Bignoniaceae)

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Volatile organic compounds (VOCs) have been described as important compounds with defensive, attractive and alelopathic functions, acting as signal between plants, or between plant and animals in antagonisms or mutualisms. *Jacaranda oxyplhylla* is a Bignoniaceae species from Brazilian savanna, pollinated by medium and large-sized bees. Its purple flowers are characterized by the presence of abundant glandular trichomes, which secrete compounds that might lead to specialized interactions with anthophilous animals. In spite of the ecological and medicinal interest in this plant, its floral scent analysis has not been performed yet. This way, we proposed to perform the characterization of floral VOCs by means of GC/MS-HS analysis in order to identify chemical compounds potentially related to pollinator attraction. Recently-opened flower samples were placed in a 20ml borosilicate glass vial and analyzed on a Thermo Scientific Focus system, equipped with an ISQ 230ST mass spectrometer and a Triplus automatic sampler. Separations were achieved by using an OV5-MS column under optimized conditions and identification of volatiles was based on the comparison of their mass spectral data and retention index with those from NIST data base and literature. Our study revealed the presence of a mixture containing one aliphatic ketone, one aliphatic alcohol, four aromatic compounds, three monoterpenes, twenty three non-terpene hydrocarbons and one thiazol derivative. The major volatile constituents and their relative percentages were unidentified hydrocarbons (42.3%) and 1-octen-3-ol (3.2%). Additional studies are underway to elucidate the chemical-biological interactions of these compounds and their role on the interactions with pollinator of *Jacaranda oxyphylla*.

PP-054. Estimation of odour active compounds of coriander after decontamination by GC/MS, SPME/GC and GC/O

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Spices are commonly used to enhance the attractiveness of aroma in foodstuffs. Unfortunately, spices became contaminated microbiologically during harvesting, drying, transport and storage. It may cause adverse sensory changes in foodstuffs, reduce their shelf life and have an adverse effect on the consumer's health. For this reason several methods of spices decontamination are used.The aim of the study was to investigate the effect of the applied steam decontamination on odour active compounds concentration in coriander *(Coriandrum sativum* L.) by used of GC/MS, SPME/GC, GC/O methods, including chiral analysis.

Hydrodistillation: in a Deryng' apparatus. 1 g sample with 0.5 ml o-xylene. Internal standard was tetradecane. Distillation was run for 3 h.

SPME/PDMS: samples 100 mg, vials 10 ml, addition of 2.5 ml deionized water and 300 µl methanol. Extraction temperature 50ºC, time 15 min.

GC/FID, GC/MS: HP 6890 with a FID detector and a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm). Agilent Technologies 5975C VL MSD, HP-5MS column (30 m x 0.25 mm x 0.25 µm). The NIST05 mass spectrum library.

GC/O-AEDA: HP 5890 with an inlet splitter and a smelling port, DB-5 column (30 m x 0.53 mm x 0.25 µm). Separated fractions were smelled in successive double dilutions of analyzed spice distillates, until the last detectable aroma disappeared. In this way aroma was referred to respective retention indexes and dilution factors (FD) for individual fractions.

Enantioselective chromatography: HP 6890 with FID detector and Rt-βDEXsm column (30 m x 0.32 mm x 0.25 µm). Enantiomeric ratios for individual compounds were determined in percent on the basis of the area of their peaks, assuming the sum of the areas for both enantiomers as 100 %.

Volatile aromatic compounds were identified and estimated in coriander before and after decontamination. The GC/O analysis showed that the primary compound responsible for the aroma in coriander was linalool, which received the highest dilution factor value (FD=256) both before and after sterilization. It was determined that the content of linalool may be estimated by a rapid SPME/GC method, for which a high correlation (0.9323) was obtained with hydrodistillation. The effect of decontamination on enantiomeric ratios main volatiles (α-pinene, limonene, γ-terpinene, linalool, camphor) were determined by chiral analysis both in hydrodistillates and SPME. No qualitative changes or losses were recorded for volatile aroma compounds in steam decontamination coriander. Also no changes were found in the composition of enantiomers of primary aroma compounds this spice under the influence of decontamination. The use of SPME allowed for rapid assessment of odor quality coriander, to be changed through this process, on the basis of measurements of linalool.

PP-055. Volatile constituents in the roots of different yarrow *(Achillea)* accessions

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Achillea species are well known medicinal plants worldwide. The flowering shoots contains essential oil as main active agent and the populations show high chemical variability. Although only the flowering shoot is usually processed as a valuable source of natural pharmaceuticals, each plant part contains biologically active substances which might have significance in the future. About the roots, only sporadic reports are known till now (Lazarević et al., 2010; Jovanović et al., 2010).

In our experiment the root volatiles of six different accessions of *Achillea* were investigated included European cultivars, selected strains and wild growing populations (5 genotypes of *A. collina* Becker and 1 strain of *A. crithmifolia* W. et K.). The plant material originates from a two-year-old living collection of yarrrow. The roots were harvested in March of 2013, before re-shooting of the plants. After purification, the air-dried, grinded roots were hydro-distilled in a Clevenger-type apparatus for 4 hours. The essential oil compounds were determined by GC-MS.

The essential oil content of the samples varied from 0.041 to 0.083 ml/100 g. The highest oil content accumulated in the roots of cultivar 'Proa', while in the Swiss variety 'Spak' produced volatiles only in traces.

The identified constituents accounted 84.21-99.75 % of the total oils. The most, forty-seven, components were identified in 'Gb 22', an *A. collina* accession of Hungarian wild origin. The main constituents of the root oil were fatty acids in each of the six genotypes, however the composition showed characteristic differences. In the five *A. collina* samples n-hexadecanol accumulated in significant concentrations (19.97-45.36 %), while other (unknown RI: 41.60, 44.61, 48.52) fatty acids were predominant in *A. crithmifolia*.

The terpenoid fraction represented 51.19 % of the oils in common yarrow genotypes, while its ratio was lower in *A. crithmifolia* (30.34 %). The main sesquiterpene constituents of the oil were β-caryophyllene, β-farnesene, β-sesquiphellandrene, vulgaron-A and curcumene-15-al. Monoterpenes occurred in the samples 11.70 % in average, with the lowest ratio (1,48 %) in *A. crithmifolia* and the highest one in cultivar 'Spak' (28,93 %). The main monoterpene compound in all the six oils proved to be the ester neryl-isovalerate.

It seems, that the composition of yarrow root oil is basically dependent on species, but also intraspecific differences occure. The composition is totally different from that of the overground parts and chamazulene is missing. These are the first results about the composition of the most popular species *A.collina*.

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PP-056. Chemical composition and antimicrobial activity of the essential oils of *Nepeta alpina* growing wild in Iran

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The increase in agricultural production can be possible with the discovery and utilization of chemical compounds for plant disease control. However, indiscriminate use of chemical compounds against microorganisms causes many negative side effects on environment and mammalian health. The hydrodistilled volatile oil from aerial parts of *Nepeta alpina* was analyzed by GC-MS. Seven compounds (95.88%) were identified of the total oil. Germacrene D (24.95%), bicyclogermacrene (17.67%) spathulenol (14.80%) and caryophyllene oxide (12.94%) were the main components. The antimicrobial activity of the aerial part essential oils of *N. alpina* is screened against Gram-positive and Gram-negative bacteria, and fungi. The best antibacterial activity was observed against *Listeria monocytogenes* with ZI (11.0 ± 0.5) mm and MIC value of 32 µg/ml.

PP-057. Composition of essential oil from roots of *Daucus littoralis* Smith subsp. *hyrcanicus* Rech. f. and its antimicrobial activity

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Daucus littoralis Smith subsp. *hyrcanicus* Rech.f (Caspian carrot) from Apiaceae family is an endemic species in north of Iran (Mazandaran and Guilan provinces) and grows wild on the sandy dunes of Caspian Sea coasts. This species has been used locally as condiment in these parts of country. The essential oils from roots of this plant have been obtained by hydrodistillation and investigated by GC and GC/MS for the first time. Average yields of roots essential oil was 2.6% (v/w). GC-MS analysis of the oil led to the identification of 29 different compounds, representing 94.4% of the total essential oil. The essential oil was characterized by high amounts of sesquiterpene hydrocarbons (67.3%). The main volatile components of the root were germacrene D (36.1%), acorenone B (19.7%) and myristicin (9.7%). Evaluation of MIC and MBC showed that the essential oils have antibacterial activity against *S. aureus* and *C. albicans* (MIC 20 µl/ml, MBC 40, 20, respectively) and *E. coli (*MIC 40 and MBC 80 µl/ml).

PP-058. Essential oil composition and antimicrobial activity of *Conyza canadensis* (L.) Cronquist from Turkey

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The genus *Conyza* Less. belongs to the family Asteraceae and consists of about fifty species all over the world. In Turkey, the genus *Conyza* is represented by three species, namely *Conyza canadensis* (L.) Cronquist, *Conyza bonariensis* (L.) Cronquist and *Conyza albida* Willd. ex. Sprengel (1,2).

In traditional medicines worldwide, *C. canadensis* is used for the treatment of diarrhea, dysentery and as diuretic agent. The plant is also reported to be used as antibacterial and anti-inflammatory (3,4). The essential oil of *C. canadensis* was mentioned as diuretic, tonic, astringent and for arresting internal haemorrhage in the United States Pharmacopoeia (5). There are number of researches on the essential oil of *C. canadensis* collected from different countries e.g. Hungary, Poland, Iran, India, Bulgaria, China, USA and Greece. Essential oils from herbs and roots of *C. canadensis* have different constituents. The major component of herb oil was limonene while the main component of root oil was (2*Z*, 8*Z*)-matricaria ester (6). To the best of our knowledge, this is the first GC-MS study of the volatile compounds of *C. canadensis* from Turkey.

In our study, the essential oil of the aerial parts and roots of *C. canadensis* collected from Selçuk, İzmir in the flowering-fruit stage was obtained using a Clevenger type distillation apparatus. The chemical composition of the essential oils was analyzed by GC and GC-MS, simultaneously. Major components of the herb oil are found elemol (21.5 %), β-eudesmol (6.3 %), caryophyllene oxide (5.2 %), α-eudesmol (5.0 %), α-muurolene (4.1 %), humulene epoxide II (3.9 %) and spathulenol (3.7 %). The root oil contains hexadecanoic (33.0 %), tetradecanoic (5.3 %) and octadecanoic (4.6 %) acids. In addition, antimicrobial activity of the essential oils was investigated by TLC-bioautography method on some bacterial and fungal strains. Both of the oils showed antimicrobial activity against the strains of *Staphylococcus aureus* ATCC 6558 and *Candida albicans* ATCC 90028, while there is no efficient compound in the oils against the strain of *Escherichia coli* NRRL B-3008.

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PP-059. Inhibitory effects of essential oil from aromatic Ugandan medicinal plants on oral bacteria

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Dental Caries (DC) and Periodontal diseases (PD) are prevalent worldwide. Most communities in low-resource settings rely on medicinal plants to prevent and treat DC and PD. Moreover, the screening of such plant extracts for biological activities has always been of great interest to scientists looking for new sources for drugs.

To test *in-vitro* inhibitory effects of essential oils (E.Os) extracts from aromatic Ugandan medicinal plants, (namely, *Cymbopogon citratus* (C.ci), *Cymbopogon nardus* (C.na), *Teclea nobilis* (T.no), *Zanthoxylum chalybeum* (Z.ch), and *Lantana trifolia* (L.tr)) that are used in traditional treatment of oral diseases in Uganda, on caries associated *Streptococcus mutans* and periodontitis associated *Porphyromonas gingivalis*.

E.Os were obtained from fresh leaves and twigs of the plants through steam distillation process. *S. mutans* was resuspended in Lactobaciilli MRS Broth and *P. gingivalis* resuspended in Peptone Yeast Glucose Medium. Inhibitory effects of the E.Os on the bacteria were assessed using broth dilution method at therapeutically realistic concentrations of 1%, 0.1% and 0.01%. Inhibitory activity was expressed as a percentage of colony forming units (CFU) in the test plate to the CFU in the control. Possible major constituents in E.Os were explored by Gas chromatography–mass spectrometry (GC-MS) analysis.

The most active E.O was from C.na. It was completely inhibitory to *P. gingivalis* at 1%, 0.1% and 0.01% concentrations. GC-MS analysis showed possible major constituents in C.na E.O as Intermedeol (43.7%); Myrcene-(10.5%); Germacrene-D-4-ol-(8.6%). C.ci, T.no, Z.ch and L.tr were inhibitory to *P. gingivalis* at 1% and 0.1% concentrations. *S.mutans* was completely inhibited by C.ci and Z.ch at 1% concentration and by C.na at 0.1% concentration.

The E.Os from the aromatics Ugandan plants show strong inhibitory effects on periodontitis associated *P. gingivalis* and limited effects on caries associated *S. mutans.*

PP-060. Analysis by the method of chromato-mass-spectrometry of the volatile constituents of *Halimodendron halodendron* growing in Southern Kazakhstan

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Halimodendron halodendron (pall.) Voss was collected in 2010, September from Kyzyl-Orda region Karmakhchinsk district Irkul village of Southern Kazakhstan. The volatiles were obtained from the crushed aerial parts (leaves, seeds) of *H. halodendron* (pall.) Voss. by hydrodistillation using a Clevenger apparatus.

The obtained volatiles were investigated by GC/MS. 48 compounds from leaves and 41 compounds from seeds of *H. halodendron* were identified. The main components of leaves volatile compounds were phytol (11.7%), linalool (11.0%), β-E-damascenone (7.8%), α-terpineol (6.5%), and hexahydrofarnesyl acetone (4.7%). The main components of seeds were: hexahydrofarnesyl acetone (10.7%) and squalene (9.3%). There were identified also phytol (4.2%), β-ionone (3.7%), 2E,4E-decadienal (2.7%), 2-butyl-oct-2-enal (2.1%).

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PP-061. Pimpinella anisum L. essential oil and combination with antifungal drugs against *Candida* species and cytotoxicity of selected compounds

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Pimpinella anisum L. (anise) of Apiaceae is an annual herbaceous plant, which grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, Spain, . The anise fruit and its essential oil are used mainly as a spicy seasoning, as a flavor additive in toothpastes and gargles, in the confectionary industry, and for the production of herb liqueurs. In medicine, the carminative, spasmolytic, and expectorant effects of the fruits and essential oil are of interest.

In the present study; commercial *P. anisum* essential oil at Pharmacopoeia Grade (PhEur) was used. The chemical compositions of the investigated oil was determined both by GC/FID and GC/MS techniques and *P. anisum* essential oil combination with antifungal drugs (oxiconazole and terbinafine) against both clinical and standard *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 66032, *Candida tropicalis* ATCC 1369 strains were screened *in vitro* by the CLSI microdilution method with Biomek 4000 automation system. *P. anisum* essential oil and antifungal drugs interaction were performed using the chequerboard assay method. Resulting fractional inhibitory concentrations were calculated and interpreted as synergy, additive or antagonism. WS1 (ATCC® CRL-1502™, human normal skin fibroblast cell line) cell line were used for cytotoxicity tests. Inhibition % was calculated each concentration of compound. IC_{50} value was calculated by non-linear regression analysis.

(*E*)-Anethole (86.4 %), ɣ-himachalene (3.85 %) and epoxy-*trans*-pseudo isoeugenol-2 methyl butyrate (1.9 %) were determined main compounds of *P. anisum*. essential oil. Predominantly, indifferent interactions were noted for *P. anisum* essential oil and antifungal drug combinations (oxiconazole and terbinafine). No antagonistic effects were observed. *In vitro* cytotoxicity of *P. anisum* essential oil, oxiconazole and terbinafine were measured in WS1 cell line. The IC_{50} values are 299.55, 27.08 and 56.68 µg/ml, respectively. DPPH antioxidant assay was also performed with standarts.

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PP-062. Insecticidal activities of *Fortunella crassifolia* Swingle peel against house fly *(Musca domestica)*

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Growing patterns of insecticidal drug resistance laid the foundation for research in exploring novel anti-insect agents from medicinal plants particularly with fumigant action for ease of application. The aim of this study was to determine the some constituents of the essential oil isolated from *Fortunella crassifolia* Swingle peel by hydro-distillation, and to test the efficacy of the essential oil on insecticidal activity against house fly, *Musca domestica*. Two *Fortunella* fractions obtained by steam distillation, Soxhlet *n*-hexane extraction, extraction with aqueous ethanol, and with ethanolic ammonia solution were evaluated *in vitro* for activity against house fly, *Musca domestica* L. These data warrant further study on identifying the components of the extracts with the highest activities. The essential oil showed potent insecticidal activity. It was suggested that the essential oil from *Fortunella crassifolia* Swingle peel might be used as a natural insecticide against house fly.

PP-063. Cold-pressed lemon essential oil by-products: enhancement throught bioactive molecules isolation

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Lemon essential oils are produced in many countries all over the world and they are widely used in food, pharmaceutical and perfume industries. Cold-pressed lemon essential oil industry generates a considerable amount of by-products (or waste) with high amounts of valuable bioactive components.

The by-products obtained from lemon transformation are represented by peels, pulps, seeds and waste water. These are known to be rich in bioactive molecules such as flavonoids, limonoids, phenolic acids, coumarins, furocoumarins, polymethoxyflavones, and carotenoids. All these compounds can be recovered from the wastes and recycled for different uses, thus reducing the costs of waste disposal as well as the amount of total waste.

This work is focused on the isolation of different limonoids (limonin, nomilin, nomiln acid glucoside, obacunone, ichangina, limonin glucoside) in lemon seeds by-products by means of a multidimensional HPLC system coupled with a photodiode array and a mass spectrometer detectors.

Limonoids isolated by the preparative LC-LC system were used to elucidate a qualitative and quantitative profile of these molecules in Cold-pressed lemon essential industry by-products (peels, pulps and seeds).

PP-064. Composition of the essential oil from *Salvia veneris* Hedge growing in Cyprus

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Genus *Salvia* is one of the largest member of the family Lamiaceae, many of which grow wild. There are several reports on the medicinal uses of some species of the genus, mainly against cold, skin infections, wounds, pharyngitis, stomatitis, stomachache, headache, memory enhancement and galactorrhoea.

The chemical compositions of many *Salvia* species have extensively been investigated by several workers revealing the presence of terpenes, tannins, flavonoids and essential oil. The studies on the essential oil composition of the species showed high variability.

Ten *Salvia* species grow wild in Cyprus, of which two are endemic. One of the endemic species, *S. veneris*, is the closest allies with the Turkish endemics called *S. cilicica, S. cassia* and *S. cyanescens*.

In this study, essential oil composition of the aerial parts of *S. veneris* collected at the flowering stage from Northern Cyprus was investigated by means GC and GC-MS. In the three oil samples analyzed, 37 compounds comprising 99.8% of the oils were characterized. 1,8-cineole (51%), camphor (9.3%), camphene (6.3%), α-pinene (5.8%) and β-pinene (5.4%) were identified as main constituents, respectively. These results clearly place *S. veneris* in CiCa type of *Salvia* oils (1).

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PP-065. Analysis of the essential oil composition of *Anthemis tricolor* Boiss. from Cyprus

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The species of the *Anthemis* genus of Asteraceae are widely used in pharmaceutics, cosmetics and food industry. The flowers of the genus have well-documented use as antiseptic and healing herbs, the main components being natural flavonoids and essential oils. The antimicrobial activity of the essential oils and different extracts from several *Anthemis* species have been reported.

In this study, the essential oil of an endemic species, *Anthemis tricolor* Boiss., collected from Cyprus during the flowering stage was obtained by hydrodistillation. Oil analyses were performed by GC and GC–MS. β-Eudesmol (14.5%), hexadecanoic acid (13.6%), spathulenol (8.0%), linoleic acid (6.7%), linolenic acid (5.5%) and γ-eudesmol (5.5%) were the main compounds. The essential oil components of *Anthemis tricolor* Boiss.were also reported previously by Karaalp et al (1).

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PP-066. Chemical composition of *Bunium alpinum* ssp. *montanum* essential oil

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The Apiaceae family includes a lot of species spread across 434 genera. Many of members are widely used in nutrition and for traditional medicinal purposes. There are not many published data referenced to the genus *Bunium.* To the best of our knowledge this is the first report about *Bunium alpinum* Waldst. & Kit. ssp. *montanum* (W.D.J. Koch) P. W. Ball. essential oil composition.

The essential oil composition of *Bunium alpinum* ssp. *montanum* (Apiaceae), obtained by hydrodistillation of aerial plant parts, was analysed by GC and GC/MS. The plant material was collected in Morača Canyon (Montenegro), in May 2012. The sample was analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) equipped with a Combi PAL sampler.

Among the twenty nine components identified in this oil (96.5 % of the total oil) (*Z*)-pentadeca-1,9-dien-4,6-diyn-3-ol (48.3 %) and *trans*-β-farnesene (34.8 %), were found to be the major constituents. It is interesting to note that two higher (*Z*)-pentadeca-1,9-dien-4,6-diyn-3-ol homologues, (*Z*)-hexadeca-1,9-dien-4,6-diyn-3-ol (6.4 %) and (*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol (synonyms: Falcarinol, carotatoxin, 0.3 %) has also been identified, which make up 55.0 %. The essential oil consisted mainly of non-terpenoid compounds (58.9 %), while the terpenoids were unevenly distributed between monoterpenoids and sesquiterpenoids (0.3 % and 37.3 %, respectively).

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PP-067. Chemical composition of *Chaerophyllum aureum* L. essential oil and head space volatiles

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The genus *Chaerophyllum* L. (Apiaceae) is taxonomically complex genus and comprises about 40 species which occur commonly throughout Europe, Asia and North America. For the reason that most members of the Apiaceae family have been used in human diet or in traditional medicine, the present study reports the chemical composition on the volatiles of *Chaerophyllum aureum* obtained from fresh aerial parts and fruits by GC and GC/MS analysis.

The plant material was collected on Vlasina plateau (Serbia), in July 2013. The samples were analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) equipped with a Combi PAL sampler and Headspace for G6501B/G6509B. In all samples, the most dominant components were monoterpene hydrocarbons. The main constituents of essential oil and headspace volatiles obtained from fresh above-ground parts of the plants in fruiting stage were: sabinene (32.2 %, 47.8 %) and limonene (25.5 %, 25.3 %) respectively. The essential oil showed high content of γ-terpinene (8.4 %) while in the HS volatiles, terpinolene was found in a significant percentage (8.2 %). Most abundant component in the fruit essential oil was limonene (26.4 %), followed by sabinene (25.6 %) and terpinolene (14.8 %), while the fruit headspace volatiles contain terpinolene as a dominant component (45.2 %). Other constituents identified in significant amount in headspace volatiles of the fruit were α-terpinene (13.1 %) and β-pinene (10.2 %). Comparing the chemical composition of hydrodistillated essential oil and headspace volatiles of the aerial parts of *C. aureum* it is obvious that the major identified compounds were the same. However, this was not the case with the composition of essential oil and HS volatiles of the fruit. Qualitative composition of both examined oils was similar but some quantitative differences were observed.

Acknowledgments

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PP-068. Comparison of GC-MS profiles of headspace volatiles and essential oils: The case of three *Thymus* samples, *Origanum heracleoticum* and *Achillea crithmifolia*

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The GC-MS analysis of hydrodistilled essential oils (EO) and head space volatiles (HS V) of the fresh or dried above-ground parts at the flowering stage of *Thymus glabrescens* Willd., *Thymus praecox* Opiz subsp. *jankae* (Celak.) Jalas (from two localities), *Origanum heracleoticum* L. and *Achillea crithmifolia* Waldst. & Kit. were done. The optimal conditions for obtaining HS V were determined: equilibrium temperature 80^{0}C, equilibrium time 20 minutes. The qualitative monoterpene composition of EO and corresponding HS V was similar for all studied samples, which could not be claimed for their quantitative composition. Namely, all components that have Adam's indices less than γ-terpinene (AI 1054) were more abundant in the HS V than in corresponding EO profiles. For some compounds difference in the prevalence was manifold, for instance: α-pinene (*T. praecox,* EO 0.9%, HS V 18.6%), myrcene (*T. praecox,* EO 3.9%, HS V 23.2%), limonene (*T. praecox,* EO 2.5%, HSV 17.8%), carvacrol (*O. heracleoticum,* EO 80.1%, HS V 6.3%), and thymol (*T. glabrescens*, EO 55.4%, HS V 4.7%). As a conclusion, HS volatiles can provide a quick insight into the qualitative composition of plant monoterpenes but could not replace the analysis of essential oils.

PP-069. Endemic Balkan parsnip *Pastinaca hirsute* Pančić: the GC-MS profile of essential oils and head-space volatiles

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Since ancient times the species of the *Pastinaca* genus – parsnips have been used in human diet as well as in traditional medicine. *Pastinaca hirsuta* Pančić is endemic to the eastern part of the Balkan Peninsula and distributed in the eastern and southern parts of Serbia and in the southern and northern parts of Bulgaria.

Considering two growth stages, the survey aims to determine and compare the composition of *P. hirsuta* essential oil and head space (HS) volatiles, obtained from fresh roots, stems, flowers and fruits. The plant was harvested at the flowering stage (June 2013) and the fruiting stage (July 2013), in the region of Vlasina Lake in Southeast Serbia (Europe).The samples were analyzed on a 7890/7000B GC/MS/MS triple quadrupole system (Agilent Technologies, USA, equipped with a Combi PAL autosampler). Agglomerative hierarchical cluster analysis (AHC) was performed by STATISTICA 8 software.

b-Pinene was one of the major components of the root and stem HS volatiles (50.6- 24.1%). (*E)*-β-Ocimene was found in a significant percentage in the stem and flowers HS volatiles (31.6- 57.3%). The most abundant constituent of the fruit HS, flower and fruit essential oils was hexyl butanoate (70.5%, 31.1%, 80.4%, 47.4% and 52.7%, respectively). Apiole, accompanied by myristicin and (Z)-falcarinol, make up over 70% of the root essential oils. γ-Palmitolactone was the major component of the steam essential oils (51.9% at the flowering stage and 45.7% at the fruiting stage). The differences in the composition of volatiles from different plant organs were noticed, while the stage of growth mainly affects the quantitative volatiles composition.

AHC analysis separates samples into two clusters (HS volatiles and essential oils). Further, the first cluster is divided into two groups and indicates on resemblance of HS volatiles of fruit, flower and stem at flowering phase as well as HS volatiles of stem at fruiting phase and both root samples. Cluster of essential oil samples is divided into three groups according to resemblance, as follows: fruit and flower essential oils, the both stem essential oils, and the both root samples.

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PP-070. Chemical composition of Hypericum rumeliacum Boiss. essential oil

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The genus *Hypericum* L. (Guttiferae/Clusiaceae/Hypericaceae) includes more than 480 species that are naturally occurring on, or which have been introduced to, every continent in the world, except Antarctica [1]. Plants of the genus *Hypericum* have been used as traditional medicinal plants all over the world [2]. The aim of this study was to perform a detailed compositional analysis of the essential oil isolated from *H. rumeliacum* and to try to establish chemotaxonomic correlation based on essential oil profiles within the members of the section *Drosocarpium* Spach (*H. rumeliacum* is a member of this section). The essential oil of aerial parts of *H. rumeliacum* obtained by hydrodistillation was analyzed by GC and GC/MS. Forty three identified compounds accounted for 98.8% of the total oil. The main components of the oil were: (*E*)-β-ocimene (18.2%), β-pinene (14.7%), (*Z*)-β-ocimene (13.0%), dodecanal (7.4%) and germacrene D (5.8%). Dominant class of compounds - terpenoids (85.3%), was unevenly distributed between mono- and sesquiterpenoids (72.6% and 11.8%, respectively). Monoterpenoids, as well as sesquiterpenoids were dominated by hydrocarbons. Non-terpenoid fraction consisted of fatty acid-derived compounds (7.9%) and alkanes (5.6%). These results along with more data on other species might be helpful in the chemotaxonomy of the genus *Hypericum* L.

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PP-071. Volatile organic compounds of the endophytic fungus *Diaphorte* sp.

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Entophytic fungi live in a symbiotic association with higher plants and may improve the host resistance to biotic and/or abiotic stresses. Natural bioactive small molecules produced by endophytes are challenging potential resources for the pharmaceutical, food and fragrance industries. In the present study, *Diaphorte* sp. (UFMGCB 9556) was isolated from Brazilian native tree *Stryphodendron adstringens* and identified by using molecular methods. The fungus was cultured in suitable liquid media for 14 days and the volatile compounds profile were determined by using headspace solid-phase micro-extraction (HS-SPME) and GC-MS techniques. The analytical results showed that, isoamyl alcohol, linalool, α-muurolene, γ-muurolene, δ-cadinene and phenylethyl alcohol were the detected main volatiles of the fungus.

PP-072. Chemical characterization and biological evaluation of the Myrtle Oil from Turkey

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Myrtle (*Myrtus communis* L.) is an evergreen shrub belonging to the Myrtaceae. *Myrtus* genus including flowering plant is represented by approximately 16 species reported in areas of the Middle East and Asia [1] . It has been used for medicinal, food and spice purposes since ancient times. The leaves are used as antiseptic, disinfectant [2]), analgesic [3], antiinflammatory agents, as a mouthwash, also for wound healing [4]. In this study, the plant material was collected from Alanya, Turkey. Essential oil from leaves of the plant was obtained by hydrodistillation using Clevenger apparatus. The essential oil was analyzed by both GC and GC-MS, simultaneously. The major compounds were identified as α-pinene (42.4 %), linalool (10.51 %), 1,8-cineole (27.08 %), and α-terpineol (4.06 %). Furthermore, the essential oil was investigated for its antioxidant activity using DPPH radical scavenging method, anti-inflammatory activity using spectrophotometric *in vitro* LOX method, anticholinesterase (ChE) activity using Elmann method, antibacterial activities using the CLSI microdilution method, also antiangiogenic activity using CAM assay. Biotransformation studies on the essential oil are going on. As biological activity results the essential oil showed significant anti-lipoxygenase activity with 72 ± 5.66 µg/ml (20 mg/ml) IC_{50} value, good antibacterial activity against *Salmonella typhimurium* with 20 µg/mL MIC value and strong anti-angiogenic activity with 1.0 ± 0.1 (50 µg /pellet) score when compared to the standards. As a conclusion the essential oil can be used as sustainable antibacterial source.

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PP-073. Chemotaxonomy and antimicrobial activity of cineole-rich essential oils from Australian *Prostanthera* Labill. species (Lamiaceae)

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Essential oils were produced from several species currently assigned to series *Racemosae* Benth. of the endemic Australian genus *Prostanthera* (Lamiaceae); *P. caerulea* R.Br., *P. cineolifera* R.T.Baker & H.G.Sm., *P. incisa* R.Br., *P. sp. aff. lasianthos* Labill., *P. sp. aff.ovalifolia* R. Br. and *P.aff. rotundifolia* R.Br., using hydrodistillation of leaves from both wild and cultivated specimens. Oils were analysed using GC-MS and NMR. Mean inhibitory concentrations were determined against a range of Gram-positive and –negative bacteria and the yeast *Candida albicans*, using microtitre plate broth dilution assays. Essential oil character varies within species, most notably with regard to *P. lasianthos*, *P. ovalifolia* and *P. rotundifolia*. Chemically, the character of these oils is almost always strongly characterized by 1,8-cineole, together with one or two main tricyclic sesquiterpene alcohols or furans on scaffolds of decahydro-naphthalene or –azulene structures, with or without a cyclopropane moiety. The main sesquiterpenes identified were prostantherol, maaliol, ledol, globulol, kessane and cis-dihydroagarofuran. Essential oils rich in the latter two furans demonstrated moderate antimicrobial activity, but those dominated by sesquiterpenols produced relatively high activity against Gram-positive species. Variation in oil character with morphology and geography suggests species complexes or heterogeneous species assemblages may be involved, highlighting the potential for chemotaxonomy to complement morphological and/or phylogenetic studies focused on taxonomic revision. Demonstrated antimicrobial activities of these essential oils is discussed in light of other pharmacological studies, underwriting the potential for cultivation and commercialization.

PP-074. The volatiles obtained from the gum and roots of *Ferula foetida* St.-Lag. with conventional and microscale techniques

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The objective of the present study was focused on the evaluation of chemical composition of volatiles and lipids from gum and roots of *Ferula foetida* St.-Lag. (Apiaceae). The gum and root were collected in Djizzakh region (Republic of Uzbekistan) in 2013. The gum and roots of *F. foetida* were subjected to hydrodistillation in Clevenger apparatus as well as Micro-Steam Distillation - Solid Phase Micro-Extraction (MSD-SPME) techniques to yield volatiles. Blue fiber with polydimethylsiloxane/ divinylbenzene adsorbent was applied for 3 min to extract volatiles from the gum and roots. Fatty acid's fraction of the gum and root was investigated after extraction by Folch method and subsequent saponification with 10% methanolic KOH. Fatty acids were partitioned by diethyl ether following acidification to pH 2, dried and converted to methyl esters using boron trifluoride–methanol solution. All the volatiles and fatty acid methyl esters were analyzed by GC/FID and GC/MS techniques.

Yields of volatiles obtained by Clevenger from gum and root were 7.5 and 0.53 ml% on dry weight, respectively. All the volatiles were predominated with sulfur containing constituents like di-sec-butyl disulfide, *trans*-propenyl sec butyl disulfide, *cis*-propenyl sec butyl disulfide. One unknown sulfur containing constituent was detected in different amount in the gum as well as in the roots. The lipids extracted from the gum and roots were found to be rich with saturated palmitic, and unsaturated acids like oleic and linoleic.

PP-075. Characterization of the essential oil and anticandidal evaluation of *Thymus pectinatus* Fisch. Et Mey. var. *pallasicus* (Hayek &Velen.) Jalas from Turkey

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Lamiaceae is one of the largest families with more than 233 genera and about 6870 species throughout the word. *Thymus* L. with 220 ssp. is centered in the Mediterranean region.[1] *Thymus* species are well known as "Kekik" in Turkish and are used as herbal tea and spices, and according to recent records, the genus is represented by 40 ssp.[2]and 17 species are endemic to Turkey. *Thymus pectinatus* var. *pallasicus* is one of the endemic varieties for Turkey. Essential oil from the air dried aerial part of the *Thymus pectinatus* var. *pallasicus* was obtained by hydrodistillation and analyzed by GC-FID and GC-MS, respectively. Analytical results showed that the main component was found to be thymol (55.5 %) along with the monoterpenes *p*-cymene (19.9 %), γ-terpinene (7.2%), carvacrol (5 %), and 1,8-cineole (%1.4). The essential oil was also evaluated for its anticandidal activity against five different standard human pathogenic strains, according to a modified *in vitro* CSLI microdilution method.[3,4] The minimum inhibitory activity of the essential oil was 250-500 microgram/ml against the tested *Candida* sp. suggesting rather weak inhibitory activity when compared to the standard antifungal fluconazole (0.5-16 microgram/ml). As *Thymus* sp. are known for their antimicrobial activities it is suggested that other clinical and pathogenic strains should be evaluated.

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PP-076. Composition of volatile oil of the roots of *Ferulago sandrasica* Peşmen & Quézel (Apiaceae) growing in Turkey and determination of its antimicrobial properties by biautography

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Ferulago W. Koch. (Apiaceae) genus is represented by approximately 50 taxa throughout the world (1). *Ferulago* species are known as "Çakşır" or "Çağşır" in Turkey and according to recent records, the genus is represented by 35 taxa in Turkey, 18 of which are endemic (2). Volatile oils obtained from the fruits (3) and leaves (4) of *Ferulago mughlae* Peşmen, an endemic species have been studied before, however to the best of our knowledge, volatile oil of the roots has not been studied. Volatile oil was obtained by hydrodistillation and analyzed by GC and GC-MS and main components were found to be limonene (28.9%), α-pinene (15.6%) and terpinolene (13.9%). Results of antimicrobial activity study via bioautography method showed that roots were active against *Staphylococcus aureus* ATCC 6558 and *Candida albicans* ATCC 90028 strains; however it was not active against *Escherichia coli* NRRL B-3008 strain.

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PP-077. Effect of thyme and eucalyptus essential oils on outer membrane protein composition of *Pseudomonas aeruginosa*

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The antimicrobial activity of essential oils is well-documented [1] but the mode of their action has still not been understood. The cell wall of Gram-negative bacteria includes the outer membrane together with its special proteins. The structural changes of outer membrane protein (OMP) composition may have an effect on the adhesive ability and pathogenic properties of bacteria. *Pseudomonas aeruginosa* is an opportunistic human pathogen responsible for several difficult-to-treat infections and for nosocomial infections as well. Previously, it has been demonstrated that cinnamon bark and clove oils can change the bacterial OMP composition [2]. The aim of our study was to examine the effect of thyme and eucalyptus essential oils on the OMP composition of *P. aeruginosa* ATCC 27853 strain.

Thyme and eucalyptus oils were obtained from a Hungarian drugstore (Herbaria). Chemical composition of the oils was analyzed with GC-MS. The minimum inhibitory concentration (MIC) values of the oils were determined by a modified tube dilution method. 0.5 and 2 x MIC concentrations of the oils were administered to the bacterial culture and incubated for 60 min. OMP preparation was performed according to ref [2]. Measurements were performed in the Protein 230 Plus LabChip Kits of the 2100 Bioanalyzer System of Agilent. After 1D and 2D SDS-PAGE the isolated proteins will be identified by MALDI-TOF/MS.

Thymol (46.3%) was the main component of thyme oil, and 1,8-cineole (84.8%) of eucalyptus oil. Both essential oils could influence the OMP composition of *P. aeruginosa*. In the case of thyme oil the protein peak with molecular weight 42.7 kDa decreased but the 79.9 kDa protein increased after 2 x MIC (3.1 µl/ml) administration of the oil. The 0.5 x MIC (1.5 µl/ml) concentration of eucalyptus oil induced the protein with 79.1 kDa.

Quantitative changes in the protein profile may contribute to the explanation of antibacterial effect of thyme and eucalyptus essential oils on *P. aeruginosa*.

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PP-078. Composition and antibacterial activity of the essential oil of *Cotula anthemoides* L. (Asteraceae)

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The Asteraceae is the largest family with more than 24 000 species belonging to over 1700 genera distributed around the globe except for Antarctica. This family contains many major ornamental and medicinal plants. The Anthemideae is one of the largest tribe of Asteraceae with more than 1740 species, predominantly distributed in Eurasia, North and South Africa, with fewer species in North America and Australia. We present here the GC and GC-MS analyses and the antibacterial activity of the essential oil of the Saharian species *Cotula anthemoides* L., belonging to this tribe. The hydrodistillation, in a Clevenger-type apparatus, of the aerial parts of *C. anthemoides* L., collected from Tiguentourine (Eastern Saharian) [1,2], yielded 0.7% of a yellowish essential oil, having an intense and penetrating odor. 41 components were identified representing 99.8 % of the essential oil mainly characterized by camphor (27.4%), santolina triene (13.0%), thujone (12.9%), camphene (10.7%) and α-curcumene (5.3%). The essential oil exhibited the best antibacterial activity against *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853 and *Proteus mirabilis* with 28 mm, 25 mm, 25 mm, inhibition zone diameters, respectively. MICs values of the essential oils were also determined by an agar dilution method. The values were ranged from 40-80µg/ml.

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PP-079. Determination of volatiles in *Lavandula stoechas* L. under *in vitro* conditions

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In this present study, we aimed to grow *Lavandula stoechas* L. under *in vitro* conditions containing various concentrations of micropropagated shoots rooted on MS medium with IBA. The flowering shoots of the micropropagated plants which were acclimatized under field conditions were used for the identification of its constituents through GC/MS after microdistillation. Accorindg to the microdistillation results, over all 22 compounds were separated and characterized. The major volatiles were identified as camphor (38.9%; 38.1%), bornyl acetate (10.6%; 10.6%), α-fenchone (9.1%; 8.6%), 1,8-cineole (4.7%; 3.8%), α-pinene (4.1%; 4.0%), linalool (3.4%; 3.5%), viridiflorol (3.4%; 4.1%) myrtenal (2.5%; 2.8%), geranyl acetate (2.0%; 2.1%), respectively.

PP-080. Effects of extraction techniques on compositions of bergamot (*Citrus bergamia)* peel essential oil

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An essential oil is a liquid, generally obtained by distillation from the leaves, stems, flowers, fruits, bark, roots, seeds, peels or other elements of a plant. Today essential oils are generally produced from more than 3 000 plants and approximately 300 of them have commercial importance. Many aromatic plants, especially citrus fruits, and their essential oils are used as flavoring agents in a wide range of food, beverage, and confectionery products and also used in the perfumery and medicine industry. The world citrus production is nearly 130 million tons per year which make it easily achievable sources for health beneficial components. The citrus fruits contain considerable amounts of peel. There were numerous studies to put forth evaluation of citrus peel and essential oil production was the most common method using it. The aim of this study was to show effects of cold press and hydro-distillation techniques on the compositions of bergamot peel essential oil. Limonene, linalool and linalyl acetate were determined as the major components. While limonene was the highest component in fresh peel essential oil (42.76%) and cold press essential oil (39.17%), the ratio of it was found as 31.05% in hydro-distilled essential oil. Linalool was the highest compound in the hydro-distilled essential oil (35.30%), the level of it was 13.89% in the fresh oil and 7.76% in the cold press oil. Linalyl acetate was the second highest components in cold pres oil as 36.21%. The linalyl acetate ratio in fresh peel and cold press oil were determined as 15.71% and 8.23% respectively. The other major components in the essential oils were γ-terpinene, β-pinene, β-myrcene, α-terpineol and geraniol.

PP-081. Evaluation of changes in lemon (*Citrus limon*) essential oil compositions obtained by different extraction techniques

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Citrus presumably is one of the most important commercial and industrial agricultural crops in the world. *Citrus* genus belongs to the large family *Rutaceae*, containing 160 genera in the seven subfamilies with many important fruit and essential oil producers. Up to now, essential oils were studied generally from the viewpoint of their flavor and fragrance chemistry to be used in foods, drinks and other products. Currently, citrus essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose use. Lemons have many important natural chemical components, including citric and ascorbic acids, flavonoids and essential oils. The goal of this study was to show effects of cold press and hydro-distillation techniques on the essential oil components of lemon peel oil. For this purpose Interdonato lemon cultivar was used. Essential oil composition was affected significantly from extraction techniques. Limonene was determined as the main component in the oil. The ratio of limonen was found as 76.35% in the fresh peel oil. After drying at 45°C in the oven, the limonene ratio was decreased to 71.49% in the oil obtained by hydro-distillation and 42.62% in the cold press oil of the dried peels. γ-terpinene and β-pinene were the other highest components, the ratios of these components were 7.80% and 6.87% for fresh peel oil and 9.74% and 7.60% for hydro-distilled oil, respectively. Linalyl acetate (32.84%) was the second highest component in the cold press oil, followed by linalool (6.81%), γ-terpinene (5.87%) and β-pinene (3.55%). From these results, there were no significant differences in the essential oil compositions between fresh and hydro-distilled lemon peel oil, on the contrary, the essential oil compositions were significantly changed using cold press production method.

PP-082. Chemical composition of the essential oil from aerial parts of *Eremostachys macrophylla* Montbr. & Auch from Northeast of Iran

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The essential oil obtained by hydrodisstillation of the aerial parts of *Eremostachys macrophylla* Montbr. & Auch., grown wild in Iran, was analyzed by GC and GC/MS. The colorless oils were obtained by hydrodistillation, using a Clevenger-type apparatus for three hours, from aerial parts in 0.18% yield (w/w). Forty-four compounds representing 91.6% of aerial parts oil of *Eremostachys macrophylla* were identified. The main components of the oil were hexadecanoic acid (27.5%), ethyl linoleate (8.5%), 6-methyl-α-ionone (8.0%), isobutyl phthalate (5.8%), α-cadinol (4.7%) and germacrene D (4.3%). The oil was rich in nonterpenoids (56.0%) and among them, oxygenated nonterpenes (53.2%) predominated over nonterpene hydrocarbons (2.8%).

PP-083. GC/MS Analysis of the essential oils from aerial parts of *Sclerorhachis platyrachis* (Boiss.) Podlech ex Rech. f. collected from Northeast of Iran

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In this study, the essential oil obtained by hydrodistillation of the aerial parts of *Sclerorhachis platyrachis* (Boiss.) Podlech ex Rech. f. (Compositae), growing wild in Sabzevar, Khorasan Razavi province (Iran), were analyzed by GC and GC/MS. The yield of total volatiles was 0.38% (w/w). Sixty- three compounds representing 89.2% of the aerial parts oil were identified. The main components of the oil were β-Pinene (17.5%) and γ-Terpinene (15.4%). The oil was rich in monoterpenoids, and among them, monoterpene hydrocarbons (48.7%) predominated over oxygenated monoterpenes (11.8%).

PP-084. Evaluation of transdermal administration of α-cyperone (4, 11-selinadien-3-one) isolated from purple nutsedge (*Cyperus rotundus*) essential oils as a new drug delivery treatment method for lowering cholesterol

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The compound α-cyperone (4,11-selinadien-3-one) isolated from purple nutsedge (*Cyperus rotundus* L.) essential oil , in this study we removed the hair from the back area of the rats and apply this compound on the skin , this compound gave us good result as an alternative drug for lowering serum cholesterol levels via the transdermal route of administration. This compound significantly decreased total serum cholesterol level in rats at ($p > 0.01$), and no histopathological changes in skin and liver were observed.

PP-085. Composition of the essential oils of *Satureja* spp. cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece)

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Eighteen populations of *Satureja montana, S. parnassica* subsp. *hellenica*, *S. pilosa* subsp. *pilosa*, *S. pilosa* subsp. *origanita* and S. *thymbra*, originated from different localities of Greece (Table 1), were cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece). After one year of cultivation, the essential oils (EOs) of their aerial parts were analyzed (Table 1). The oils were obtained by hydrodistillation in a modified Clevenger-type apparatus (1) and their chemical analyses were performed by GC and GC-MS. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (2) and those described by Adams (3), as well as on comparison of their retention indices (4) and with literature values (3).

Oxygenated monoterpenes constitute the main fraction of 15/18 EOs (51.1%- 89.6%). Some constituents are relevant even in different percentages in almost all samples: carvacrol, thymol, *p*-cymene, γ-terpinene, linalool, borneol, *cis*-sabinene hydrate, spathulenol, caryophyllene oxide. It is noteworthy that most *Satureja* species produce high amounts of carvacrol/thymol/*p*-cymene or linalool or geraniol/geranyl acetate, which are related biosynthetically (5).

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PP-086. Ultraperformance convergence chromatography: expanding selectivity for the chromatographic laboratory

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Supercritical fluid chromatography (SFC) is a fifty year old technique, which uses CO_2 in a supercritical phase as the main solvent, the elution strength being tune by adding co-solvents or by modifying the operating pressure or temperature. The technique has obvious advantages, but suffered from several limitations, linked to the difficulty to design instruments able to handle a compressible fluid, with sufficient reproducibility and sensitivity. For that reason, SFC was popular mainly for applications for which it brings a clear separation improvement, like for the separation of chiral molecules, or in preparative mode, due to important low solvent consumption and associated cost saving, and the low toxicity of the eluents.

In the recent years, important developments were made in the field of HPLC, with the development of sub-2micron particles, and new chromatographic systems holistically designed to take full benefit of the smaller particle. This so called UPLC or UHPLC technique opened the way to developments in the SFC domain, removing the reproducibility and sensitivity barriers.

Supercritical CO_2 presents interesting properties for the chromatographer. Unlike normal phase LC eluents, it is miscible with the entire eluotropic series of solvents. In addition, both traditional normal phase and reversed-phase column chemistries can be utilized. The application domain is extremely large, limited mainly by the solubility of the analytes which must have some solubility in organic solvents. So, most of the LC application domains and some of the GC domains are covered. This is why the term “convergence”, used by Calving Gidding (1) about high pressure GC, was retained to describe this chromatographic technique.

Similarly to UPLC, UltraPerformance Convergence Chromatography (UPC^2) requires a holistically designed chromatographic system that utilizes liquid CO_2 as a mobile phase to leverage the chromatographic principles and selectivity of normal phase chromatography while providing the ease-of-use of reversed-phase LC.

In this contribution, we will explain the principles of the technique, detail the solvents/columns compatibility range, and illustrate it with examples chosen in the pharmaceutical, food, environment and chemical materials domains.

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PP-087. Essential oil from Algerian Thyme obtained by microwave accelerated steam distillation: chemical composition and antimicrobial activities

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Thymus pallescens de Noé [1] belongs to the *Lamiaceae* family. It is an aromatic plant well known in Algerian folk medicine and largely used for its sedative, antispasmodic and anthelmintic properties. The problem raised by the resistance of bacteria to antibiotics has encouraged the search for alternative natural molecules for therapeutic use.

The aim of the present study was to investigate the antimicrobial activities of essential oils (EO) from Algerian thyme, obtained by Microwave Steam Distillation (MSD) against bacteria and fungi. The chemical compositions and the yields of essential oils obtained by MSD and SD (Steam Distillation) were compared.

MSD, developed in our laboratory has been performed in a batch reactor (Bergamo, Italy). An electrical steam generator and a condenser placed outside microwave oven are connected to a cartridge containing plants via Pyrex connecting tubes. The condenser is connected to a receiving Florentine flask which is preferably a separating funnel to enabling the continuously collected of condensate EO and water [2].

The EO were analyzed by gas chromatography coupled to mass spectrometry (GC/MS). The relative percentage of the components was calculated from gas chromatography with flame ionization detection (GC-FID) using the same conditions of GC/MS analysis.

The antimicrobial activities were tested by diffusion on agar medium method. Standard discs of antibiotic were used as positive controls and the discs imbued ethanol as a negative control (solvent employed to dissolve the EO). The minimal inhibitory concentration (MIC) was determined by broth dilution method.

The results show that the yields obtained by the both methods were similar, the difference concerned the extraction times (5 minuts for MSD and 20 minuts for SD). The chemical composition of the oils performed by the two processes is broadly comparable since the same compounds were found with a few differences in relative amounts for some of them. Thus, carvacrol were the major compound in both cases with a relative amount more abundant in the essential oil extracted by SD 86.7% against 77.8% in the oil obtained by MDS. The contents of ortho-cymene and eugenol were more significant in the oil improved by MSD (4.5% against 0.2% for ortho-cymene and 2.3% against 0.3% for eugenol). In the published literature, carvacrol, thymol and para-cymene were reported to be the major compounds in *Thymus* species. Our findings suggest the probable occurrence a new chemotype which contain ortho-cymene. It is the first time that the ortho-cymene compound is reported in the Algerian *Thymus* species. Its presence has recently been reported by Hussain et al. [3] in *Thymus linearis* and *T. serpyllum* species.

The EO varied in their antimicrobial activities. The fungal strains were more sensitive than bacteria. The Gram-positive bacteria exhibited higher sensitivity to the tested oils. *E. coli* was the most sensitive among the Gram-negatives whereas *Listeria* was the least resistant among pathogens.

The extraction method by microwave provides significant interest in the sense that it can gain time which induces a reduction of the consumption of energy and thus a reduction of the costs of exploitation.

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PP-088. Fractional hydrodistillation and biological evaluation of essential eils from *Crithmum maritimum* L.

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In some previous studies, it has been demonstrated that the final composition of the essential oils can be influenced by the distillation time and some biological activities increased according to the proportion of some types of compounds such as monoterpene alcohols and phenolic derivatives contained in the tested essential oil fractions (1).

The aim of the present study was particularly the identification of the bioactive principles of the essential oils from the aerial parts and the roots of *Crithmum maritimu*L. (2) during their fractional extraction by hydrodistillation. The antioxidant, antibacterial and cytotoxic activities of the essential oils and their collected fractions were also studied.

Our results showed that the main constituent of the oils was dillapiole, detected at increasing percentages (67.7-100%) in all the collected fractions during the extraction process. The antioxidant features of all the isolated fractions (F_1-F_9) of the two oils were also evaluated using DPPH, ABTS, reducing power and paraoxonase assays. The results showed that the IC_{50} (DPPH) of F_9 (aerial parts and roots) were 0.042 ± 0.001 mg/mL and 0.038 ± 0.001 mg/mL, respectively. Moreover, the oil roots exhibited strong growth suppression particularly against *Staphylococcus aureus* and *Enterococcus faecalis*. The fraction F_9, the aerial parts and the roots essential oils were also tested for their cytotoxic activity and interesting results were noted.

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PP-089. Access to novel bioactive isoxazolines from unpurified dillapiole from the essential oil of *Crithmum maritimum*

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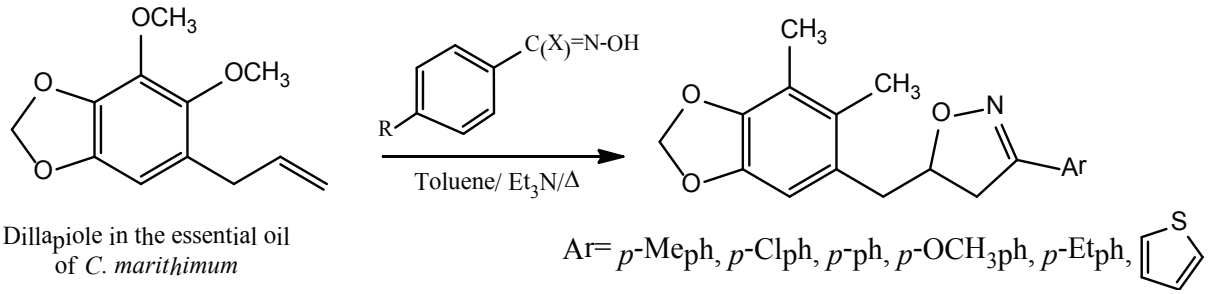
The 1,3-dipolar cycloaddition has been the subject of intense research over the last decade, due to its great synthetic value. Indeed, the cyclo-addition is the most effective process to the synthesis of five-membered heterocycles, which are difficult to be prepared with other means. Furthermore, it gives access to several substances with pronounced biological activities (1). As part of our research on a bioactive five-membered heterocycles, we proposed to investigate the behavior of the allylic double bond of the unpurified Dillapiole contained in an appreciable amount (19.7-26.8%) in the essential oil of *Crithmum maritimum L.*, (2) towards different arylnitriles oxides as a dipole. The reaction was regiospecific and leaded to a series of new dillapiole isoxazolines.

The structures of all the newly synthesized compounds were elucidated by spectroscopic means (^{1}H, ^{13}C NMR and MS).

The antimicrobial, the antioxidant [DPPH and paraoxonase (PON 1) assays] and the cytotoxic activities of all the synthesized heterocycles were investigated and discussed.



Dillapiole in the essential oil of *C. maritimum*

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PP-090. Inter- and intra-population diversity of carvacrol and thymol in eight populations of *Satureja bachtiarica Bung.*

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Satureja bachtiarica Bung. is one of the endemic species growing wild in Iran. It is a well-known aromatic plant which is usually used as a flavoring agent in foods and as a traditional medicinal herb. In this research, inter- and intra-population diversity of carvacrol and thymol, as major compounds in most species of *Satureja* genus, were assessed in eight populations of *S. bachtiarica.* 10 to 15 individuals were used for each population. Significant diffrences were observed for carvacrol and thymol among and within populations. Within populations, thymol was varied from 30.09-65.89 % in Ilam, 11.37-77.19 % in Eghlid, 0.44-26.02 % in Semirom, 0.10-59.71 % in Sisakht, 8.68-70.21 % in Sepidan, 0.05-6.11 % in Shahrekord, 2.10-11.21 % in Yazd and 3.42-44.43 % in Kalar. Also, carvacrol was varied from 1.33-9.99 % in Ilam, 0.90-65.93 % in Eghlid, 16.60-61.98 % in Semirom, 3.37-82.37 % in Sisakht, 0.17-40.40 % in Sepidan, 43.86-67.91 % in Shahrekord, 63.78-83.12 % in Yazd and 2.73-57.52 % in Kalar. Among populations, mean thymol content was the highest (44.32 %) in Ilam and the lowest (1.97 %) in Shahrekord. While, Ilam showed the lowest mean carvacrol content and Yazd had the highest (72.50 %) and follwed by Shahrekord (58.05 %). According to these results, there was a wide variability for carvacrol and thymol among and within the studied populations, indicating the existence of different chemotypes.

PP-091. Scents from Brazilian Cerrado: The essential oil from the leaves and flowers of *Hyptis suaveolens* (L.) Poit. (Lamiaceae).

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The Cerrado is a savannah-like vegetation occurring in Central Brazil, covering almost 2 million km^2. The Cerrado is very varied in form, ranging from dense grassland to an almost closed woodland with a canopy height up to 15 m. More than 12,000 plant species have been cataloged in this biome, including several aromatic plant families, such as Lamiaceae, Verbenaceae and Asteraceae. Studies indicate that the Cerrado is an important center of biodiversity, being considered one of the 25 most important biodiversity hotspots of the world. The huge biodiversity from this biome may be a great source of fascinating natural scent (1,2). A research project has been started in order to investigate the volatile chemical composition of the rich flora from the Cerrado. Hyptis suaveolens (L.) Poit is a herb, 50-100 cm tall, abundant and with a strong scent. Samples were harvested in April 2013, at the Ermida Dom Bosco preservation area, Brasília, DF.

Flowers and leaves from six individuals of a population were collected in Brasilia, Brazil. A voucher specimen was deposited in the herbarium of Embrapa Genetic Resources and Biotechnology (registry: CEN 82850). Fresh leaves (59.6 g) and flowers (113.3 g) were subjected to hydrodistillation separately in a Clevenger-type apparatus for 2 hours each. The oils were analyzed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems, both with HP-5MS fused silica capillary columns (30 m x 0.25 mm x 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL/min. Oven temperature was raised from 60 to 240°C at 3°C/min. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (3,4).

Oil yields were 0.42% and 0.05% for flowers and leaves, respectively. In the oil from the flowers 56 compounds were identified, corresponding to 95.9% of the oil, whereas in the oil from leaves 68 compounds were identified (93.6% of the oil). The leaf oil was rich in sesquiterpenes (70.9%), and the major components were bicyclogermacrene (10.2%), (E)-caryophyllene (7.3%), γ-muurolene (5.6%), epi-α-cadinol (4.9%) and 1,8-cineol (4.8%). The oil from the flowers oil was rich in monoterpenes (71%). Its major compounds were sabinene (25.9%), 1,8-cineole (17.7%), bicyclogermacrene (7.2%), β-pinene (6.9%), limonene (5.3%) and γ-muurolene (5.2%).

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PP-092. Scents from Brazilian Cerrado: Chemical composition of the essential oil from the flowers of *Hoehnephytum trixoides* (Gardner) Cabrera (Asteraceae).

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Cerrado (Brazilian savannah) has one of the highest concentrations of endemic species on the planet and it is one of the most threatened by human activities. Cerrado has very many aromatic plant families and 1.5% of all endemic species on the planet. More than 12,000 plant species have been cataloged in this biome, many of which had never had their volatile composition studied (1,2). A research project has been started by Embrapa in partnership with other research centers in order to investigate the volatile chemical composition of the rich flora from this biome (3). *Hoehnephytum trixoides* is a subshrub, 1 m tall, with abundant yellow flowers. Flower samples were harvested in August 2013, during the dry season at IBGE ecological reserve in Brasília, Brazil.

Flowers from five individuals of a population were collected in Brasilia, Brazil. A voucher specimen was deposited in the herbarium of the Genetic Resources and Biotechnology (registry: CEN 82877). Dried flowers (133 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours. The oil was analyzed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems, both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL/minute. Oven temperature was raised from 60 to 240°C at 3°C/minute. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (4,5).

The essential oil was obtained in 0.7 % yield. Thirty-nine compounds were identified, corresponding to 97.1% of the oil. The major compounds present were β-pinene (33.7%), δ-3-carene (22.9%), α-pinene (8.9%), sabinene (6.7%) and bicyclogermacrene (5.6%).

Acknowledgments

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PP-093. Phytochemical composition and biological assays of the aerial parts of *Thymelaea hirsuta* essential oil from Tunisia

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The present study aimed to appraise the main constituents, antimicrobial and cytotoxic activities of the aerial parts of *Thymelaea hirsuta* Endl. essential oil isolated by the hydro-distillation. The essential oil was analyzed by GC–MS and bioassays were carried out. A total of twenty-two compounds, representing 98.46% of the oil, were identified and the analysis revealed that, the major components were heptane (34.20%), followed by germacrene D (15.66%), γ-eudesmol (14.25%), citronellyl formate (12.04%), trans-caryophyllene (3.92%), δ-cadinene (3.08%), and β-bourbonene (2.93%). The antimicrobial activity of the oil was evaluated against eight bacterial and three fungal pathogenic strains. Gram-positive bacteria were noted to be more sensitive to the oil than Gram-negative bacteria and yeasts. In vitro cytotoxicity evaluation against HeLa cell lines showed that the essential oil possessed moderate cytotoxicity on human tumor cells, with high IC_{50} value of 175 µg/mL. Overall, the results indicate that the *Thymelaea hirsuta* essential oil has a number of attractive properties that might open new promising opportunities for the control or prevention of a wide range of microbial infections and cancers with further investigation to determine its active components.

PP-094. Geographic origin influence on Tunisian *Citrus aurantium* L. leaves essential oil chemical composition

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Sour orange *Citrus aurantium* L. is a well known tree as a *Citrus* rootstock. Petit grain essential oil is obtained by leaves hydrodistillation. Sour oranges orchards plantations are spread in North–East of Tunisia. The present study aimed to determine the effect of raw material geographic origin on quality of leaves essential oils. Various batches of sour orange leaves from three different regions (Grombalia, Menzel Bouezelfa, and Mornag) were hydrodistillated and analysed by GC and GC/MS. Those regions have different climatic and edaphic characteristics. Variable amounts of components were detected for each region petit grain. The main components for all essential oils were linalool (16.7% for Grombalia and 10.9 for Mornag) and linalyl acetate (49.4 % for Mornag, 73.1% for Menzel Bouzelfa and 69.7% for Mornag). The other components were mostly monoterpenes. Grombalia leaves essential oil differs from the other petit grain qualitatively and quantitatively. Furthermore, Menzel Bouzelfa petit grain had the lowest yield during the distillation. These results showed the impact of the various soils and climatic characteristics on the quantitative and qualitative parameters of *Citrus aurantium* L. petit grain.

PP-095. Volatiles from nine Apiaceae species cultivated in Poland

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Plants of Apiaceae family are well known as coumarins' and essential oils' sources, which are used widely in medicine and folk medicine. The aim of our study was the chemical analysis of volatiles from nine plants of Apiaceae family, which are cultivated in the Botanical Garden of the Medical University of Lublin.

The plant material was the aerial parts and fruits of *Silaum silaus* (L.) Schinz & Thell., *Seseli devenyense* Simonkai and *Ferula assa-foetida* L, *Glehnia littoralis* Fr. Schmidt ex Miquel, *Seseli libanotis* and *Heracleum dulce*, the fruits of *Torillis japonica* and *Orlaya grandiflora* Hoffm as well as the herba of *Peucedanum luxurians.* The air dried and powdered plants were submitted to hydrodistillation and the obtained essential oils have been chemically analysed through GC and GC/MS.

The analyses showed as the most abundant constituents from the aerial parts of *Silaum silaus* myrcene 14%, together with methyl isoeugenol 27% and methyl eugenol. The two latters were the same also for the herb of *Ferula assa-foetida* (26% and 24% respectively).

Seseli devenyense showed β-sesquiphellandrene 18%, caryophyllene 8% and amorphene 8% as main compounds; while *Seseli libanotis* showed the presence of α-pinene 7% together with β-phellandrene 6%. Germacrene B appeared as major constituent in *Glehnia littoralis*, while from the aerial parts of *Heracleum dulce* a lot of coumarins have been identified reaching 60% of the total oil.

From the fruits of *Orlaya grandiflora* the analyses showed the presence of β-caryophyllene 18% and δ-cadinene 10%; while from *Torillis japonica* 1,6-germacradien-5-ol 38%, β-elemene 18% and β-caryophyllene 11% as main compounds. Finally from the aerial parts of *Peucedanum luxurians* the analyses showed the presence of *trans*-β-farnesene 16% and germacrene D 13% among the most abundant constituents.

The antimicrobial activities of all the essential oils were also assayed against Gram negative and positive bacterial as well as against human pathogenic fungi exhibited very interesting biological profile.

PP-096. Chemical profile of volatiles of 12 representative Greek honeys-Biological activities

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In the framework of our studies on Greek bee-keeping products (honey, propolis, royal jelly and pollen) and as it has been observed an increasing interest in the use of honey for the treatment of bacterial infections as well as in traditional Mediterranean food, we report in this study the chemical analyses, the antimicrobial evaluation and the pollinic spectrum of twelve representative honey samples produced in different regions of Greece (from forests, islands, of different % of thyme, pine, fir, orange, etc). In the framework of typification of Greek honeys based on their pollen, pollinic spectra (which include the identification of the kind of pollen and their percentages) were obtained by Louveaux's quantitative microscopical analyses of our samples.

Their volatiles through GC-MS and their chemical profiles through classical isolation procedures were studied. In all samples the alkanes are the abundant chemical category (30-75%), followed by alkenes (5-25%), fatty acids (1-28%) and alcohols (0.5-18%).

It is noteworthy the existence of *E*-4-(1,2,4-trihydroxy-2,6,6-trimethylcyclohexyl)-but-3-en-2-one, hydroxymethylfurfural, 3-hydroxy-4-phenyl-2-butanone and 3,4,5-trimethoxybenzaldehyde in all thyme samples, which they could be characterized as chemical markers of this honey quality; caffeine and 8-hydroxylinalool was detected in the studied orange honey, which are characteristic compound of orange type honey.

Also phenylacetic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, vanillin, *p-anisic acid* and anisaldeyde have been identified in some honey samples, as these compounds have been referred to give a characteristic odor.

Furthermore, all of the studied honey samples were assayed against Gram negative and positive bacterial as well as against human pathogenic fungi exhibited a strong and broad spectrum of antimicrobial activity against all assayed microorganisms.

Acknowledgments

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PP-097. Rosemary (*Rosmarinus officinalis* L.) essential oil composition along an altitudinal gradient in the Requena-Utiel uplands (Western Valencia, Spain)

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Rosemary essential oils are recognized having several therapeutic applications. The diverse composition of the essential oils of different rosemary's ecotypes in a given area may modify their level of activity. This preliminary study intends to highlight the complex relationships between chemical composition and biotypes and/or chemotypes. The chemical composition of the essential oil of *Rosmarinus officinalis* L. was obtained for three wild populations sampled in the Requena-Utiel uplands (Western Valencia, East Spain) in February and distributed in an altitudinal gradient (400, 900 and 1200 m.a.s.l.). The relationship between bioclimatic indexes, soil characteristics and oil composition were determined through linear discriminant analysis (LDA) to both ecological and essential oil data set components. Twenty-eight main compounds were identified in the oils. The essential oil composition of the three plant populations appeared quite different and allowed to identify three different chemotypes. The Eucalyptol chemotype (36% eucalyptol, 17% camphor, 13% α-pinene) showed a positive relationship with higher mean annual temperature, higher minimal temperature of the coldest month, and low nutrient content of soil, which were characteristics of the lowest altitude. At higher altitudes, the camphor chemotype (32% camphor, 24% eucalyptol, 11% α-pinene) related to the higher soil nutrient content and water balance in plants and soils, and higher minimal temperature of the coldest month. Another chemotype found at high altitude characterized by similar proportions of Eucalyptol and camphor. Rosemary plants with an oil composition with camphor contents above 24% resist to freezing and grow at altitudes between 900 and 1200 m.

PP-098. Volatile constituents of Turkish Bitter orange leaf extracts with different extraction methods.

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Bitter orange (*Citrus aurantium* L.) is cultivated coast of the Mediterranean region of Turkey. Bitter orange leaf or petitgrain oil possesses more desirable organoleptic properties than other leaf oils obtained from the other Citrus trees (lemon, mandarin, sweet orange, bergamot, etc.), However the production of leaf oil is limited (1). The main producer of bitter orange leaf oils are France, Italy, Spain and Paraguay. It is widely used in perfumery for the sweet and fresh note. Because bitter orange essential oil has resistance of alkaline medium, it is suitable for the production of soaps,. Researchers studied on composition of bitter orange leaf and peel essential oil from different countries (2-5). Bitter orange leaves were collected from southern Turkey (BATEM Antalya) in December 2013 and their leaf extract were obtained by SCFE and hydrodistillaton (HD) method. After SCFE extraction, winterization process applied on samples for precipitation of wax and undesired residues.

The volatile constituents of extracts was determined by GC/MS analysis and 48 compounds identified in HD extract. Identification of individual constituents was based on comparison of their GC Retention indices (RI) on the apolar column determined relative to the retention time of a homolog series of n-alkanes (C_8-C_{18}) with linear interpolation. NIST and Wiley commercial spectral libraries were used for computer matching. Further identification was made by comparing mass spectra with reported by Adams(6). According to HD extract results Citrus aurantium oil is composed of 92,48 % of oxygenated components. 6,77 % of remained part of it is composed by monoterpene hydrocarbons. 0,64 % of it is composed by sesquiterpene hydrocarbons.

The main components of the Turkish bitter orange leaf oil were linalyl acetate (48.23 %), linalool (28.07 %), α-terpineol (5.53 %), geranyl acetate (4.82 %), limonene (1.78 %), neryl acetate (2.59 %), myrcene (2.06 %), which constituted altogether about 93.08 % of the whole oil.

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PP-099. Sage and thyme essential oils in laying hens diets

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Experimental studies during the past three decades found beneficial influence of essential oils (EO) on lipid metabolism, ability to stimulate digestion, antimicrobial, antioxidant and anti-inflammatory properties. Consumers prefer natural products with health benefits because they are perceived as safer and more nutritious than food containing additives of a non-natural origin. The objective of this study was to examine the dietary effect of two concentrations of *Salvia officinalis* and *Thymus vulgaris* EO on antioxidant and immunological parameters, intestinal integrity and oxidative stability of eggs.

At 34 weeks of age, 120 Lohman Brown laying hens were randomly divided into five groups of 24 birds each. Each group consists of 6 replicates. A replicate was a cage with 4 birds. The first group was fed basal diet (BD), second and third with BD supplemented with 0.05% or 0.1% of sage EO and third and fourth group recieved 0.05% or 0.1% of thyme EO. Data were analyzed for the effect of treatment (sage and thyme), concentration (0.05% and 0.1%) and interaction of treatment x concentration using two-way analysis of variance ANOVA with the level of significance set at $P<0.05$.

Thyme oil decreased malondialdehyde (MDA) concentration in liver and kidney and superoxide dismutase (SOD) in red blood cells, increased activity of glutathione peroxidase (GPx), intestinal integrity and immunoglobulin A concentration in duodenal mucosa, total antioxidant status (TAS) in plasma and phagocytic activity in blood. MDA concentration was increased in yolk on 30 day of eggs storage, this result indicated a temporal response on the oxidative deterioration in eggs.

Sage oil decreased MDA concentration in kidney and duodenal mucosa and SOD in red blood cells. Total antioxidant status in plasma and MDA concentration in yolk already on 20 day of eggs storage were increased.

Fatty acids contents of yolk in eggs were also investigated and we didn´t find any positive effect of both EO.

These results demonstrated that thyme oil shows more effective potential to improve intestinal barrier integrity as well as evoking an immune response in hens, than if their diets are supplemented with sage oil and that these both additives improved antioxidant status of laying hens and enhanced oxidative stability of egg yolk lipids during the refrigerated storage.

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PP-100. Essential oil contents of black and white *Myrtle cultivars*

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Myrtle plants are mostly found as a wild plant in Mediterranean maquis. They have white and black coloured fruits. Myrtle oil is obtained from leaves of wild myrtle plants. But it is not clear that how the oil content is varied in the leaves of cultivated black and white myrtle cultivars which are grown for bigger sized fruits. White myrtle with bigger fruit size called "Hambeles" is grown for fruits in Mediterranean coastal side of Turkey. Newly founded black myrtle cultivar with bigger fruit size is called "Yakup". Aim of this study was to compare essential oil contents of Hambeles and Yakup mrytle cultivars. Essential oils were extracted from leaves by solvent free microwave extraction (SFME) method. Major components of myrtle oil of Yakup and Hambeles cultivars were 38,7% and 33,9% for 1,8-cineole; 30,6% and 29,3% for α-pinene; 8,3% and 13,9% for linalool; 6,0% and 5,5% for limonene and 4,2% and 4,75% for linalyl acetate,respectively. Essential oil content of leaves of myrtle cultivars have more or less similar percentages for both cultivars. Whereas, α-pinene contents of cultivated ones were higher than that of wild plant which was measured as 5.3% via SFME method in a former study(1). Results of this study indicated that myrtle can be cultivated without any decreases in essential oil content. Thus, negative impacts of myrtle plant harvest on nature can be decreased by cultivation of myrtle plants.

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PP-101. Chemical variability of *Phagnalon sordidum* essential oil from Corsica

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The genus *Phagnalon* L. belongs to the *Asteraceae* family and includes about 36 species. It is widespread throughout Northeastern tropical Africa, the Macaronesian region, the Mediterranean basin, the Irano-Turanian and Saharo-Arabian regions. To our knowledge only two studies have been conducted on the essential oils of *Phagnalon* genus and we reported here for the first time the chemical composition of the *P. sordidum* (L.) Reichenb. essential oil.

The aim of the work was to investigate the chemical composition of *P. sordidum* essential oil and to characterize the interspecies variations in essential oils from 20 Corsican sample locations. The chemical compositions of *P. sordidum* essential oils were carried out using GC/RI and GC-MS. The study of chemical variability was performed using Principal component analysis (PCA) and cluster analysis (CA).

Analysis of *P. sordidum* essential oils allowed the identification of 76 components accounting for 82.3-96.6 % of the total mixtures. Among them, the main components were β-pinene (1.0-20.3%), myrcene (0.3-4.9%), *p*-cymene (0.3-7.2%), limonene (0.6-8.4%), linalool (1.0-6.8%), decanal (2.5-5.5%), thymol (1.3-25.7%), (*E*)-β-caryophyllene (2.9-31.4%), germacrene-D (0.6-8.8%), caryophyllene oxide (1.0-5.6%) and hexadecanoic acid (0.3-6.3%). Statistical analyses allowed to determinate two main groups GI and GII differentiated by the amount of hydrocarbon compounds which accounted for 60% for the samples of GI vs only 44% for those of GII. These results could be linked to the introgression phenomena which occur between *P. sordidum* and *P. saxatile* in Corsica Island.

PP-102. Volatile and flavonoid compositions for quality assessment of liqueur prepared from peels of *Citrus medica* L. "Corsican"

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The volatile and flavonoid compositions of *Citrus medica* L. "Corsican" peels were studied according to the maturity of the citron fruits measured using growing degree-days (GDD). Quantitative variation with the stage of development of fruits cultivated in Corsica were observed using gas chromatography (GC), GC-mass spectrometry (MS), and liquid chromatography-MS/MS. Thirty volatile compounds were identified in peel essential oil. Limonene and γ-terpinene were the major compounds. The volatile compositions of citron commercial liqueurs were also characterized by high amounts of monoterpene hydrocarbons with the same two major components. The main flavonoid components of citron fruits and derived liqueurs were rutin and neohesperidin. This chemical characterization could be used for quality assessment and/or certification of botanical origin of food products from *C. medica* "Corsican".

PP-103. Individual monitoring to study organ and diurnal variations in *Mentha longifolia* L.

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Growing aromatic plants as a source of bioactive compounds and extracts requires the production of raw materials with a standardized chemical composition. For this purpose, besides the selection of most suitable chemotypes, organ variations and phenotypic plasticity should be taken into account. This co-occurrence of variability sources results in a foreseeable high intra-populational chemodiversity. This way, a sampling process consisting of mixing material from randomly selected plants in wild populations may sometimes lead to little reliable data regarding the environmental factors affecting chemical composition variability.

With regard to *Mentha longifolia* L. a large amount of chemotypes has been characterized; nevertheless, scarcely information is available concerning diurnal and organ variations in volatile composition. The goal of this work is to carry out a first approach to diurnal variation for leaves and inflorescences in *M. longifolia* by means of individual monitoring as a way to bear in mind the intrapopulational variability. A total amount of 36 samples were processed from 3 individuals which were monitored during 2 days with different meteorological conditions. The samples (< 1 g) were collected from leaves and inflorescences at 8 AM, 2 PM and 8 PM, kept at once in sealed vials containing dichloromethane and frozen at -18ºC in such a way that samples handling was reduced to the maximum. The extraction was performed by means of an ultrasound assisted method at low temperature in order to avoid the formation of artifacts.

Since individual monitoring, itself, can not involve any statistical analysis, only great differences have been taken into account. So, the mentioned sources of chemical variability become patently clear from the obtained results. Two individuals show α-terpineol acetate and carvone acetate as major compounds. No organ and diurnal variations were observed in neither of both individuals. Pulegone was found the major compound in the third individual, although considerable amounts of 1,8-cineole, isomenthone, trans-isopulegone, carvone acetate (only in inflorescences) and sesquiterpenes were also observed. Some great changes affecting major compounds have been registered in this individual, both regarding organ differences and diurnal variations. Nevertheless, the most interesting issue may be to consider the differences between the meteorological conditions. Indeed, from these data, the influence of a high temperature and dryness period in diurnal variation could be highlighted. On the other hand, the rest of data shows a noticeable consistency which supports the analytical reliability.

From the methodological point of view, the sampling and extraction process employed can be considered a suitable, fast and cheap way to perform this type of analysis with an enough number of individuals to make feasible a statistical approach. In the same way, the correlation between these data and the ones from volatile emissions analyzed by SPME should be established in order to consider the ecological significance of volatile content in flowers and leaves.

PP-104. Effect of drought stress on essential oil composition of *Thymus vulgaris* L. (Chemotype 1,8-cineole) from a population of Valencia (Spain)

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Drought stress is one of the most important abiotic factors affecting essential oils composition. The Mediterranean climates are characterized by the irregularity in rain and a typical drought period in summer. Given that in these countries aromatic crops such as *Thymus* species, sage, etc. are located in dry-farming lands, the potential effect of drought stress in essential oils composition becomes particularly relevant.

Phenolic and no-phenolic chemotypes of *Thymus vulgaris* L. have been described in literature, the same way that the ecological factors with which they are related. Nevertheless, less attention has been paid to 1,8-cineole chemotype, typical of Iberian Peninsula. As a part of a wider project to study soil and climate influence in essential oil composition of *T. vulgaris* (chemotype 1,8-cineole), the content of this communication refers to effect of drought stress in plants from a wild population located in Valencia (Spain). Two samplings were carried out in Serra (Valencia) on May 9, 2013 and August 1st, 2013. From the climatic point of view, two well defined periods could be defined: a relatively cool and wet spring followed by a hot and very dry summer, in such a way that the occurrence of drought stress can be accepted.

Five individuals were collected and analyzed separately in each one of the samplings. The essential oil was extracted at once from fresh material by hydrodistillation with a Clevenger type apparatus for 2 h. Identification and quantification of the chemical composition were performed by GC-MS and GC-FID with dodecane as internal standard. A total of 31 compounds were identified accounting for 99.25 %-92.86 % of total composition. 1,8-cineole (59.26-18.27 %) was found the main component. Other major compounds belonging to monoterpenic fraction were camphor (5.06-12.11 %), myrcene (1.88-9.00 %), borneol (2.87-7.08 %), beta-terpinene (1.87-5.85%) and camphene (1.06-5.80%). Regarding the sesquiterpenic fraction, beta-caryophyllene (0.50-5.40-%), beta-selinene (0.30-4.14%), spathulenol (0.12-8.38 %) and caryophyllene oxide (0,42-8,77 %) were the major compounds. An oxygenated sesquiterpene was tentatively identified as 4,4,11,11-tetramethyl-7-tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, showing also a great seasonal and individual variability (0-17.03%).

Analysis of variance (ANOVA) and Tukey's HSD multiple-range test at $P<0.05$ were used to consider significant differences in average values of the components among the sampling periods. Despite the very high intrapopulational variability, some clear and statistically significant differences were found. The most relevant one concerns to the main component (1, 8-cineole) whose rate shows a high increase during the drought period, the same way the oxygenated monoterpenes in whole. On the contrary, significant decreases were observed in sesquiterpenic fraction.

Although promising data have been obtained, they should be cautiously considered. The whole project in which this work is included is based on a wider range of edaphic and climatic features.

PP-105. Influence of Corn Mint (*Mentha canadensis* L.) and Spearmint (*Mentha spicata* L.) essential oils on human psychophysiology

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Mint species are widely used in cosmetic industry due to the fresh aroma and flavor, *e.g.* as additives in dental care products. The different main components, though, are responsible for their different scope of application in pharmacy (1, 2).

In the present study, the influence of the essential oils of two different mint species namely *Mentha spicata* L. and *Mentha canadensis* L. from a commercial source were tested on the psychophysiological parameters after inhalation. GC and GC/MS analyses showed that the used spearmint oil contained 61.8% of *R*-(−)-carvone, 23.7% of limonene and 1.4% of 1,8-cineol. The main components of the corn mint oil were menthol (34.8%), menthon (23.6%) and isomenthon (15.6%) followed by 2.9% of limonene.

In a "within subjects-effect experiment" with repeated measures design, 32 participants (16 male and 16 female) aged between 18 - 35 years were tested in three sessions each. Using an aroma lamp, in two sessions either spearmint oil or corn mint oil were administered for 40 min., another session served as control using pure water. The order of the administered odors was counterbalanced. At the beginning, and the end of each session a mood questionnaire (MDBF) was filled, also blood pressure and pulse of each participant was measured. Additionally, the participants had to rate the oil and water for pleasantness and familiarity. The data indicated no significant influence of either mint oils on subjective wellbeing compared to the control condition. An ANOVA statistical evaluation according previous applied methods (3), revealed significant differences in blood pressure between the tested oils. Systolic blood pressure remained constant in the spearmint condition while it significantly decreased under the influence of corn mint. Gender specific differences could also be observed in blood pressure. Both mint oils were significantly more familiar to females.

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PP-106. Component analysis and comprehensive antioxidant activity of Ginger (*Zingiber officinale* Roscoe) essential oil

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GC-MS-FID analysis of the tested ginger oil resulted in the identification of 71 compounds. The main components of this Ecuadorian oil are citral (geranial 10.5% & neral 9.1%), α-zingiberene (17.4%), camphene (7.8%), α-farnesene (6.8%) and β-sesquiphellandrene (6.7%) in contrast to other commercial ginger oil qualities which are typically high in sesquiterpenes and low in citral. The antioxidant activity of the oil expressed by IC_{50} in descending order is: $OH^{\bullet}$ (0.0065 µg/mL) > chelating capacity (0.822 µg/mL) > $ABTS^{\bullet+}$ (3.94 µg/mL)> xanthine oxidase inhibitory effects (138.0 µg/mL) > $O_2^{\bullet}$ (404.0 µg/mL) > DPPH (675 µg/mL). Lipid peroxidation inhibition by the essential oil in both stages, i.e. hydroperoxide formation and malondialdehyde formation, was less efficient than the inhibition by BHT. *In vivo* studies of ginger oil in *Saccharomyces cerevisiae*, show significant dose-dependent increase in antioxidant marker enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), which created the possibility of blocking the oxidation processes in yeast cells. Ginger oil in concentrations of 1.6 mg / mL increase the viability of the cells to the oxidative stress induced by exogenous addition of H_2O_2.

PP-107. Melissopalynological and volatile analysis of Corsican ”automne maquis” honey from *Arbutus unedo* L. habitat

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The *Arbutus unedo* L. (strawberry tree) (Ericaceae) honey was a typical Mediterranean product and its principal production is found in Sardinia and Corsica [1]. The strawberry tree honey has a light amber color, with medium-to-high olfactory and aromatic intensities described as phenolic, coffee grounds, bitter and pungent [1]. The taste of the honey is characterized by a very pronounced bitterness and astringency [2]. In Corsica, the honey predominated by *A. unedo*, was classified as “autumn maquis” honey since obtaining “Appellation d’Origine Contrôlée” designiation [3]. The aim of this work was to characterize the volatile compositions of Corsican “autumn maquis” honey certified by melissopalynological analysis. The relationship between volatile fractions of honey and those of *A. unedo* flowers was also determined in order to establish a chemical relationship between the nectariferous resources and the corresponding honey.

In the current study, 30 Corsican “autumn maquis” honeys were characterized by the typical combination of *A. unedo* and other autumnal taxa as *Hedera helix*, *Smilax aspera*, *Rosmarinus officinalis* and two *Asteraceae* pollen forms. Corsican origin was certified by the diversity of the taxa’s biogeographical origins and significant presence of *Castanea sativa* and *Quercus* sp. Volatile fractions of “autumn maquis” honeys and *A. unedo* flowers were studied using HS-SPME, GC and GC-MS. The volatile composition of honey was characterized by 27 compounds amounting to 72.9–82.4% of the total composition. The main compounds were isophorone (34.8%) and 3,4,5-trimethylphenol (27.1%). This latter compound is reported for the first time in *A. unedo* honey. The main compounds of *A. unedo* flower were 4-oxoisophorone (18.9%), 2-heptanone (8.0%), β-isophorone (6.3%), and 2-pentylfuran (6.2%). Both *A. unedo* flower and “autumn maquis” honey exhibit a high content of isophorone derivatives like isophorone, 4-oxoisophorone and β-isophorone. In addition, the *A. unedo* flowers displayed a higher content of linear compounds (32.9%), while the honeys was rich in phenolic compounds (39.7%).

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PP-108. Antioxidant activity and chemical composition of *Senecio inaequidens,* an invasive species from Corsica

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The genus *Senecio* is one of the largest of the Asteraceae family that includes more than 1500 species all around the world [1]. In Corsica Island, 10 species including 3 endemics are reported [2]. Among them *S. inaequidens* DC. (small leaved groundsel) are a perennial plant 20-100 cm high that blooms from March to December. It is native of South Africa and considerate as an invasive species. Phytochemical investigation on the solvent extract of the plant were recently reviewed [3] and described pyrolizidic alkaloid, eremophilane, furanoeremophilane and polyphenolic compounds. We recently reported the chemical composition of the essential and its anticorrosive activity [4] and to the best of our knowledge, it was the unique report.

The aim of the work was to investigate the anti-oxidant activity of the *S. inaequidens* essential oil and its fraction and sub fraction in order to identify compounds or classes of compounds responsible of the activity. Chemical analyses were performed using a combination of GC-IR, GC/MS and NMR and antioxidant potential was investigated by assessing $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ as the reducing power of ferric ion. Analyses were performed on the whole essential oil, the hydrocarbon fraction and 5 oxygenated fractions.

Analysis of *S. inaequidens* essential oils allowed the identification of 60 components which accounted to 98.7 % of the total amount. Among them, the main components were myrcene (21.4%) (Z)-β-ocimene (17.6 %), α-pinene (12.5%), limonene (8.1%) and cacalohastine (6.8 %). The anti-radical scavenging and reducing power of essential oil exhibited an interesting activity and the maximum-activity was measured for the oxygenated fraction dominated by monoterpene alcohols.

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PP-109. Biological activities of the essential oils of *Tanacetum abrotanifolium* Druce and proposed chemotype of *T. macrophyllum* (Waldst. & Kit.) Schultz Bip.

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Water-distilled essential oils from aerial parts of *Tanacetum abrotanifolium* Druce, and *T. macrophyllum* (Waldst. & Kit.) Schultz Bip. from Turkey were investigated for their insecticidal and biological activity. Highest contact toxicity against *S. granarius* was observed for the leaf oil of *T. macrophyllum* (88.93%). Significantly lower but considerable activity was also observed for *T. abrotanifolium* flower oil (81.30%; $P < 0.05$). The flower oil of *T. macrophyllum* also showed considerable fumigant toxicity against *L. minor* at 10 mg/mL application concentration (61.86 %) when compared with other samples at the same concentration. The highest DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity (47.7%) and phosphomolybdenum reducing activity was observed also for the flower oil of *T. macrophyllum* at 10 mg/mL concentration. However observed DPPH scavenging and PRAP activities were moderate for this oil when compared with positive controls at the same concentration. The essential oils were also analyzed by gas chromatography and gas chromatography/mass spectrometry. The flower and stem oils of *T. abrotanifolium* were characterized with camphor 35.2%, (*E*)-sesquilavandulol 19%, 1,8-cineole 13.5%. and hexadecanoic acid 41.8%, (*E*)-sesquilavandulol 16.2%, tetradecanoic acid 6.6% respectively. The flower and leaf oils of *T. macrophyllum* were characterized with *γ*-eudesmol 21.5%, (*E*)-sesquilavandulol 20.3%, copaborneol 8.5% and copaborneol 14.1%, 1,8-cineole 11%, bornyl acetate 9.6%, borneol 6.3%, respectively. Agglomerative hierarchical cluster analysis of the qualitative and quantitative data obtained from the essential oil composition of the *T. macrophyllum* essential oil from the present research and previous reports pointed out that two different chemotypes could be proposed with current findings which are *p*-methyl benzyl alcohol/ cadinene and eudesmane chemotypes. Investigated *T. macrophyllum* oils could be categorized as the eudesmane chemotype.

PP-110. DPPH Scavenging, PRAP activities and essential oil composition of edible *Lathyrus ochrus* L.

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Essential oil of edible *Lathyrus ochrus* L. from Cyprus was investigated by simultaneous GC, GC/MS analyses under the same conditions. Trace amount of oil (0.01> mL) was obtained by hydro distillation of 200 g fresh plants and trapped in 1 mL *n*-hexane. Twenty components were detected representing 91.5 ± 0.5 % of the oil. The main components of the oil were phytol 49.4 ± 0.4 %, hexadecanoic acid 20.6 ± 0.7 % and pentacosane 4.2 ± 0.1 %. Essential oil solution (1% oil: *n*-hexane) afforded low DPPH scavenging activity (9.28 ± 1.30 %) when compared with positive controls α-tocopherol (89.13 ± 0.54 %) and BHT (90.81 ± 0.35 %) at the same concentrations. Antioxidant activity of the oil was determined using a new HPTLC-PRAP assay. The oil solution (1% oil: *n*-hexane) afforded low reducing activity of phosphomolybdenum complex (594.85 AU) when compared with positive controls α- tocopherol (837.03 AU), BHT (819.70 AU) and negative control (48.43 AU).

PP-111. In vi*tro* antibacterial activity of essential oils against multidrug-resistant strains

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The growing antibiotic resistance causes major health problems both in hospital-acquired infections and daily medical practice. Therefore, the discovery of new alternative treatments against resistant pathogens is quite important nowadays. Essential oils (EOs) are complex, volatile herbal substances, which could be promising and affordable therapeutic alternatives or could support medical therapy.

The aim of our study was the microbiological evaluation of some medically applied essential oils (cinnamon bark, clove, thyme, scots pine, and eucalyptus) by different *in vitro* techniques.

The essential oils were obtained from a Hungarian drugstore (Herbaria) and they were analyzed with GC-MS. The antibacterial activity of these oils was tested against methicillin-resistant *Staphylococcus aureus* (MRSA 4262), *Pseudomonas aeruginosa* (ATCC 27853) and multidrug-resistant *Pseudomonas aeruginosa* (34205) with broth dilution and direct bioautography. In the case of broth dilution method, 5% emulsion was prepared from each EO with 10% solution of Tween 80 and nutrient broth. Control tubes were also prepared containing solution of Tween 80 and bacterial cultures. All measurements were made in triplicate. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the essential oils were expressed in µl/ml. The antibacterial effect of EOs was also determined with TLC-bioautography, which is an appropriate and accurate method for investigation of complex extracts. The essential oils were diluted with absolute ethanol. An aqueous solution of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma-Aldrich Ltd.) was used for the visualization of inhibition zones. As positive control, adequate antibiotics (vancomycin, ciprofloxacin or polymyxin B) were applied to the plates. The antibacterial activity was expressed in the diameter (mm) of inhibition zones.

On the whole, all EOs were effective against at least one of the investigated bacteria. Cinnamon, clove, and thyme oil were the most effective oils. The EO of pine was moderately effective and only against *P. aeruginosa* ATCC strain. Cinnamon and thyme oil showed better inhibitory effect (MIC: 0.39 µl/ml equally) than eucalyptus oil (MIC: 6.25 µl/ml) against MRSA. In TLC-bioautography, cinnamon oil produced the largest inhibition zone (8 mm) against multidrug-resistant *P. aeruginosa*, while against MRSA clove oil was the most effective (9.5 mm). In comparison, we found that the effect of the EOs was lower than the effect of control antibiotics.

In our further studies, we are planning to investigate other potentially effective EOs, to extend our research including other pathogens and *in vitro* techniques (e.g. vapour-phase method), and to determine the mode of action of the effective oils *in vivo* (mouse) models.

Acknowledgments

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PP-112. Chemical composition of essential oils of cultivated *Melissa officinalis* subsp*. altissima*

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Melissa officinalis L. is a perennial herbs and it is a member of the Lamiaceae (mint) family. It is native to the Mediterranean region and leaves of *Melissa officinalis* are used as a herbal tea for their properties in folk medicine. Three subspecies, *M. officinalis* L. subsp. *officinalis*, *M. officinalis* L. subsp. *altissima* (Sibthr. et Smith) Arcang and *M. officinalis* L. subsp. *inodora* (Bornm.) Bornm. are known. Although, *M. officinalis* subsp. *officinalis* are studied extensively, only a few reports on *M. officinalis* subsp. *altissima* were found. In this study, seeds of *M. officinalis* subsp. *altissima* were collected from the wild flora of Antalya (Serik-Akbas, altitude 220 m). Seedlings were grown in greenhouse condition and then they were transferred to experimental field of Akdeniz University located in Antalya, Mediterranean Region of Turkey. Essential oils obtained by hydrodistillation of the dried leaves of three *M. officinalis* subsp. *altissima* samples were investigated by GC-MS. In total, twenty-three constituents were identified, representing 92.5–94.7% of the oils. The oils of all samples were rich in sesquiterpenes such as caryophyllene oxide (44.5-33.3%), β-copaene (10-8.72%) and caryophyllene (5.57-8.26%). Caryophyllene oxide was the major component in all three samples (44.5-33.4-38.58%, respectively).

PP-113. Essential oils of selected plants growing in Tanzania

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Guided by ethnobotanical literature and availability from natural sources, in the framework of our research on odoriferous Tanzania plants [1,2], used as edibles or spices as well as in folk medicine, we report herein the analysis of four essential oils.

All the species *Bidens magnifolia* Sherff, *Bothriocline amplexicaulis* (Muschl.) Wild & G.V.Pope, *Conyza welwitschii* (S. Moore) Wild and *Blumea alata* (D. Don) DC *(syn= Laggera alata)* belong in Asteraceae family and they grow wild in Tanzania.

Hydro-distilled essential oils from the aerial parts of those plants were investigated by GC and GC/MS. Major compound in all samples was spathulenol (14-46%)) followed by δ-cadinene (*B. magnifolia*), α-calaconene (*C. welwitschii*), aromadendrene (*B. alata*) and caryophylla-3,8(13)-dien-5-ol (*B. amplexicaulis*). The chromatograms from the three of the samples, except of *B. amplexicaulis*, are similar with a large number of hydronaphthalenes as γ-muurolen, δ-cadinene, cis-calamenene and α-calacorene.

The most of these plants, due to their high content of spathulenol, which demonstrate significant repellent activity [3], are used as repellents and mosquitocides [4].

Furthermore, the essential oils were investigated for their antimicrobial activity, by the agar dilution technique against Gram negative and Gram positive human pathogenic bacteria and fungi and they showed a very interesting broad antimicrobial spectrum of activities.

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PP-114. Headspace solid phase microextraction procedure for mastic gum -antimicrobial activity

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Mastic is a well-known natural resin from the trunk and branches, of *Pistacia lentiscus* var. *chia* (Anacardiaceae), which is grown as endemic only in the Greek island of Chios. It has been used in traditional Greek medicine for various gastrointestinal disorders while the plant has been mentioned by famous ancient Greek physicians (Dioscorides, Theophrastos, etc) recommending its healing properties.

In the framework of re-evaluation of important Greek natural products using new techniques, we present the chemical analysis of mastic gum through Headspace Solid Phase Microextraction (HSPME), which combines sampling free from organic solvents, applying to complex matrices; it is economic, sufficiently fast and friendly to the environment and to our knowledge is presented for first time, on crude natural product.

The volatile profile of natural crude mastic gum was detected based on HSPME and Gas Chromatography–Mass Spectrometry (GC-MS). Through this analysis α-pinene (25.6%), verbenone (14.0%), β-cymene and verbenene appeared as the most abundant constituents, representing 58% of the total, among the 27 identified volatile components of the mastic.

Moreover, the crude extract of mastic, as well as, its acidic and neutral fractions was assayed against a panel of 11 human and food pathogenic Gram (+) bacteria and fungi showing a promisssing antimicrobial profile confirming the traditional uses of mastic gum as antiseptic and/or food preservative.

Acknowledgments

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PP-115. Variability of chemical composition and biological activities of *Allium triquetrum* L. essential oils

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The present work describes the chemical composition and evaluates the antimicrobial properties of the essential oils from the Tunisian *Allium triquetrum* L. It is particularly interesting study because there are no reports on of this species in any sector and with specific chemical composition. The chemical composition of four essential oil samples (flowers, stems, leaves and bulbs) of *A. triquetrum*, obtained separately by hydrodistillation were analyzed by combination of GC-FID and GC-MS. Altogether, 45 compounds were identified representing 90.5-95.3% of the total oil content. The chemical composition of bulbs oil was characterized by a high proportion of sulfurous compounds (81.9%) among which dipropyl trisulfide (11.7%) and di-2-propenyl trisulfide (10.0%) were the predominant compounds. The oxygenated sesquiterpenes represent the major fraction (79.2%) in stem oil giving *T*-cadinol (26.8%), α-eudesmol (11.4%) and β-eudesmol (16.8%) as the main components; they were present also in leaf oil. We also noted the presence of some alkane compounds such *n*-nonadecane (13.3%) in flower oil. Some compounds identified in this plant have not been reported about other species of *Allium* such elemol, β-copaen-4-α-ol, globulol, viridiflorol, guaiol, eremoligenol, α-eudesmol, β-eudesmol and pimaradiene. Furthermore, the isolated oils were evaluated for antibacterial and antifungal activities. All the oils exhibited significant *in vitro* antibacterial activity, especially against *Enterococcus faecalis* and *Staphylococcus aureus*. The oil obtained from the stem, leaf and bulbs exhibited an interesting antibacterial activity, with a Minimum Inhibitory Concentration (MIC) of 31.25µg/mL against *S. aureus*. It was found also that stem and bulbs oil showed the highest growth inhibition of almost fungi especially against *Fusarium solani* (75.33and 71.33%, resp.) even greater than the positive control: benomyl (69.33%). The results indicate that the essential oil of *A. triquetrum* contains chemical compounds with good potential for application in natural health products and in the protection of agriculture products.

PP-116. Evaluation of in vitro cytotoxic and antitumor activity of the essential oil from *Sideritis perfoliata*

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The genus *Sideritis* (Lamiaceae) is represented by 46 species in Turkey. *Sideritis perfoliata* L. is known as "adacayi, dagcayi, and Kandil cayi" and consumed as tea in in different regions of Turkey (1,2). The purpose of this study was to investigate the cytotoxic and antitumor activity of essential oil from *Sideritis perfoliata* against one *normal* human *cell* line *(*NIH) *and six* human *tumor cell* lines (K562, PC-3, MCF-7, HeLa, A549, HT-29). Antitumor activity of *Sideritis perfoliata* essential oil were reported as moderate on C32 and ACHN *tumor cell* lines, and also as inactive on LNCaP and MCF-7 cell lines in a previous study (3). To the best of our knowledge, in the *present study*, we have reported for the *first* time cytotoxic and antitumor activity of *Sideritis perfoliata* essential oil on other six different cell lines except for MCF-7. According to our study, essential oil of *Sideritis perfoliata* at 100 µg/ml did not *show any cytotoxic and* antitumor activity against *both normal and tumor* cells. However, previous studies have shown that Limonene, previously *reported as major constituent* of the essential oil of *Sideritis perfoliata,* has anticancer effects (1,4). As a result, changes in the chemical profile of essential oil of *Sideritis perfoliata*, depending on location and seasonal variations, could influence their anticancer activity. Therefore, it is important to determine the chemical composition of essential oil of *Sideritis perfoliata* to correlate with their antitumoral activity. Furthermore; Essential oil of *Sideritis perfoliata* did not show toxic effect on NIH and this result clearly might indicate this species could be safely consumed as tea.

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PP-117. Headspace analysis of *Bauhinia rufa* (Bong) Steud. (Leguminosae) flower scent

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Plants release several volatile organic compounds (VOCs), which may have protective and signaling effects that can act in biological interactions. *Bauhinia rufa* is a small tree widely distributed throughout Brazilian savannas. Its white flowers smell unpleasantly and are pollinated by bats. Phytochemical analysis has been done only with leaves of this species due to its medicinal importance. Our study focused on characterizing *Bauhinia rufa* floral scent in order to understand more deeply the signals potentially related to pollinator attraction. This way, we proposed to perform the chemical characterization of floral VOCs by means of GC/MS-HS analysis in order to identify chemical compounds potentially related to plant-pollinator interaction. Recently-opened flower samples were placed in a 20 ml borosilicate glass vial and analyzed on a Thermo Scientific Focus system, equipped with an ISQ 230ST mass spectrometer and a Triplus automatic sampler. Separations were achieved by using an OV5-MS column under optimized conditions and the volatiles identification was based on the comparison of their mass spectral data and retention index with those from NIST data base and literature. Our study revealed that floral scent of *Bauhinia rufa* comprises a complex mixture of at least 24 compounds: six monoterpenes, one aldehyde, thirteen non-oxygenated sesquiterpenes and four oxygenated sesquiterpenes. Additional studies are underway to elucidate the role of these chemical compounds on the biological interaction with pollinators.

PP-118. Antibacterial activity of the essential oil from *Mentha viridis* L.

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Plant species that belong to the *Mentha* genus and the Lamiacea family are characterized by their complexity. The presence of essential oils in these species is proven, and these oils are used in cosmetics, pharmaceuticals, food products, confectionery and alcoholic beverages [1]. The use of essential oils in the control of micro-organisms is a natural alternative in the search for new substances to control diseases that are caused primarily by bacteria. Research shows that intensive and indiscriminate use of synthetic substances favor the rise of resistant micro-organisms, cause negative effects to the environment and to the organisms that are exposed to these products [2].

The antimicrobial activity of the essential oil from *Mentha viridis* L. against *Escheria coli* and *Staphylococcus aureus* bacteria was evaluated.

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus [3]. The qualitative analysis of the essential oil was performed by gas chromatography coupled to a mass spectrometer. The bioassay utilized the diffusion test in agar medium with dilution of the essential oil in DMSO to give concentrations of 500, 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 µL mL^{-1}. DMSO was used for the negative control, and the antibiotic chloramphenicol was tested as the positive control [4]. The diameters of the haloes were measured seven days after the initiating the experiment.

The principal compounds found in the essential oil from *Mentha viridis* L. were linalool (40.70%), carvone (13.52%) and α-terpinene (8.56%). The inhibitory effect of the concentrations of the essential oil on the growth of the bacteria was 0 mm for the negative DMSO control and 25 mm for the chloramphenicol control (1000 µL mL^{-1}) for both bacteria. The diameters of the halos observed for *E.* coli with each concentration were 8.0 mm for 500 µL mL^{-1}; 8.5 mm for 250 µL mL^{-1}; 8.5 for 125 µL mL^{-1}; and 6.0 mm for 62.5 µL mL^{-1}. No inhibition of the growth of *E. coli* was observed for the concentrations of 31.3, 15.6, 7.8 and 3.9 µL mL^{-1}. The diameters of the halos observed for *S. aureus* were 11 mm for 500 µL mL^{-1}; 8.0 mm for 250 µL mL^{-1}; 8.0 mm for 125 µL mL^{-1}; 8.0 mm for 62.5 µL mL^{-1} and no inhibition of *S. aureus* growth at concentrations of 31.3; 15.6; 7.8 and 3.9 µL mL^{-1}. An effect of the essential oil from *Mentha viridis* L. on the micro-organisms tested was observed at a concentration as low as 62.5 µL mL^{-1}.

Acknowledgements

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PP-119. Hemolytic activity of the essential oil from *Mentha pulegium* (L). L. in liquid medium

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The use of plants and their products as medications must be closely associated with their rational application based on the appropriate choice of the active ingredients and their dosage and the adverse effects associated with these drugs. While disclosure of the use of natural products has grown tremendously in recent years, products from plants may have toxic components [1]. Intravascular rupture of red blood cells (RBCs) after administration of certain substances can raise the level of hemoglobin in plasma, leading to dysfunction of vascular endothelial cells and resulting in vascular thrombosis [2].

The hemolytic potential of the essential oil from *Mentha pulegium* (L) L. against human erythrocytes was evaluated.

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus [3]. The qualitative and quantitative analysis of the essential oil was performed by gas chromatography associated with mass spectrometry. To perform the hemolytic assay, human erythrocytes were diluted in phosphate buffer at a 2% concentration. The essential oil was also diluted in buffer solution to give concentrations of 0.6, 1, 2, 3, 4, 5 and 10 µL mL^{-1} The controls were composed of erythrocytes in PBS (mechanical hemolysis control) and erythrocytes in distilled water (total hemolysis control). Readings were taken on a spectrophotometer at 412 nm [4].

The principal compounds found in the essential oil of *M. pulegium* were pulegone (50.01%), menthol (31.90%) and menthone (16.56%). The hemolysis of 100% of the erythrocytes was observed at all the concentrations tested. The lipophilicity of the essential oils furnish the capacity to separate layers of polysaccharides, phospholipids and fatty acids and to interfere with the permeability of the membrane [5]. The medium and the concentration of the hematocrit are essential factors for the evaluation of hemolytic activity, and these factors are responsible for considerable variations in activity.

Acknowledgments

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PP-120. Study of the antibacterial activity of essential oils and macerated ginger: *Zingiber officinale*

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Zingiber officinale L, commonly known as ginger in Algeria (Zinjabile , Skenjbir) which is a rhizomatous plant cultivated throughout Asia, South-East , China and some parts of Japan, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times.

The extraction of essential oils from the dried and fresh rhizomes gave the successive values of 0.6 % and 0.8 %, and the preparation of the maceration for 12 hours requires grating rhizomes to crush internal oily cells.

The evaluation of the antimicrobial activity of the oil and the macerate against *Pseudomonas aeruginosa , Escherchia coli, Staphylococcus aureus Klebsiella* sp, *Enterobacter* sp by the disc diffusion method was conducted. The results obtained were comparable with the tested reference compounds.

PP-121. Spasmolytic and antimicrobial activity of 5-phenylpentyl isothiocyanate, a new glucosinolate autolysis product from horseradish (*Armoracia rusticana* P. Gaertn., B. Mey. & Scherb., Brassicaceae)

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In continuation of our previous studies of volatile glucosinolate (GLS) autolysis products originating from cruciferous plant species [1], we focused our attention on horseradish (*Armoracia rusticana P. Gaertn., B. Mey. & Scherb., Brassicaceae)*, a plant species known since ancient times, widely used in traditional medicines and cuisines of many nations [2]. Nowadays, this plant is cultivated for its roots and leaves; roots are used widely as a spice due to sharp, burning and bitter mustard-like aroma originating mainly from GLSs breakdown products; leaves are frequently used as a salad, mixed with other vegetables [3].

Several GLS breakdown products have been identified by GC and GC-MS analyses of the autolysates (above- and underground parts), including one new natural product, 5-phenylpentyl isothiocyanate. In order to confirm the tentative structure assignment and to obtain sufficient amount of the pure isothiocyanates (ITCs) for biological studies, a series of ω-phenylalkyl ITCs (benzyl, phenethyl, 3-phenylpropyl, 4-phenylbutyl and 5-phenylpentyl) were synthesized starting from commercially available chemicals. The corroboration of the identity of 5-phenylpentyl ITC suggests the existence of a 5-phenylpentyl GLS in the aerial parts of this species as one of the possible “mustard oil” precursors.

In addition, the horseradish autolysates, together with the five synthesized ω-phenylalkyl ITCs were screened for their spasmolytic and antimicrobial activities. The assays revealed a strong activity of the tested samples in both assays. These finding are important because they open a possible new field of application of horseradish in diarrheas of infective genesis.

Although horseradish above-ground parts are already used to some extent as a salad, due to the above-mentioned beneficial effect on human health, from this point of view, increased consumption of horseradish leaves is recommended, maybe as a relish, spices or in the form of a remedy.

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PP-122. Further antibacterial *Geranium macrorrhizum* L. essential oil constituents and the synthesis of epoxygermacrones

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Geranium macrorrhizum L. (Geraniaceae) is a plant species highly valued in ethnopharmacology of the Balkan region [1]. It possesses a wide range of beneficial effects and activities such as antimicrobial, hypotensive, spasmolytic, astringent, cardiotonic, antioxidant, and sedative properties [2]. *Geranium macrorrhizum* has been established as a prolific source of diverse secondary metabolites. Up to now, there are over 300 metabolites reported only for the essential oils of this plant species. The essential oil of the aerial parts of the plant is mostly composed of a germacrane sesquiterpene ketone, germacrone. A number of germacrone-derived compounds have been isolated or detected in this taxon as well ([2] and references cited therein).

Antimicrobial activities of *G. macrorrhizum* secondary metabolites have been lately investigated by our research group [2-4]. In a 2010 study [2], high and selective activities of *G. macrorrhizum* essential oils (of both aerial parts and rhizome) towards *Bacillus subtilis* have been observed, however, full details on the active principles responsible for the noted high antimicrobial action were not known. Germacrone contributed greatly to the activity of the oil, but it was obviously not the only active agent [2].

Our research turned to the search for other active principles of *G. macrorrhizum* aerial parts oil. In that sense, a series of bioassay-guided chromatographic separations were performed. As a result, two (minor) compounds were isolated which we failed to detect in the 2010 study, 1,10-epoxygermacrone and 4,5-epoxygermacrone. The isolated compounds were fully elucidated by GC/MS and NMR (1D- and 2D-NMR spectra), structures were confirmed by synthesis starting from germacrone and then the substances were submitted to a microdilution-susceptibility assay to determine the minimal inhibitory concentrations (MICs) against six bacterial (both Gram-positive and Gram-negative) and one fungal strain. Both compounds showed much higher antibacterial activities than germacrone against *B. subtilis* (germacrone was tested alongside *G. macrorrhizum* oils, and the results were published in [2]). 1,10-Epoxygermacrone was 1,250-fold more active toward *B. subtilis* than the parent ketone (MICs values determined were 4.3 and 43 nmol/ml for 1,10- and 4,5-epoxygermacrone, respectively). The observed activity of the two epoxides at least partially justifies the ethnomedicinal use of this plant species.

Acknowledgments

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PP-123. Essential oil composition of three endemic *Tripleurospermum* species from Turkey

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The genus *Tripleurospermum* Schultz Bip. is represented in Turkey by 24 species and altogether 26 taxa. 11 species are endemic in Turkey. Microdistilled essential oils of the dried flowers of three endemic species were analyzed by GC-FID and GC/MS. All the oils were characterized by the occurrence of matricaria esters as main constituents.

The oil of T. *callosum* (Boiss. et Heldr.) E. Hossain contained (2*Z*,8*Z*)-matricaria ester (81.5%), (2*E*,8*Z*)-matricaria ester (4%), (*Z*)-β-farnesene (2.3%) and (2*Z*,8*E*)-matricaria ester (1.4%) as main constituents. The oil of *T. heterolepis* (Freyn et Sint.) Bornm. was characterized by the occurrence of (2*Z*,8*Z*)-matricaria ester (78.0%), (*Z*)-β-farnesene (5.2%), (2*E*,8*Z*)-matricaria ester (3.4%), cis-cadin-4-ene (2.9%), β-sesquiphellandrene (2.1%), (2*Z*,8*E*)-matricaria ester (1.3%) as major components. Main components in the oil of *T. hygrophilum* (Bornm.) Bornm. were (2*E*,8*Z*)-matricaria ester (43.3%), 2,8-matricaria ester (32.2%), 8*Z*-2,3-dihydromatricaria ester (8.5%), (2*Z*,8*Z*)-matricaria ester (6.3%), (*Z*)-β-farnesene (2.2%).

PP-124. Essential oil constituents of *Phlomis pungens* Willd. from Azerbaijan

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The genus *Phlomis* as perennial herbs of Lamiaceae consists of more than 100 species distributed in Africa, Asia and Europe (1, 2). In Azerbaijan, *Phlomis* is represented by 6 species (3).

In this work, hydrodistilled essential oils from leaves and aerial parts of *P. pungens* Willd. from three different localities (Galalti, Altiagach, Nakhchivan) of Azerbaijan were studied by GC and GC/MS. Oil yields in the samples were less than 1%.

More than forty five constituents were identified in the oils. The percentages of the main components in the oils were found as germacrene D (19.4 – 46.4%), hexadecanoic acid (8.1 – 36.2%), (*Z*)-β-farnesene (3.1 – 9.2%) and hexahydrofarnesyl acetone (1.3 -7.6%).

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PP-125. Composition of the essential oil of *Hypericum aviculariifolium* Jaub&Spach subsp. *depilatum* (Freyn&Bornm.) Robson var. *bourgaei* (Boiss.) growing in Turkey

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The genus *Hypericum* L. is represented by 96 species, 47 taxa endemic in Turkey. *Hypericum aviculariifolium* Jaub&Spach subsp. *depilatum* (Freyn&Bornm.) Robson var. *bourgaei* (Boiss.) (Clusiaceae) is endemic in Turkey. The essential oil obtained by hydrodistillation of *Hypericum avicularii folium* subsp.*depilatum* var. *bourgaei* collected from Antalya,Turkey.

Essential oil of *Hypericum avicularii folium* subsp.*depilatum* var. *bourgaei* from Turkey was analysed by GC and GC/MS, simultaneously. Hexadecanoic acid (28.0 %), lauric acid (11.3%), myristic acid (9.7%) and caryophyllene oxide (8.7 %) were main constituents.

PP-126. Effects of salicylic acid application on quantitative and qualitative yield of cumin under irrigated and rain-fed cultivation

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Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species[1] that accumulates in many plant species under drought stress [2]. Increasing in Salicylic acid in this situation can increase plant drought resistance [3]. An experiment was conducted as split-plot in irrigation and rain-fed condition in order to evaluate response of cumin quality and quantity to Salicylic acid in 2011 at Sabzevar Islamic Azad University. The main plot was planting type (rain-fed and irrigated) and the sub plot was type of application of Salicylic acid (seed-treating, seed-treating+ foliage spraying, spraying at flowering and without Salicylic acid application). Seed treating and spraying with Salicylic acid was consisted of application 100 mM of this solution. The results showed that irrigation increased economic yield, biological yield, number of lateral branch and number of umbrella per plant of cumin while, essential oil percentage was not affected by irrigation. Seed application+ spraying of Salicylic acid was superior than other application methods in yield and yield components increasing while, seed application had no significant effect on economic and biological yield of cumin. In general results showed that application of Salicylic acid as seed treated+ spraying or only spraying of them specially in dry land cultivation are better than seed application for increasing cumin yield.

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PP-127. The influence of foliar application of zinc fertilizers on glandular trichomes, essential oil content and composition of holy basil *(Ocimum sanctum)*

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Holy basil (*Ocimum sanctum*) belongs to Lamiaceae family. It has been used in pharmaceutical industry from ancient times due to its valuable medicinal properties. In order to evaluate the effect of foliar application of zinc fertilizers on glandular trichomes, essential oil content and composition, an experiment was conducted based on randomized complete block design with three replications. The treatments were nano zinc chelate (0, 0.5, 1 and 1.5 $gr.l^{-1}$) and zinc solfate (1 and 1.5 $gr.l^{-1}$) fertilizers. The plants were harvested at full bloom stage and dried in shade. The essential oil was extracted by a clevenger apparatus. The size and number of glandular trichomes were measured by light microscope. The results showed that the effect of foliar application of zinc fertilizers on distribution and size of glandular trichomes and also essential oil content of holy basil were significant ($p \geq 0.01$). The highest values of glandular trichomes number (21.7, 17.4) and glandular trichomes size (0.3, 0.3 mm^2 at lower and upper epidermis, respectively) were obtained in plants sprayed with 1.5 $gr.l^{-1}$ nano zinc chelate. The plants treated by 1.5 $gr.l^{-1}$ nano zinc chelate had highest essential oil content (2.1%). Eugenol (25.6-34.7%), 1,8-cineole (21.9-23.9%) and methyl chavicol (12.5-13.4%) were main essential oil components of holy basil. Over all, foliar application of plant with 1.5 $gr.l^{-1}$ nano zinc chelate is recommended to increase essential oil content of holy basil.

PP-128. Molecular characterization and phytochemical evaluation of *Artemisia* species from Iran, a valuable potency for producing of some metabolites

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Iran has 33 species of *Artemisia*, which two of them (i.e. *A. melanolepis* Boiss. and *A. khorassanica* Podl.) are endemic distributed in most part of the country. Phytochemical evaluation of *Artemisia* species from Iran revealed artemisinin, as a new antimalarial drug, and also some other terpene synthases that produce sesquiterpenes which play important roles in plant defense, antifungal, antibacterial, antioxidant, anthelmintic and anti-inflammatory activities. We found many common artemisinin and terpene biosynthesis pathway in different *Artemisia* species as what reported in *A. annua* using quantitative PCR. Karyological data on *Artemisia* species from Iran, revealed two basic chromosome numbers, x = 8, 9, each with diploid, tetraploid and hexaploid levels. In addition, phylogenetic tree using the internal transcribed spacers (ITS) and external transcribed spacer (ETS) of nuclear ribosomal (nr) DNA cautiously confirm the monophyly of the genus *Artemisia* from Iran. These species have a valuable potency for producing of some metabolites that can be useful in medicine.

PP-129. The effects of fennel shoot aqueous extract on different germination aspects of *Lolium* sp. and *Vicia* spp.

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One of the most important prerequisites for achieving sustainable agriculture is to make fields environments closer to natural ecosystems via reducing their dependence on chemical inputs especially pesticides. In this case, allelopathy has a high potential for controlling weeds in fields.

In order to investigate the allelopathic effects of fennel aqueous extract on germination aspects of *Lolium* sp. and *Vicia* spp. weed seeds, two separate experiments were conducted based on completely randomized design with 3 replications at Tarbiat Modares University in March 2014. In order to prepare the fennel aqueous extract stock, 100 gram of fennel shoot dry matter was milled and then poured in distilled water. The mixture was shaken 24 hours at lab temperature. Then the mixture was filtered via filter paper and the different extract concentrations were made from it. Extract concentration treatments for both experiments were as below: 0, 20, 40, 60, 80 and 100 percent of stock.

The results showed that with increment in extract concentration, the germination aspects of both weed seeds were affected significantly. Germination percentage and rate and also radicle and plumule lengths for both weeds were significantly reduced with increment in fennel extract concentration.

PP-130. Alismol and geranyl linalool: Two chemotypes of essential oils of *Daucus carota* subsp. *sativus* from Algeria

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Chemical composition of the aerial parts and roots essential oils from eight different locations of Algerian *Daucus carota* L. subsp. *sativus* DC. , cultivated carrot, have been investigated, using GC/RI and GC-MS. Essential oils allowed the identification of eighty-six compounds, constituting 90.7–97.6% of the total essential oils. The main components of aerial part collective essential oil were alismol C71 (15.2 %), (E)-β-caryophyllene C53 (10.1 %), myrcene C16 (9.6 %), α-humulene C55 (9.5 %), β-ionone C57 (5.2 %) and spathulenol C67 (4.2 %). The main components of root collective essential oils were geranyl linalool C80 (27.3 %), myristicin C59 (7.3 %), pentacosane C86 (5.6 %) and bornyl acetate C38 (3.8 %). Intra-species variations of the chemical compositions of essential oils from eight Algerian sample locations were also investigated using statistical analysis.

The samples were separated in 2 groups by hierarchical cluster analysis, according to the composition of the corresponding essential oils. To our knowledge, this is the first report of alismol and geranyl linalool as major compounds in *D. carota* subsp. *sativus* essential oils.

PP-131. Chemical composition, antioxidant and antifungal activities of roots essential oil of *Carthamus arvensis* from Algeria

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Chemical composition of the roots essential oils of Algerian *Carthamus arvensis* has been investigated, from the first time, using GC/RI, GC-MS and NMR. Biological activity of the essential oil was assayed for their antioxidant and antifungal activities. Essential oil of *C. arvensis* was dominated by 2-(3-phenyl-1-propynyl)-furan exceeds 90 % of total essential oil composition. Exact structure was elucidated by ^{1}H NMR, ^{13}C NMR, DEPT and two dimensional NMR techniques (HSQC and HMBC) as well as the literature NMR chemical shift database.

This compound was established only in *Carlina* genus and commonly named carlina oxide. To our knowledge this is the first report of carlina oxide in *Carthamus* genus essential oils. The antioxidant activity was evaluated as a free radical scavenging capacity (RSC). RSC was assessed measuring the scavenging activity of the essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH.) and (OH.) radicals. Essential oil exhibited very strong RSCs, reducing the DPPH radical formation (IC_{50}) of 0.35µg/mL. In addition, the essential oil presents the highest capacity to slow the rate of oxidation of linoleic acid and Beta-carotene (> 100 % for 1µg/mL) even better than from synthetic antioxidant BHT. Other hand, essential oil showed strong in vitro antifungal activity based on the inhibition zone and minimal inhibitory concentration values against the pathogen (*Aspergillus niger* and *Penicillium italicum*). *Citrus sinensis* fruits infected by *Penicillium italicum* pathogen were treated in vivo with essential oil. Very low concentrations were needed for the absence of orange infection and very low disease incidence.

2-(3-phenyl-1-propynyl)-furan

PP-132. *Enterococcus faecalis* and *Candida albicans* biofilm Inhibited by *Cinnamomum cassia* essential oil solution as a root canal irrigant

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Cassia essential oil was analyzed by GC/MS. The oil prepared in 20% ethanol, was evaluated for antimicrobial activity against *Enterococcus faecalis* and *Candida albicans* biofilm. Eradication was evaluated at three time intervals and compared with 2% chlorhexidine digluconate and 2.5% sodium hypochlorite. *Cassia* essential oil, consisting mainly of 75% cinnamaldehyde, has shown an interesting antimicrobial activity against studied microbial species in their both phenotypes planktonic and biofilm. At *in vitro* irrigation assay, *Cassia* essential oil at 1.25% killed all *Enterococcus faecalis* and *Candida albicans* viable cells in mature biofilm for 30 seconds of exposure only, 2.5% sodium hypochlorite for five minutes and 2% chlorhexidine digluconate for one minute. At planktonic state, chlorhexidine digluconate was more antimicrobially active than *Cassia* essential oil and sodium hypochlorite.

PP-133. Chemical composition of the essential oils of *Juniperus oxycedrus* and *Juniperus phoenicea* from Algeria

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The essential oils from the leaves of *Juniperus oxycedrus* and *Juniperus phoenicea* were obtained by hydrodistillation, and investigated by gas chromatography (GC) and gas chromatography /mass spectrometry (GC/MS). The two oils were of α-pinene (32.1– 50.5%) chemotype. δ-3-Carene was the second major constituent (14.5-30.4%). α- cedrol (4.5-7%) was the third main compound in the two oils. The oil from *J. oxycedrus* was composed of about 31 compounds representing 97.1% of the total composition of the oil. *J. oxycedrus* oil composed mainly of monoterpenoids which amounted to 83.4%, of which 80.6% was monoterpene hydrocarbons. The sesquiterpenoids accounted for about 13.7% of the total oil composition. The oil from *J. phoenicea* was composed of about 44 compounds representing 98.8% of the total composition of the oil. *J. phoenicea* oil composed mainly of monoterpenoids which amounted to 90.0%, of which 85.6% was monoterpene hydrocarbons. The sesquiterpenoids accounted for about 9% of the total oil composition. Of the remaining components, the contents of α-phellandrene (4.0- 6.5%), α-terpinolene (3.8- 5.6%) and β-myrcene (2.7- 3.9%), were significantly high in both oils. We report here the comparative study of the chemical composition of the oils from the two species of *Juniperus*. This study will contribute to the knowledge of a local product that could improve the use of Algerian *Juniperus*.

PP-134. Determination of difference in nitter orange (*Citrus aurantium*) peel essential oil compositions obtained with cold pess and hydro-distillation

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Many naturally occurring compounds present in different parts of plants such as fruit, leaves, roots, barks etc. In this scope essential oils are mostly used natural compounds. They have antimicrobial, antioxidant, anticancer effects and so on. Citrus essential oils are complex mixtures of approximately 400 compounds whose ratios can be changed according to specific citrus cultivar, ecological conditions, extraction methods etc. Citrus essential oils, obtained as by-products of the citrus processing, are one of the most widely used essential oils in the world. They are used in foods, medicines, cosmetics and cleaning products. They could be obtained by cold press, hydro-distillation etc. In this study, the effects of cold press and hydrodistillation applications on essential composition of bitter orange peel oil were investigated. Additionally changes in essential oil compositions by drying were presented. Essential composition was affected significantly from extraction techniques. Limonene was determined as the main component of bitter orange essential oil. Its ratio was found as 94.48% in the fresh peel oil. After drying at 45°C in the oven, the limonene ratio was determined as 94.00% in the dried peel oil using hydro-distillation and 94.65% in cold press oil of the dried peel. The other highest components were β-myrcene, linalool, β-pinene, αpinene in descending order. From these results, although there were no significant differences between cold press and hydro-distilled essential oil compositions, the essential oil compositions of oil obtained by cold-press were more similar to fresh peel oil than the oils obtained by hydrodistillation.

PP-135. Changes in compositions of *Origanum majorana* and *Origanum syriacum* essential oils depends on the drying Temperatures

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The genus *Origanum* L. is represented in Turkey by 24 species and, 16 of them are endemic, the ratio of endemism in the genus is 66.7%. Oregano plays a primary role among culinary herbs in Turkey trade. Although cultivation of oregano has been increased recently, the majority of raw material in essential oil industry was covered from wild collection. Essential oil compositions of *Origanum* spp have been extensively investigated. Because of its huge genetic diversity, the studies have been continued at different perspective such as adaptation, cultivation, processing, health effects etc. *Origanum* essential oils are characterized by a number of main components which are implicated in the various plant odours. Carvacrol is generally predominant component in the essential oil. In the production of essential oil, plants generally dried in shade and hydro-distillation process was used. In this study; effects of different drying temperatures as 35, 45 and 55°C were determined by analyzing essential oil content and compositions of *Origanum majorana* L. and *Origanum syriacum* L. using Clevenger apparatus and GCMS-FID, respectively. Plants were cultivated at the institute field, nearly 30 m altitude, harvested at their flowering periods in the second year after planting. The essential oil content of *O. majorana* and *O. syriacum* were calculated as 5.41 and 2.52% at 35°C, 5.49 and 2.71% at 43°C and 5.29 and 2.21% at 50°C, respectively. The main essential oil component was carvacrol and its levels were 67.57 and 60.88% at 35°C, 70.43 and 65.73% at 43°C and 68.79 and 69.31% at 50°C, respectively for *O.majorana* and *O.syriacum*. The other major components were *o*-cymene, γ-terpinene, borneol and α-terpinene in descending order found all drying temperatures in two plants. According to the results, 43°C was found as the most suitable drying temperatures in this study.

PP-136. Effects of drying techniques on essential oil compositions of *Origanum onites* and *Origanum vulgare*

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Turkey is an important gene center for the family *Lamiaceae*, which comprises annual or perennial plants, spread especially in the Mediterranean region. The genera *Thymus*, *Origanum*, *Satureja*, *Thymbra* and *Coridothymus* which are found in this family and called as generally "kekik" in Turkey, have great importance both economically as well as distribution areas. Besides pharmaceutical industry, medicinal and aromatic plants have been used in food industry as tea, spices and flavour, in the production of cosmetic products and cleansing agents. Due to the high water content, fresh spices can be easily spoiled via microbiological or biochemical reactions. The shelf life of spices can be extended with drying but this can cause loss of volatile oils depending on drying temperature and time. The composition, especially volatile oil content and quality, of medical and aromatic plants are changed with harvest and post-harvest treatments. In this study; effects of different drying techniques on the essential oil content and composition of *Origanum onites* (called as İzmir Kekiği) and *Origanum vulgare* (called as İstanbul Kekiği) were analyzed. Plants were harvested at their flowering periods in the second year after planting, dried in shade and in oven at 35°C, essential oils were obtained using hydro-distillation techniques and essential oil compositions were analyzed using GCMS-FID. The raw materials were cultivated at the institute field, nearly at 30 m altitude. The essential oils yields of *Origanum onites* and *Origanum vulgare* were calculated as 2.97 and 3.43% for dried in shade, 2.89 and 3.12% dried in oven, respectively. Carvacrol was the main component of essential oils and ratio of it was 45.14 and 53.96% for shade dried plant and 46.50 and 49.86% for oven dried *Origanum onites* and *Origanum vulgare* essential oils respectively. The other major components were similar for both plants essential oils and these were *p*-cymene, γ-terpinene, borneol, caryophyllene and αterpinene in descending order.

PP-137. Determination of essential oil components of some medicinal and aromatic plants produced in the Hatay Region

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The aim of this study was to assess the chemical composition of essential oils isolated by hydro-distillation from eleven plants *Mentha piperita* L. (nane), *Feoniculum vulgare* Mill. (rezene), *Melissa officinalis* L. (melisa), *Thymus sp*. L. (kekik), *Lavandula angustifolia* L. (lavanta), *Myrtus communis* L. (mersin), *Salvia lavandulifolia* Vahl. (adaçayı), *Ocimum basilicum* L. (fesleğen), *Rosemary officinalis* L. (biberiye), *Laurus nobilis* L. (defne) and *Eucalyptus globulus* L. (okaliptüs). Gas chromatography coupled with FID and MS of essential oil revealed pulegone(62.5%), *trans*-anethole(89.3%), limonene(63.3%), carvacrol(37.4%), linalool(34.7%), eucalyptol(50.6%), eucalyptol(52.1%), and eucalyptol(67.8%) to be major oils' components, respectively.

PP-138. Evaluation of Dittany of Greece, chemical analysis-therapeutic properties

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Dittany of Crete consists of the dried flowering herb of *Origanum dictamnus* L. (Lamiaceae). The plant is native and endemic to the island of Crete, where it grows wild. Various names have been attributed to the plant, among them dictamnos, probably derived from "Dicti" + "thamnos". "Dicti" is the name of Cretan mountain where Zeus was raised up by the goat Amalthia and "thamnos" means shrub in Greek [1].

Recently (July 2013 by Dr Chinou as rapporteur, EMA/HMPC/200429/2012) a monograph for Dittany was approved among EU countries [2], giving the opportunity, to register it as a traditional herbal medicinal product. In this monograph, qualitative and quantitative composition and pharmaceutical forms (infusion, decoction for oral, cutaneous use) are described.

In the framework of the re-evaluation of chemistry and therapeutic properties of Greek plants with potential commercial interest, we present the chemical analysis of the essential oil and water extracts (infusions) of three samples (organic and conventional cultivation from the Greek market).

The chemical composition of the essential oils from *Origanum dictamnus* was analysed by GC and GC–MS carvacrol, thymol, p-cymene together with γ-terpinene were identified as major constituents. The water extracts were produced according to the exact posology for medicinal use described in the monograph and they were chemically analyzed and their total polyphenols were determined for the first time [1]. The antimicrobial activity of the essential oil as well as the extracts of all samples were assayed against Gram negative and positive bacterial and against human and food pathogenic fungi, and were exhibited a very interesting biological profile with MICs' values from 0.10 to 1.10 mg/ml.

Acknowledgments

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PP-139. Chemical composition of the essential oils of eight aromatic-medicinal plants from Dzungarian Gobi from Outer Mongolia

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Chemical composition of the essential oils of eight aromatic-medicinal plants from Dzungarian Gobi from Outer Mongolia[1] has been investigated using GC-MS analysis.

An international literature review has revealed that the chemical composition of their essential oils have not been described in any previous study[2,3].

The hydro distilled essential oils obtained from dried plant materials were analyzed by GC-MS[4]. The results are summarized in table:

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Table: Aromatic-medicinal plants from Dzungarian Gobi from Outer Mongolia		
Species	**Collection site**	**Main components % of oil**
Brachanthemum mongolicum Krasch (Asteraceae, September)	Dzungarian Gobi. Uushgiin-Us (September)	camphor (34.94%), 1.8-cineol (20.55%), borneol (6.35%), camphene (3.53%), chrysanthenone (3.43%), p-cymol (2.18%), α-thujone (1.73%), cis-chrysanthenol (1.07%), chrysanthenon –epoxid (1.00%), bornyl acetate (0.94%)
Mentha arvensis L (Lamiaceae, July, September)	Dzungarian Gobi. Oasis of Bulgan (September)	menthone (30.2-42.0%), pulegone (30.5-41.3%), isomenthon (4.5-5.8%), limonene (4.8-5.0%), menthol (2.8-4.5%), piperitone (1.3-4.4%), methyl acetate (2.0-2.6%)
Hyssopus cuspidatus Boriss (Lamiaceae)	Dzungarian Gobi. Mountains, Khavtag-Ula	terpineol (20.0%), limonene (16.9%), (Z)-β-ocimene (12.17%), β-pinene (6.11%), linalool (6.0%), cis-carveol (5.88%), pinocarvon (3.18%), cis-pinocamphone (3.12%), sabinene (3.11%), pinocarveol (3.13%), myrtenol (3.12%), isopinocamphone (2.50%), (E)-β-ocimene (2.45%), 1.8-cineol (1.60%), myrtenal (1.60%), myrcene (1.10%)
Ziziphora pamiroalaica Juz (Lamiaceae, September)	Dzungarian Gobi. Baitag Bogd of mountains Range	pulegone (63.19%), menthone (14.40%), piperitone (6.05%), isopulegone (2.54%), caryophyllene oxide (1.69%)
Chenopodium botrys L (Chenopodiaceae, September)	Dzungarian Gobi. Mountains, Khavtag-Ula, Takhin-Shar Range	elemol (16.96%), β-eudesmol (14.81%), α-eudesmolyl acetate (5.87%), T-muurolol (5.11%), γ-eudesmol (5.01%), γ-cadinene (4.50%), globulol (1.72%), spathulenol (1.25%), guaiol (2.10%), α-eudesmol (1.24%), α-terpineol (1.19%)
Ferula ferulaeoides (Stend) Kor (Apiaceae, September)	Dzungarian Gobi. Ongonin Khudo of Oasis Bulgan	guaiol (58.76%), E-nerolidol (10.16%), β-farnesene (3.02%), limonene (2.85%), α-cadinol (2.07%), myristicine (1.74%), bornylacetate (1.51%), γ-terpinene (1.40%), β-bisabolene (1.37%)
Larix sibirica Ldb (Pinaceae, September)	Dzungarian Gobi. Khargait-gol of Baitag Bogd-Range	***In the oil of needles***: 3-δ-carene (18.4-30.9%), α-pinene (9.1-13.1%), β-pinene (5.4-10.3%), limonene (2.8-6.1%), terpinolene (2.3-3.8%), myrcene (1.0-2.6%), γ-terpinene (1.3%); ***In the oil of resin***: α-pinene (43.2%),3-δ-carene (19.0%), β-pinene (14.6%), myrcene (2.6%), p-cymene (1.42%), limonene (1.10%), pinocarveol (1.0%)
Glycyrrhiza uralensis Fisch (Leguminosae, September)	Dzungarian Gobi. Oasis Bulgan	palmitin acid (11.10%), terpinen-4-ol (13.81%), tetradecanol (10.74%), 1.10-decandiol (9.01%), γ-terpinene (4.32%), phenthulfuran (3.44%), α-terpinene (2.53), thymol (2.25%), phthilade (2.10%), myrcene (1.67%), (E,E)-2.4-decadienal (1.4%), isophytol (1.35%), 1.8-cineol (1.0%), nerol (0.70%)

PP-140. Antioxidant activity and chemical composition of essential oils *Mentha rotundifolia* from Algeria isolated by microwave and hydrodistillation

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The Essential oil of *Mentha rotundifolia* (L.) Huds growing wild from East Algeria (Naciria at 60 km in East of Algiers) obtained by hydrodistillation (HD) and a microwave distillation process (MD) have been analysed by means of GC-FID and GC/MS in combination with retention indices. In total, 54 constituents were identified (accounting for 96.7 and 95.6% in HD and MD oils, respectively).

The main components were piperitone oxide (25.1 and 29.1% in HD and MD oils, respectively), piperitenone oxide (8.9 – 11.8%), terpinen-4-ol (9.3 – 3.4%), β-caryophyllene (5.4 – 7.3%), allo-aromadendrene (5.3 - 6.4%) and D-germacrène (5.4 – 7.1%). In comparison with HD, MD allows to obtain oil in a very short time, with the reduction of solvent used similar yields, comparable qualities and substantial savings of energy.

The antioxidant activity was determined according to the ability of the tested samples to scavenge the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH*). The essential oil were slightly active (32.6 and 21.8% in HD and MD oils, respectively) comparing with BHT (64.7%).

PP-141. Chemical composition and antimicrobial activitiy of *Pinus pinea* from Algeria

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Pinus pinea L (Pinaceae), is known in the Tlemcen region under the vernacular name Zgougou. Pine oils are used as flavoring additives for food and beverages, fragrances in cosmetics and scenting agents in household products [1,2]. Chemical composition of essential oils extracted by hydrodistillation from aerial parts of *P. pinea* (needles, twigs and buds), growing in West Northern of Algeria has been investigated by GC/RI and GC/MS. 56 compounds, representing 94.8% of the total collective oil, were identified. Essential oil was dominated by hydrocarbon compounds. The major compounds from twenty oils stations were: limonene (44.4-73.8%), α-pinene (3.4-11.2%), trans-caryophylene (1.7-8.1%). The intra-species variations of the chemical compositions of *P. pinea* aerial parts essential oils from twenty Algerian sample locations were investigated using statistical analysis. Essential oils samples were clustered in 2 groups by hierarchical cluster analysis, according to their chemical composition. The oils were investigated for their antimicrobial activity against twelve bacteria and three fungi. A promising antimicrobial potential was observed especially against *Klebsiella pneumoniae* ATCC 700603, *Bacillus cereus* ATCC 10876 and *Candida albicans* ATCC 444. The results presented may suggest that *P. pinea* essential oils possess antimicrobial property, and therefore, can be used as natural preservative ingredients in food and/or pharmaceuticals.

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PP-142. Chemical composition of the essential oil of *Ferulago phialocarpa* Rech.f. & H. Riedl., an endemic medicinal plant from Iran

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The genus *Ferulago* belongs to the family Apiaceae (Umbelliferae) and comprises about 35 to 40 herbaceous species, which are widely distributed in the temperate zone of both hemispheres, especially in Central Asia and Mediterranean area. In this study, chemical composition of the essential oils of *Ferulago phialocarpa* Rech.f. & H. Riedl, an endemic medicinal plant from Iran, was analyzed by GC and GC-MS technique. Hydro-distillation of the inflorescence essential oil of *F. phialocarpa* resulted in an oil with 0.89% yield (*w/w*) on the base of the dry weight of plant materials. In total, twenty-nine components have been identified constituting about 96.1% of oil composition. The principal fraction of the essential oils was found to consist of monoterpene hydrocarbons (60.81%) with *α*-pinene (43.38%) as the major component. Oxygen-containing monoterpenes (9.07%) constituted another major fraction of the oil with *cis*-chrysanthenyl acetate (5.91%) as the main constituent.

PP-143. Chemical composition, antioxidant and antifungal activities of essential oil and hydrosol extract of *Mentha aquatica* from Algeria

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Chemical composition of the aerial part essential oils and hydrosol extract of North Algerian *Mentha aquatica* has been investigated, using GC/RI and GC-MS. Biological activity of the essential oil and hydrosol extract was assayed for their antioxidant and antifungal activities. GC and GC/MS analysis of *M. inodora* essential oil from 18 stations allowed the identification of 34 compounds accounting for 92.2 % of collective essential oil. The main components were α-terpinyl acetate (52.1 %), 1-oct-3-enyl acetate (5.7 %), α-terpineol (5.3 %), 7-dihydro-7-acetoxy-carvone (5.1 %) and carvone (4.6 %). To our knowledge this is the first report of α-terpinyl acetate as major compound in *M. aquatica* essential oils. Intra-species variations of the chemical compositions of essential oils from twenty-four Algerian sample locations were also investigated using statistical analysis. The antioxidant activity was evaluated as a free radical scavenging capacity (RSC). RSC was assessed measuring the scavenging activity of the essential oils on 2,2-diphenyl-1-picrylhydrazil (DPPH.) and (OH.) radicals. Essential oil exhibited very strong RSCs, reducing the DPPH radical formation (IC_{50}) of 40.90µg/mL and 2.95µg/mL respectively for essential oil and hydrosol extract. In addition, the essential oil presents good capacity to slow the rate of oxidation of linoleic acid and Beta-carotene with IC_{50} of 40.87µg/mL and 0.51µg/mL respectively for essential oil and hydrosol extract. In the other hand, essential oil and hydrosol extract showed strong in vitro antifungal activity based on the inhibition zone and minimal inhibitory concentration values against the pathogen (*Aspergillus niger* and *Penicillium italicum*). *Citrus sinensis* fruits infected by *Penicillium italicum* pathogen were treated in vivo with hydrosol water. Very low concentrations were needed for the absence of orange infection and very low disease incidence.

PP-144. *Penicillium italicum* control and antifungal activity of essential oil and hydrosol extract of *Thymus capitatus* L. against four fungal pathogens of *Citrus sinensis* L.

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Essential oil and hydrosol extract of *Thymus capitatus* L. from Algeria were tested for antifungal activity against four phytopathogenic fungi causing the deterioration of *Citrus sinensis* fruits. Chemical composition of the aerial part essential oils and hydrosol extract of North Algerian *Thymus capitatus* has been investigated using GC/RI and GC-MS. Essential oil was dominated by carvacrol (69.6 %), *p*-cymene (12.4 %) and γ-Terpinene (4.3 %). Hydrosol extract was dominated by carvacrol (95.1 %). Essential oil and hydrosol extract showed strong *in vitro* antifungal activity based on the inhibition zone and minimal inhibitory concentration values against the pathogen (*Aspergillus niger*, *Aspergillus oryza*, *Penicillium italicum* and *Fusarium solani*). *Citrus sinensis* fruits infected by *Penicillium italicum* pathogen were treated in vivo with essential oil, hydrosol extract and hydrosol. Very low concentrations were needed for the absence of orange infection and very low disease incidence. This activity can be correlated with chemical composition of essential oil, hydrosol extract and hydrosol which are rich in carvacrol component (More than 69%). Essential oil and hydrosol of *Thymus capitatus* could be used for management of this pathogen as alternative source of ecological fungicides or an ideal alternative to control *P. italicum* infection during oranges storage.

PP-145. Chemical variability and biological activities of essential oils of *Micromeria inodora* (Desf.) Benth. from Algeria

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Chemical composition of the aerial part essential oils of North Algerian *Micromeria inodora* (Desf.) Benth. has been investigated, from the first time, using GC/RI and GC-MS. Biological activity of the essential oil was established against fourteen species of micro-organisms involved in nosocomial infections using paper disc diffusion and dilution agar assays. GC and GC/MS analysis of *M. inodora* essential oil allowed the identification of 86 compounds accounting for 95.8 % of collective essential oil. The main components were *(E)*-sesquisabinene hydrate (20.9 %), α-terpinyl acetate (19.8 %), globulol (4.9 %), caryophyllene oxide (4.3 %), β-bisabolol (2.9 %) and *(E)*-7-epi-sesquisabinene hydrate (2.6 %). Intra-species variations of the chemical compositions of essential oils from twenty-four Algerian sample locations were also investigated using statistical analysis. The samples were separated in 2 groups by hierarchical cluster analysis, according to the composition of the corresponding essential oils. The chemical variability could be attributed to growing conditions and environmental factors. The study of the antimicrobial activity of Algerian essential oil demonstrated a good activity especially against Gram-positive strains such as *Staphylococcus aureus, Bacillus cereus*, *Bacillus subtilis* and *Enterococcus faecalis,* and moderate activity against yeasts (*Candida albicans*). Those results chose that *M. inodora* essential oil can be considerate as promising source of natural products with potential against various nosocomial community and toxic infections.

PP-146. Development of self-emulsifying drug delivery systems of *Alpinia galanga* essential oil: Effect of surfactant and co-surfactant

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Using natural materials instead of chemical substances for the use in human and animals for safety aspects has increased. This study is our main part of the development of the self-emulsifying drug delivery systems (SEDDS) of plant essential oils using for aquatic animal behavior modification.

The aim of the present work was to investigate the effect of surfactant and cosurfactant on formation and stability of the SEDDS of *Alpinia galanga* Willd. essential oil.

The oil was obtained by hydrodistillation of fresh rhizome of *A. galanga*. The obtained oil was firstly investigated using GC-MS. Pseudoternary phase diagrams of the oil were substantially constructed using a water titration method. Tween20, Tween80, or TritonX-100 was used as a surfactant whereas ethanol, iso-propanol, or propylene glycol was used as a co-surfactant. The stability test of the selected SEDDS of *A. galanga* oil was done at 45°C for 30 days.

Was found that 1,8-cineole and 4-allylphenyl acetate are the main components of *A. galanga* oil. Results demonstrated that TritonX-100 in combination with ethanol or iso-propanol yielded the largest monophasic area in the phase diagrams. The SEDDS composed of 20% oil and 80% surfactant mixture of TritonX-100 and ethanol or isopropanol at a ratio of 2:1, 2:0.6, or 2:0.4 were selected for the stability test and the results showed that the aqueous system composed of TritonX and isopropanol in a ratio of 2:1 was the most stable with a significantly smaller particles size than others ($p<0.05$).

It was concluded that the type of a surfactant and a cosurfactant as well as their composition ratio played an important role on the formation and stability of the SEDDS of *A. galanga* essential oil.

PP-147. Do analytical parameters affect the GC-MS separation and quantification of compounds of essential oils – case of *Hypericum hirsutum*?

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Essential oils isolated from different *Hypericum* species are widely analyzed, regarding to the healing properties of the St. John's wort. However, at least 10 GC-MS methods are described in the literature, with different analytical parameters. Also, wide range of other methods is described for analysis of essential oils applied for research of aromatic plants which are not applied for *Hypericum* representatives. Thus, the aim of the study was to compare five methods randomly selected from the literature with purpose to evaluate whether analytical parameters affect separation and quantification of compounds present in the essential oil of *Hypericum hirsutum* L., Hypericaceae. The run time of 5 analytical methods was in range from 35.0 to 73.3 minutes. In all applied methods wide range of differences in analytical parameters (initial temperature, flow rate, use of ramps, injection and detector temperature, split/splitless mode, pulsed split mode…) were used. The identification of compounds was based on the mass spectra which were compared with the NIST/NBS Wiley library of mass spectra, as well as with literature data. Even in the separation and quantification of major compounds present in the essential oil notable variations were observed. Caryophyllene oxide ranged from 13.82 to 20.42%, τ-muurolol from not detected to 8.27%, spathulenol 4.57 – 6.13%, cadalene 3.36-5.69%, aromadendrene 3.98 – 7.11%, longifolene from not detected to 4.02%, allo-aromadendrene 1.58 – 3.58% and α-pachoulene from 0.55 to 5.12%. Furthermore, some of compounds present in the percentage lower than 1 or 2% were not detected in all aplied methods (2-undecanol, α-costol, epizonarene, α-humulene, β-oplopenone, valerenal).

PP-148. Determination of essential oil components in *Melissa officinalis* L. under in vitro conditions

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Lemon balm (*Melissa officinalis* L.) of the Lamiaceae family is a perennial herbaceous having both medicinal and aromatic importance. This present study aimed to determine the compounds from essential oils of the plant using *in vitro* induced calli and micropropagated shoots. The essential oil was isolated by microdistillation of this economically important plant from seeds obtained from Germany. The main components were determined by GC/FID and GC/MS from callus as geranial (43.4 %), neral (26.8 %), alloaromadendrene (8.5 %), geranyl acetate (7.4 %), 6-methyl-5-hepten-2-one (3.5 %), β-caryophyllene (3.1 %), respectively. The main compounds from herbage were geranial (44.8 %), neral (30.3 %), geranyl acetate (5.7 %), 6-methyl-5-hepten-2-one (3.5 %), β-caryophyllene (2.3 %), alloaromadendrene (1.6 %), respectively. The results obtained are in correlation with the previous studies.

PP-149. Volatiles of *Trinia glauca* L. Dumort. (Apiaceae)

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Trinia Hoffm., a genus of the plant family Apiaceae (Umbelliferae), comprises around ten species, distributed mostly in Europe. *Trinia glauca* (L.) Dumort. (syn. *T. vulgaris* DC. and *T. stankovii* Schischk.), commonly known as honewort, is widespread in western, central and southern Europe and south-west Asia. Ants are believed to be important pollinators of *T. glauca* [1]. We noticed that *T. glauca*, wild-growing on the slope of mountain Suva planina (south-eastern Serbia), emitted a characteristic amine odor and that it was frequently visited by ants. That motivated us to analyze the volatiles of that particular plant population and possibly identify compounds that attract ants to *T. glauca*. The volatiles of this plant species have been the subject of only two previous studies that identified 84 constituents in total [2,3]. Herein, we report the results of a detailed analysis of the aerial parts essential oil of *T. glauca*. GC and GC/MS analysis of the hydrodistilled essential oil allowed the identification of 220 constituents, representing 90% of the detected oil constituents. The main oil components were (*Z*)-falcarinol (10.6%), bicyclogermacrene (8.0%), germacrene D (7.4%), δ-cadinene (4.3%), β-caryophyllene (3.2%), tricosane (3.0%), germacrene B (2.9%) and γ-muurolene (2.9%). It is interesting to note that the composition of *T. glauca* from Turkey [2] was very similar to our oil chemical profile in all respects but the major constituent-(*Z*)-falcarinol in our case and germacrene D with 19.6% in the Turkish oil. (*Z*)-Falcarinol is believed to protect the roots of carrots (*Daucus carota*, another Apiaceae taxon) from fungal diseases [4]. A number of minor constituents (pyrazine derivatives, aliphatic esters of short-chain alcohols, etc.) identified in the oil were previously demonstrated to be ant-related substances (pheromones involved in foraging and food gathering) [5].

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PP-150. Volatile secondary metabolites of *Conocephalum conicum* (L.) Dum. from Serbia

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Conocephalum conicum (L.) Dum., Conocephalaceae, or Scented Liverwort, is one of the most common of the thallose (leaf-lacking) liverworts [1]. It is very common in Asia, North America, North Africa and Europe [2]. *Conocephalum conicum* is rich with essential oil and has a distinctive smell when crushed [2]. Composition of *C. conicum* essential oil has been studied many times [3]. Monoterpene hydrocarbons and oxygenated monoterpene derivatives have been found as essential-oil constituents, together with sesquiterpene hydrocarbons, alcohols and lactones. Many of these compounds, like pinguisane-type sesquiterpenoids or sacculatane-type diterpenoids, have not been found in any other plants, fungi or marine organisms and represent significant chemotaxonomic markers [4]. Up to now, several different chemotypes of this liverwort were identified [1,3]. They were differentiated by their flavonoid constituents [5] and by some characteristic volatile components [1,3]. However, according to a thorough literature search, data on the chemical composition of the essential oil of a Serbian population of *C. conicum* does not exist to date. For this reason, detailed chemical analyses of the volatile secondary metabolites of two populations of *C. conicum* from Serbia (localities near Niš and Vlasina lake) were conducted in order to provide an insight into the chemistry of this species in Serbia. Analyses (by GC and GC-MS) of the two essential oils hydrodistilled from dry liverwort material enabled the identification of 168 constituents, representing 98.1 and 99.2% of the total oils. The most abundant constituents of the oils were 1-octen-3-ol, aristola-1(10),8-diene and conocephalenol. These first results suggested that the oils from Serbian populations differ significantly from those previously reported [1-4]. This was additionally corroborated by an agglomerative hierarchical cluster analysis (AHC) and principal component analysis (PCA). This allowed us to assess the chemotaxonomical usefulness of such chemical data in differentiating *C. conicum* populations and to corroborate the existence of a new chemotype from Serbia.

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PP-151. Polypharmacological properties of *Choisya ternata* Kunth essential oil constituents. Esters of N-methyl anthranilic acid - a new panacea?

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Methyl- (MA) and isopropyl- (IA) esters of *N*-methyl anthranilic acid, found in *Choisya ternata* Kunth (Rutaceae) essential oil, were previously confirmed to have a wide range of useful and at first glance different pharmacological properties [1]. Almost all until now proven activities of MA and IA can be connected to animal awakens [1]. This polypharmacology (i.e. panacea-like properties) could be, at least partially, rationalized as the result of the possible interaction of these compounds with histaminergic neurons in the central nervous system, the major waking centre of the brain. However, the potential involvement of histamine receptors (H1-H4) in the pharmacological activity of MA/IA has not been directly studied previously.

Possible MA/IA interactions with H1 receptor complex were investigated *in vivo* (3 months old female Wistar rats), using a standard model of dye extravasation (Evans blue). The levels of histamine-induced dye extravasation in the absence, and under the influence of standard receptor antagonists and MA and IA were measured and compared. The obtained results indicated that, similar to the standard drugs and in a wide dose range, both MA and IA produce a dose dependent decrease in dye extravasation when compared to the control ($p<0.0001$). As H1 receptors are predominantly involved in vasodilatation in the presence of histamine, the results of *in vivo* experiments suggested that the studied anthranilates might be H1 antagonists. In order to further provide proof of the histaminergic action of the two anthranilates (more precisely the interaction with the H1 histamine receptor), a set of *in silico* experiments were performed: standard drugs (H1 antagonists (*E*/*Z*)-doxepine and chloropyramine), MA and IA (MM+ optimized geometries) were docked into the histamine H1 receptor complex using AutoDock Vina 1.1.2 software [2]. Although the whole span of the H1 receptor was searched (blind docking), the obtained results strongly suggested that IA and MA bind to the doxepine/chloropyramine pocket (two energetically comparable orientations were found) and interact with Trp428A, Phe432A, Ser111A, Tyr108A, Phe199A amino acid residues (calculated binding energies were comparable to that of the chloropyramine best docking pose). Also, the possible interaction of gastroprotective MA/IA with H2 receptors should not be overlooked (H2 receptors are involved in both gastric acid secretion and inflammatory response).

These experiments provide a link between the different pharmacological properties demonstrated previously [1] for the two essential oil constituents (MA and IA) and point to a histaminergic pathway as their key mechanism of action.

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PP-152. Volatile compounds of *Coffea arabica* L. green and roasted beans

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Coffee is one of the most popular beverages around the world, constituting a significant portion of daily beverage intake in many Western countries. With the advancement of the research on antioxidants and health, coffee has been recognized as a rich source of dietary antioxidants with potential to improve health (1). The pleasant aromas released during the grinding of roasted coffee beans are as attractive as the aromas of fresh brewed coffee (2). Solid-phase microextraction (SPME) offers an alternative sampling technique for the analysis of volatile organics. This method is based on the adsorption of analytes on a polymeric stationery phase deposited on a fused-silica fiber via a partitioning effect between the adsorbent and the sample matrix. The adsorption is provided by immersing the precoated fiber in a liquid sample or exposing it to the headspace above a liquid or solid sample (3). It was investigated along with this study, volatile contents of green and roasted beans of *Coffea arabica* L. (Rubiaceae). Volatile constituents were trapped with headspace solid-phase microextraction (HS-SPME) technique and analyzed by GC-MS. The main components for green coffee were identified as isoamylalcohol (10.4%), hexanal (10.4%) and hexocosane (8.2%), while furfurylalcohol (13.6%), furfurylacetate (10.7%) and 5-methyl furfural (9.27%) were identified as the main components of the roasted coffee. In conclusion, using HS-SPME-GC-MS, we were able to quantify different volatile compounds belonging to different chemical classes as alcohol, alkane and aldehyde derivatives in green coffee beans; furan, pyrazine, pyrrole and pyridine derivatives in roasted coffee beans.

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PP-153. Malaysian aromatic plants as herbal, health care and personal care ingredients

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Malaysia is one of the 12 mega diversity country which is richly endowed with high tropical forest diversity. More than 2000 species of plants in the Malaysian forest have been reported to have therapeutic value and some of these belong to the aromatic families such as Annonaceae, Lauraceae, Myrtaceae, Rutaceae, Thymelaceae and Zingiberaceae. Long recognized for its healing, fragrance, nutritional and therapeutic properties, the essential oils have been used in traditional medicinal preparations and today, the herbal industries are including herbs and essential oils into their formulations. To date, FRIM have a collection of more than 120 species which were deeply investigated for its potential. Knowledge on the chemical and biological activities of essential oils have enhanced the importance of aromatic plants as useful sources of aroma chemicals with the potential to be incorporated into cosmetics, fragrances, health care and personal care products. The chemical constituents, bioactivity profile, formulation and commercialisation of some selected essential oils will be discussed in this paper.

PP-154. Essential oil composition and antibacterial activity of the lemon grass (*Cymbopogon citratus*): comparison of solvent free microwave extraction (SFME) with hydrodistillation (HD)

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Solvent-free microwave extraction (SFME) for the isolation of essential oil from lemon grass (*Cymbopogon citratus)* has been compared with the conventional hydro-distillation (HD) in terms of yield, composition and antibacterial activity. The yield of the essential oil obtained from 30 min of SFME was found to be similar to that of HD for 180 min. The compounds of the essential oil extracted by SFME and HD were determined as gerenial (49.4%- 51.4 %), neral (30.6%, 29.4%), β-myrecene (5.7%, 5.8%), geraniol (2.3%, 2.3%), geranic acid (1.3%, 1.2 %), and 6-methyl-5-hepten-2-one (0.43%, 1.06 %) respectively, by GC-MS analysis.

The standard disc diffusion method recommended by CLSI (1) was used to determine the antibacterial properties of EOs. The results given at Table 1 showed that the EO of lemon grass had a wide spectrum of antibacterial activities against two Gram-positive and four Gram-negative bacterial species used in this study.

Bacterial species	HD (mm)	SFME (mm)	Antibiotic control (mm)
S. aureus ATCC 25923	36	35	38 (P)
E. faecalis ATCC 29212	25	36	24 (VA)
E. coli ATCC 25922	18	21	22 (AMC)
K. pneumoniae ATCC 13883	15	21	18 (CAZ)
S. marcescens ATCC 8100	18	34	32 (MEM)
P. aeruginosa ATCC 27853	11	18	33 (MEM)

Table 1. Antimicrobial activity of *C.citratus* EOs against the bacterial strains tested based on the disc diffusion method (P, Penicillin G (10 units); VA, Vancomycin (30 mg); AMC, Amoxycillin/clavulanic acid 2: 1 (30 mg); CAZ, Ceftazidime (30 mg); MEM, Meropenem (10 mg)).

SFME seems to be a more advantageous extraction technique for the isolation of essential oil from lemon grass compared to HD, due to reduced extraction times, higher antibacterial activity and using no solvent (2).

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PP-155. Antibacterial evaluation of *Mentha piperita* L., *Lavandula angustifolia* L. and *Eucalyptus globulus* Labill. essential oil combinations against *Streptococcus* sp.

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Streptococcus is round-shaped Gram positive bacterium that usually grows in the upper respiratory tract, on mouth and skin which may cause serious diseases. Additionally in recent years *Streptococcus* sp. have also developed resistance towards antibiotics such as penicillin, erythromycin and amoxicillin, which has become serious problem.

Concurrently, natural antimicrobial substances obtained from different sources have withdrawn attention and significant revival. Essential oils have been used for a long time due to the antimicrobial properties and have great potential as antibiotic alternatives. Also it is well known that antimicrobial activity can be enhanced using essential oil combinations resulting in synergy.

In the frame of this study, we aimed to evaluate the synergistic antibacterial effect of different concentrations of *M. piperita*, *L. angustifolia* and *E. globulus* essential oil combination against pathogenic *Streptococci* which cause serious diseases in humans. The essential oils were obtained from commercial sources. Phytochemical components of the essential oils have been analyzed by GC-FID and GC-MS techniques, simultaneously. Essential oil combinations were prepared such as 1:1 (v:v) ratio. *In vitro* antibacterial activity of essential oils were carried out by microdilution method against *Streptococcus pyogenes, S. pneumoniae, S. mitis, S. mutans* and *S. sanguinis* standard strains. Resulting antibacterial effects were evaluated for fractional inhibitory concentrations (FICs) antagonist, ineffective, additive and synergistic effects.

According to the GC-FID and GC-MS analysis results of *M. piperita*, *L. angustifolia* and *E. globulus* essential oils major components were determined as menthol (42.8 %), linalool (38.8 %) and 1,8-cineole (80.2 %), respectively. As a result of the antibacterial activity experiments, essential oil combinations have been identified as “additive effective combinations” when compared to the standard antibacterial agents with MIC values of (0,002-4 mg/ml) FICs (1-4), suggesting that essential combinations are a good way of enhancing antimicrobial activity.

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PP-156. Effects of drying and extraction methods on the yield and composition of wild *Hyptis suaveolens* essential oil and their antimicrobial activities.

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Hyptis suaveolens (L.) Poit. (Lamiaceae) is a fast growing perennial herb often found in dense clump along roadsides in Thailand. The plants give out strong characteristic smell especially in the hot weather. Thai folks used to grow the plants around the poultry cages to keep the chicken away from the flus. Thai folk healers used the plant juice to wipe the child tongue under certain ailments. This plant has wide distribution in many tropical countries with many traditional uses such as jaundice, abscesses, hemorrhoids, anal oral candidiasis. The leaves are used as skin disinfectant and as a carminative, the seeds are used for gastrointestinal disorder etc. There are variation in the composition of the essential oil which may be related to the biological effects. At least four types of H. *suaveolens* can be summarized from earlier works according to its major constituent i.e. sabinene, β-caryophyllene, 1,8-cineol, and germacrene-D. From seven studies on the compositions of H. *suaveolens* essential oil from different locations, 3 out of 7 were sabinene dominant type, 2 studies reported β-caryophyllene dominant type, 1,8-cineol and germacrene-D were also reported as the major component in certain locations. This work reports for the first time abietatriene dominant H. suaveolens from the north-eastern part of Thailand in contrast to earlier work from the northern part of Thailand which reported sabinene as the major component. The essential oil was prepared by hydrodistillation from the fresh plant material (F, 0.02 % v/w fresh wt) and from the dried plant materials (D) i.e. drying under the sun (D1, 0.04 % v/w dry wt) and oven dried (D2, 0.06 % v/w dry wt). The components of the oils were recorded in a GC-MS and showed different major peaks of F and D samples. Antimicrobial activities were also tested against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Candida albicans.* Various extracts were also prepared from the F and D samples using soxhlet extraction apparatus yielding hexane extract, dichloromethane extract and methanol extract. Comparison of antimicrobial activities were made and it was found that the activity of the essential oil obtained by hydrodistillation of the aerial parts was better than those obtained from either leaves or stem. The distilled oil gave antimicrobial activities better than all extracts, but among the extracts; hexane extract was more active than the rest. Drying at 50o C in an oven gave better yield and higher activity of essential oil than the fresh material or drying under the sun. The increased activity could be due to the increase in the amount of abietatriene and caryophyllene-oxide in the oven dried sample. The high content of diterpenes suggest the use in aromatherapy as a refreshing base note that can be used to fix and add depth to perfumes.

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PP-157. Determination of essential oil components and antibacterial activity of *Laurus nobilis* leaves collected every month in winter

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Laurel (*Laurus nobilis*. L). is a very important medicinal and aromatic plant, which belongs to the *Lauraceae* family .The essential oil of the leaves has antibacterial and antimicrobial properties. The aim of the study is determining the variation in essential oil components month by month in winter conditions. In this study, *Laurus nobilis* leaves were collected from collection garden situated in Aksu campus of Antalya Bati Akdeniz Agricultural Research Institute. The plants were dried in shade and then essential oil rate, essential oil components and antibacterial activity analyses were done in every month of November-March period. Essential oil rates of leaves were determined as 3,5%, 3,03%, 2,5%, 3,5% and 2,87%, respectively. The main essential oil components were found as 1,8-cineole, α-terpinyl acetate, sabinene, α-pinene and β-pinene. The highest 1,8-cineole rate were determined as 57,18% in February, the lowest rate were determined as 54,23% in January. The highest α-terpinyl acetate rate were determined as 10,42% in February, the lowest rate were determined as 8,38% in December. The highest sabinene rate were determined as 10,40% in March, the lowest rate were determined as 8,89% in January. For winter season, it was seen that the February month has the highest essential oil ratio and 1,8-cineole percentage.

PP-158. Biological activities and composition of the *Crithmum maritimum* L. essential oil

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Crithmum maritimum L. (Apiaceae) is an edible plant that is consumed in salads or as pickles in Cyprus. In the rocky coasts of Cyprus this plant grows in very harsh conditions at the sea level in the rock cracks. In our screening study for a safe, nature friendly insecticide alternatives that could be used in agriculture we have studied the insecticidal activity (contact toxicity) of the essential oil of *C. maritimum* against *Spodoptera exigua* (Hübner) (Lepidoptera; Noctuidae) larvae at different development stages (3rd, 4th and 5th instar larvae). Hydro-distillation of 100 g *Crithmum maritimum* aerial parts afforded 0.22 mL essential oil. According to the GC, GC-MS analysis results of the essential oil it was composed of γ-terpinene 39.3%, β-phellandrene 22.6%, carvacrol methylether 10.5% and (Z)-β-ocimene 8.2%. In our activity studies 100 µL/mL essential oil was used with 1,2 and 4 µL/larvae doses for 3rd, 4th and 5th instar S. exigua larvaes respectively. The toxicity of the oil against the S. exigua larvaes were evaluated after 24 hours. *C. maritimum* essential oil afforded 89% mortality against the 3rd instar larvae however the mortality was decreased to 50% in the 4th and none in the 5th instar S. exigua larvae. The essential oil was also evaluated for AChE and BChE inhibition. The essential oil of *Crithmum maritimum* afforded a promising insecticidal activity that prompted us to investigate the active principles of the oil and activity of the oil against other pests which is the subject of our ongoing research.

PP-159. Composition and antibacterial activity of *Euphrasia rostkoviana* Hayne essential oil

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Eyebright, *Euphrasia rostkoviana* Hayne (Orobanchaceae), is a medicinal herb traditionally used in Europe for centuries. Decoctions of flowering aerial part are used against dry cough or purulent skin lesion and especially as eyewash to treat conjunctivitis and blepharitis. The herb has been reported to contain essential oil, however, the composition and bioactivity of the oil is unknown. Therefore, in this study, we investigated the chemical composition and antibacterial activity of the eyebright essential oil against *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, S. epidermidis* and *Pseudomonas aeruginosa*.

The oil was extracted by hydro-distillation using Clevenger-type apparatus yielding 0.02% (w/v) of yellowish-brown oil. GC-MS analysis revealed more than 60 constituents, with n-hexadecanoic acid (18.47 %) being the most abundant component followed by thymol (7.97), myristic acid (4.71), linalool (4.65), anethole (4.09), linolenic acid (3.81), hexahydrofarnesyl acetone (3.16), lauric acid (2.79), α-terpineol (2.39) and borneol (2.39). Despite the recent report on antimicrobial activity of *E. rostkoviana* extracts (1), our antimicrobial assay, performed by broth microdilution method, did not show inhibitory activity against any of the bacterial species tested at the highest concentrations tested (512 µg/mL).

According to our best knowledge, this is the first report on the composition of *E. rostkoviana* essential oil.

Acknowledgments

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PP-160. Biologically active *trans*-sabinol esters from the essential oil of *Achillea falcata* L. (Asteraceae)

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Achillea falcata L. (syn. *A. damascena*) is an endemic Mediterranean taxon from the Compositae plant family used in the traditional medicine of many nations [1]. The composition of the essential oil [1-3], as well as the content of flavones [4] and alkamides [5] of *A. falcata* was investigated to date. GC-MS analyses of the essential oil of *A. falcata* from Syria in our hands revealed the presence of *trans*-sabinol and *trans*-sabinyl acetate amounting to 30.5% and 10.7% of the aerial parts' and roots' essential oil, respectively. The composition of this oil differed significantly from the ones in the three previously published reports [1-3]. This motivated us to perform a more detailed compositional study which led to the identification of a homologous series of rare and, in some instances, new natural esters of *trans*-sabinol (formate and tiglate). The identity of these esters was confirmed by synthesis starting from *trans*-sabinol, isolated by chromatography from the same oil. All esters were fully spectrally characterized (MS, IR, UV–Vis, 1D and 2D NMR). To assess the safety and potential beneficial pharmacological activity of the major constituents of *A. falcata* essential oil (*trans*-sabinol and its acetate), as well as of the two new *trans*-sabinyl esters and one more (senecioate), these compounds were subjected to a number biological/ pharmacological assays. The acute toxicity was evaluated using brine shrimps (*Artemia* sp.) and *A. falcata* constituents showed low to moderate toxicity. Additionally, the esters were tested for acetylcholinesterase inhibitory activity. A number of esters showed a relatively high percent of inhibition (ranging from 20 up to 40%) in the tested concentration range. The synthesized *trans*-sabinyl esters, as well as *trans*-sabinol, demonstrated a dose-dependent antinociceptive activity in the hot plate, tail immersion and acetylcholine-induced writhings tests suggesting their influence on both the peripheral and central nervous systems. The peak of the antinociceptive effect of the tested substances was between 15 and 30 minutes after the application. The short time course of action of *trans*-sabinol and its esters indicated that they could be rapidly metabolized. However, it is possible that the observed antinociceptive activity could be secondary to a sedative and/or ataxic action of the applied substances.

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PP-161. Composition of the essential oil of *Centaurea behen* L.

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Centaurea genus is represented with 192 taxa in Turkey, 114 of which are endemic. It is known as "peygamber çiçeği, zerdali dikeni, coban kaldiran, timur dikeni" in Turkey. Many species of the genus *Centaurea* have traditionally been used for their antirheumatic, diuretic, choleretic, stomachic, astringent, cytotoxic, antibacterial, antipyretic and tonic properties. The essential oil composition of some *Centaurea* species from Turkey have been investigated. Generally, germacrene D, hexadecanoic acid,caryophyllene and caryophyllene oxide were reported to be the major volatile components in the earlier studies.

In this present study, the essential oil of *Centaurea behen* L. collected Elazığ, Turkey has been analyzed by gas chromatography (GC) and GC/mass spectrometry (GC/MS) techniques. A total of 26 components were identified in the essential oil. The main constituents of the oil have been revealed as follows: hexadecanoic acid (32.7%), germacrene D (14.8%), and phytol (12.3%).

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PP-162. Antifungal activity and potential use of essential oils against *Fusarium culmorum and Fusarium verticillioides*

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Essential oils of bay leaf, cinnamon, clove and oregano and oregano were tested *in vitro* and oregano essential oil *in vivo*, against two foodborne fungi belonging to the dominant mycobiota of stored rice, *Fusarium culmorum* and *Fusarium verticillioides*, collected from the Albufera rice-producing Mediterranean area near Valencia (Spain).

Chemical composition was identified by gas chromatography-mass spectrometry. Essential oils presented a high percentage of oxygenated components: 78.8% in bay leaf (eucalyptol 51%); 90.3% in clove (eugenol 89.8%); 92% in cinnamon (eugenol 60% and eugenyl acetate 18.3%); 71.8% in oregano (carvacrol 49.6% and thymol 21.2%). Monoterpenes and sesquiterpenes were: 18% in bay leaf, 9% in clove, 5% in cinnamon, 25% in oregano. This research showed that essential oils have a great potential to control both fungal pathogens. In the *in vitro* test, the essential oils of cinnamon, clove and oregano reduced fungal growth by 90% and almost 100%, being oregano the most effective essential oil to inhibit fungal growth. The effect of the oregano essential oil on fungal development in inoculated rice grains was tested *in vivo* by exposing inoculated and control rice grains (kernels) to essential oil vapour. The obtained results demonstrated its effectiveness in rice grain conservation. The essential oils of oregano, clove and cinnamon provide an alternative for controlling *Fusarium culmorum* and *Fusarium verticillioides* in rice, cereals and stored products.

PP-163. Identification and biological activity of allylmethoxyphenyl esters from *Anthemis segetalis* Ten. (Asteraceae) essential oil

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The available phytochemical data on *Anthemis segetalis* Ten (syn. *A. brachycentros* J. Gay ex W.D.J. Koch, *Cota brachycentros* J. Gay, *C. segetalis* (Ten.) Holub), a wild-growing taxon with a South-East European natural distribution (Italy and the Balkan Peninsula), is scarce [1]. Previous chemical analyses (GC and GC–MS) of *A. segetalis* essential oil–a complex mixture of more than 150 different compounds-revealed the presence of three structurally related constituents, most probably esters of allylmethoxyphenol and isomeric pentanoic and 2-pentenoic acids [1]. There were no previous records on the occurrence of such esters as constituents of any species belonging to the genus *Anthemis*. To determine the exact structure of these tentatively identified minor essential-oil constituents, we have synthesized a small combinatorial library of 54 regioisomeric allylmethoxyphenyl pentanoates and 2-pentenoates (49 completely new compounds). GC–MS in combination with 1D- and 2D-NMR analyses of the library compounds provided unambiguous data that led to a straightforward identification of the mentioned *A. segetalis* constituents as eugenyl angelate, 2-methylbutanoate and 3-methylbutanoate (0.21, 0.22, and 0.13 mg/100 g of fresh plant material, respectively).

Anthemis segetalis can easily be mistaken, by (inexperienced) herb collectors, for either *Matricaria chamomilla* L. (German chamomile) or *Anthemis nobilis L.* (syn. *Chamaemelum nobile* (L.) All; Roman chamomile); these two taxa are among the oldest, still widely used and well documented medicinal plants of worldwide recognition. Moreover, all taxa having a morphological similarity with either Roman or German chamomile are recognized as equally possessing (only) beneficial properties. For this reason, we decided to assess the safety and potential beneficial pharmacological uses of eugenyl angelate, 2-methylbutanoate and 3-methylbutanoate (new *A. segetalis* essential-oil constituents) and several other library compounds (these were tested to provide relevant data for a SAR (structure–activity relationship) analysis). We have studied the effect of these compounds in several models of toxicity (acute toxicity against *Artemia salina*, cytotoxicity against two cell lines (fibroblast and melanoma)), as well as their acetylcholinesterase inhibitory and antibacterial activities. *Anthemis segetalis* constituents showed low-to-moderate activity in all tests. The obtained results suggest that the intake of these compounds in naturally available amounts, on their own, would probably not represent a risk to human health but the possible adverse interactions with the plant matrix should not be neglected.

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PP-164. Chemical analysis of *Scandix balansae* Reut. ex Boiss. (tribe Scandiceae) fruit essential oil and a "low-level" chemotaxonomic analysis of the plant family Apiaceae

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Scandix balansae Reut. Ex Boiss. (Scandiceae, Apiaceae) is an endemic plant species found on the slopes of the Caucasus Mountains in north Turkey [1]. To the best of our knowledge, there are no previous studies on either the volatile or nonvolatile secondary metabolites of this species. Analyses by GC and GC/MS of an essential-oil sample obtained from dry fruits of *S. balansae* allowed the identification of 81 components, comprising 91.4% of the total oil composition. Interestingly, the major identified volatile compounds were medium-chain-length *n*-alkanes, i.e., tridecane (6.7%), pentadecane (13.4%), and heptadecane (19.3%), and a long-chain homolog nonacosane (7.6%). A number of minor oil constituents, among them tetradecyl 3-methylbutanoate, and octadecyl 2-methylpropanoate, 3-methylbutanoate, and pentanoate, turned out to have a restricted natural occurrence not only in umbellifers but also in the Plant Kingdom, whereas the last ester is a new natural compound in general. The identity of these rare plant constituents that present excellent chemotaxonomic marker candidates for *Scandix* species was unambiguously confirmed by coinjection of the oil sample with appropriate standards, which were synthesized for this purpose and fully characterized (^{1}H- and ^{13}C-NMR, IR, MS).

Despite its long taxonomic history, the family Apiaceae Lindl. (Umbelliferae Juss.) still awaits a modern classification. Therefore, we decided to explore the possible applicability of the essential oils' compositional data in the taxonomy of Apiaceae. The multivariate statistical analysis (MVA) of chemical-composition data of volatile secondary metabolites is a powerful tool used on many occasions to resolve taxonomical uncertainties on different levels. The tribe Scandiceae Spreng. (subfamily Apioideae Seem) is an excellent candidate for the "low-level" taxonomical analysis (focused on selected genera and tribes of a plant family, with the goal to provide more details and to give a deeper insight into the differentiation of these plants [2]) of Apiaceae–diversity with respect to ecology, floral and fruit morphology and anatomy, and umbel structure [2]–and genus *Scandix* is one of the genera constituting the core of the tribe. For that reason ("low-level" taxonomical analysis of Apiaceae), the herein studied and additional 58 oils obtained from Scandiceae taxa were compared using multivariate statistical analyses (MVA). Agglomerative hierarchical clustering and principal component analysis of the chemical data on the volatiles of 59 samples of Scandiceae, performed in this work, exhibited a close relationship of Scandiceae genera with other Apiaceae genera. MVA demonstrated that the evolution of the volatiles' metabolism of Scandiceae taxa was neither genera-specific nor follows their morphological evolution.

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PP-165. Chemical composition, antioxidant and antimicrobial activities of *Mentha rotundifolia* essential oil

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Actually, most investigations of current research focused on the study of the biologically active compounds from natural molecules. The objective of this study was the evaluation of the chemical composition and the antioxidant activity of *Mentha rotundifolia* (L) Huds. essential oil, a medicinal plant belonging to the Lamiaceae family, largely used in Algerian folk medicine. The essential oil extracted by hydrodistillation from the aerial parts of the plant using a Clevenger- type apparatus yielded 0.7 % (v/w). This essential oil was analyzed by GC and GC/MS, forty three compounds were identified, representing 100% of the total essential oil. The major constituent was trans- piperitenone oxide (66.39%). To cross-validate the antioxidant activity of the essential oil three tests have been carried out, DPPH radical- scavenging assay (DPPH test), ferric reducing power assay (FRAP), ferrous ion chelating. Concerning the DPPH test, the IC_{50} was 1755± 0,006 µg/ ml. whereas for the second test the EC_{50} was 1625.5 ± 0.004 µg /ml. However, the ferrous ion chelating test showed no activity of the essential oil. This oil seems to have weak antioxidant activity when compared to the standard antioxidant (BHA and EDTA). This is probably due to the nature of the constituents which lake phenol compounds Antimicrobial effect of the essential oil was evaluated by agar diffusion assay against six microbial strains, three bacteria and three fungi. The results showed that the EO has a strong antifungal activity and a moderate antibacterial activity.

PP-166. GC/MS, GC/O and sensory aroma analysis of novel components for chicory coffee

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Chicory coffee is identified with the category of *wellness* beverages, i.e. a group of products connected with a healthy lifestyle (*health, harmonious life, energy and beauty at all ages*). Chicory coffee is a solution for those, who like the taste and aroma of coffee, while their health state prohibits them to consume caffeine. In view of its nutritional properties chicory coffee is recommended for individuals on slimming diets and patients suffering from gastrointestinal diseases, diseases of the pancreas and liver, and heart diseases. Chicory coffee is recommendable due to its health-promoting properties and consumers should be attracted to this product by its aroma. Aroma attributes of chicory coffee result not only from the raw materials used, but first of all the production process. Raw materials are subjected to roasting, which leads to considerable changes in the appearance and chemical composition of the product and as a consequence result in the formation of its aroma.The aim of the study was aroma estimation of novel raw materials for chicory coffee (roasted artichoke, hawthorn and lovage) containing high level of chlorogenic acid which may be an aroma precursor.

Novel raw materials after their preliminary preparation were roasted in a device by Probat Werke BRZ-2 (Emmericham Rhein).

SPME/DVB/CAR/PDMS: samples 0.5 g, vials 10 ml, addition of 2 ml deionized water. Extraction temperature: 70°C, time 40 min.

GC/MS: 5975C VL MSD (Agilent Technologies) with a HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). Mass spectra were compared with data from the NIST05 library.

GC/O: isolation of volatiles by the simultaneous distillation and extraction method (SDE) in Lickens-Nickerson apparatus was used. The determination of key aroma compounds was conducted on HP 5890 with an inlet splitter and a smelling port with DB-5 column. Separated fractions were smelled in successive double dilutions of analyzed distillates, until the last detectable aroma disappeared. In this way aroma was referred to respective retention indexes and dilution factors (FD) for individual fractions.

Profile sensory analysis of control chicory coffee with addition of novel components: the flavor descriptors were selected in several previous experiments according to the sensory international dictionary of terms. 10 assessors participated in analysis, 14 attributes for each sample.

The most aromatic novel materials for coffee substitute was roasted artichoke, which produced the highest number of volatiles during roasting particularly those important in coffee aroma such as 2-methoxy-4-vinylphenol. The GC/O analysis showed that the primary compound responsible for the aroma in roasted artichoke was 2-methoxy-4-vinylphenol with a spicy, smoky, clove aroma, while the roasted hawthorn key aroma compound were 2-ethyl-3,5-dimethylpyrazine and maltol which have highest FD 128. The analysis of roasted lovage distillate showed that a decisive influence on the flavor had α-selinene and 3-n-butylphthalide, then furfural. Profile sensory analysis of control chicory coffee with 20% addition of novel components showed that roasted artichokes and hawthorn improves flavor of chicory coffee (lovage changed it into selery-like).

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PP-167. Determination of lemon balm (*Melissa officinalis* L.) volatiles composition by different methods

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Lemon balm (*Melissa officinalis* L.) is valuable medicinal plant which is native to southern Europe (Mediterranean) and western Asia and commonly cultivated, in Poland on area 5000 ha. Lemon balm herb (*Melissa herba*) and lemon balm leaves (*Melissa folium*), components of herbal mixtures, are used as a digestive, antispasmodic and antiviral. Lemon balm essential oil (*Melissa oleum*), obtained from leaves by hydrodistillation, consists mainly of citronellal (2-40%) and citral (10-30%), which is a mixture of two monoterpens: geranial and neral. The main aim of the study was to choose the fast and precise method of lemon balm volatiles determination for the use in genotype selection.

Plant material was obtained in 2013 from eight lemon balm strains which were the part of breeding project done in Institute of Natural Fibres and Medicinal Plants in Poland. The herb was collected in full vegetative growth in the second year of cultivation. The herb was dried in a shaded, well ventilated place.

The volatiles were estimated by three different isolation methods. The first method was extraction of dry leaves material by methyl tert-butyl ether (MTBE) containing 10 µg/ml isobutylbenzene as an internal standard, and then analysis by GC/MS. Quantification of the compounds was based on total ion chromatographic peak size, as related to the internal standard.

As the second one methods was steam distillation of essential oils, done in Deryng's apparatus from 2g sample; internal standard was tetradecane (solution in o-xylene). Distillation was run 3h.

At last SPME was applied: conditions of the estimation were elaborated in this work: fiber – PDMS, samples 0.5g vials 10ml. Extraction temperature was 60ºC, time 20 min.

GC/MS: 5975C VL MSD with capillary column HP-5MS of 30m x 0.25mm x 0.25µm (by Agilent Technologies) was used. Mass spectra were compared with data from the NIST05 library.

All the methods showed differentiation between estimated lemon balm strains in relation to concentration of main volatiles eg. range of geranial level was 10-30% and neral 5-15% of the total components.

All the three methods can be used for volatiles estimation in Lemon balm herb samples and allowed to identified the same main aroma compounds: citronellal, neral, geranial, caryophyllene, caryophyllene oxide. However, the obtained data were not equal, particularly when compare volatiles quantity. SPME and extraction seems to be better for isolation more volatile components than hydrodistillation which is considered, however, as a method of showing the actual proportions of the quantitative components of essential oils.

Monitoring of main lemon balm volatiles can be done in a quick and simplest way by SPME/GC method (green chemistry).

PP-168. Characterisation of volatile compounds of *Prunus spinosa* L.

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Prunus spinosa L. (sloes), is a tree in the family Rosaceae. Sloes opens usually white, sometimes pink flowers in the spring. The leaves are small but abundant numbers. Fruits grow up dark purple in color and to be in a large number in one branch. This fruit is used in various ways in alternative medicine today. Solid phase microextraction (SPME) was used to analyze the volatile compounds in sloes. Fourteen compounds, representing 99.96% of the sloes oil contents, were isolated and analyzed using GC-FID/MS. The most aboundant chemicals identified were aldehydes (59.58%), monoterpene hydrocarbons (19.25%) and monoterpenoid (19.09%). Common components of sloes identified were eucalytol, thymene, limonene, hexanal and benzaldehyde. According to our study sloes has highly volatile components.

PP-169. Characterisation of volatile compounds of *Capparis spinosa* L.

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Capparis spinosa L. grows mainly in areas where the Mediterranean climate, in around and Tokat in Central Anatolia, in the southeastern provinces and Eastern Blacksea. *Capparis spinosa* is a thorny plant like bush structure upright and recumbent growing. It enjoys phosphorus, potassium, and calcium-rich calcareous and clayey soils. It is a plant that likes sun and spontaneously grows in slopes south-facing. Our country has different climatic and ecological conditions, rich plant flora. Also, it has a great economic potential in terms of medicinal and aromatic plants cultured and collected from nature. Solid phase microextraction (SPME) was used to analyze the volatile compounds in *Capparis spinosa.* Seventeen compounds, constituting 93.5% of the oil composition of *Capparis spinosa* were characterized using GC-FID/MS. The most abundant chemicals identified were aldehydes (18.2%) and monoterpene hydrocarbons (4.4%). Common components of *Capparis spinosa* identified were benzyl alcohol, octanoic acid, benzoic acid, α-terpinolene, carvacrol, zingerone and 4-fluoro benzaldehyde. Our results suggest that *Capparis spinosa* L. is a potential source of volatile components.

PP-170. Effect of *p*-cymene treatment on *in vitro* and *in vivo* leukocyte activity

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The CYM is a secondary metabolite, belongs to class of monoterpene and is found in essential oils from plants, food and in several spices. May present important biological activities as antimicrobial, antinociceptive and anti-inflammatory. Furthermore, it is an important intermediate used in pharmaceutical, flavor and aroma industries and for the production of fungicides and pesticides. In this study, we investigated the effects of CYM *in vitro* and *in vivo* on leukocyte activity. In the cell viability assay, CYM (3, 10, 30, 90 µg/mL) had low cytotoxicity. To evaluate the direct effect of CYM on *in vitro* neutrophil chemotaxis, different concentrations were applied (1, 3, 10, 30, 60 and 90 µg/ml). The chemo-attractants fMLP (10^{-6} M) and leukotriene B_4 (LTB_4; 10^{-8} M) were used. CYM significantly reduced (P<0.05) neutrophil migration in response to fMLP stimulation (60.2%, 64.1%, 69.1% and 58.1%, respectively.), but not toward LTB_4 stimulation. Since CYM treatment did not affect the viability of neutrophils in the concentrations tested, our data suggest that CYM have a direct effect on inhibition of *in vitro* neutrophils migration. The effects of CYM pretreatment on the *in vivo* of inflammatory cells migration was evaluated by peritonitis carrageenan-induced. After 4 h of inflammation induction, there was an increase in the number of peritoneal exudate leukocytes (11,53±1.18×10^6 cells/cavity) compared with the control group (5,37±0.20×10^6 cells/cavity). The animals pretreated with CYM (100, 200 and 400 mg/kg) presented a significant reduction of leukocyte infiltration into peritoneal cavity (35,2%, 39,8% and 22,9 %, resp.) compared with untreated animals, and this response was mainly attributable to a reduction of the number of polymorphonuclear leukocytes infiltration. The *in situ* effect of CYM pretreatment (100 and 200 mg/kg) evaluated by real time microscopic analysis for rolling and adhesion events of leukocytes in the mesenteric microcirculation resulted in a significantly decrease in the number of rolling (41.1% and 62.6%, respectivelly) whereas CYM pretreatment, in all tested doses, a significantly decrease in the number of adherent (55,8%, 72,6% and 56,5%, respectivelly) cells was observed. CYM pretreatment resulted in a significantly decrease in the number of rolling (100, 200 mg/kg) and in the number of adherent leukocytes (100, 200, 400 mg/kg) to the perivascular tissue. The phagocytic index was increased significantly in concentrations of CYM (3, 10, 30 µg/ml). Treatment with CYM (10, 90 µg/ml) also reduced TNF-α levels but did not alter TGF-β and IL-10 levels in fMLP-stimulated neutrophils. In conclusion, CYM may be considered as a potential agent for treatment inflammatory injury, however, further studies are necessary.

PP-171. Effects of anethole in nociception experimental models

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Recently we demonstrated that oral anethole administration in experimental animals inhibited the formation of inflammatory exudate and migration of leukocytes in models of pleurisy induced by carrageenan and ear edema induced by croton oil; antihypernociceptive activity in models of acute and persistent inflammatory pain induced by carrageenan and Complete Freund Adjuvant (ACF), respectively. It was also demonstrated that anethole reduces the recruitment of neutrophils in experimental models of *in vitro* chemotaxis and *in situ* microcirculation, in addition to immunomodulatory activity through a reduction of the migration of lymphocytes and macrophages induced by sheep erythrocytes antigens. Some of these effects have been related to the inhibitory effect of anethole on the production or release of inflammatory mediators, such as prostaglandins, nitric oxide, interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-17. Additionally, we demonstrated that anethole did not alter the plasma levels of transaminases (aspartate transaminase and alanine transaminase; i.e., markers of hepatic lesions) or morphological and histological profiles of hepatic tissue when administered for 7 days. Despite of these findings, there were need to continue the studies on the antinociceptive activity of anethole. Therefore, our aims in this study were investigated the role of anethole in various experimental models of nociception. The animals were pretreated with anethole (62.5, 125, 250 and 500 mg / kg) one hour before the experiments. To eliminate a possible sedative effect of anethole the open field test was conducted. Anethole (62.5, 125, 250 and 500 mg/kg) showed an antinociceptive of writhing model induced by acetic acid, in the second phase of the formalin test (125 and 250 mg / kg) in the test of glutamate (62.5, 125 and 250 mg / kg) and expresses pain induced by ACF (250 mg / kg). In contrast, anethole was not able to increase the latency time on the hot plate and decrease the number of flinches during the initial phase of the formalin test in none of the doses tested. It was also demonstrated that treatment with anethole in a single dose or daily for 7 days did not alter motor activity in the open field test. Therefore, these data showed that anethole exerts a peripheral antinociceptive effect without causing sedation. This antinociceptive action may be the result of a reduction on the production or release of inflammatory mediators. The results suggest that the anethole could be an interesting therapeutic alternative in inflammatory and painful diseases.

PP-172. Chemical characterization of the essential oil of *Baccharis reticularia* DC. from three locations of Distrito Federal, Brazil

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Several species of the genus *Baccharis* are known and widely used due to its pharmacological properties. *Baccharis reticularia* DC. is an aromatic shrub, native and endemic to Brazil, whose chemical and biological properties have not been reported yet. The aim of this work was the characterization of the essential oil of this species coming from three distinct locations in the Federal District, Brazil. The essential oil from the dried leaves and flowers of 10 plants of each site was extracted by hydrodistillation in a modified Clevenger apparatus and analysed by gas chromatography (GC-FID) coupled to mass spectrometry (GC-MS). The contents of essential oil obtained ranged from 0.74% to 0.98% and did not differ statistically among the populations. The major compounds (> 5.0%) found in the essential oil of three populations of *B. reticularia* were beta-pinene, beta-phellandrene, bicyclogermacrene, germacrene D, spathulenol and kessane. There were significant variations in the relative percentage of the chemical constituents of the essential oil of the three populations. The Canonical Discriminant Analysis of the chemical profile of the essential oil of *B. reticularia* allowed to distinguish three populations.

PP-173. Biological activity of the essential oil of *Lippia origanoides* and *Syzygium aromaticum* against wood decay fungi

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The preservatives used today in the woods, both inorganic and organic, have high toxicity (1). The control of wood decay fungi and other pests with essential oils is quite promising due to their low toxicity to humans and animals and, also, to their biodegradability (2). The objective of this work was to evaluate and compare the biological activity of the essential oil of *Lippia origanoides* Kunth and clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), against two wood decay fungi: White-rot (*Trametes versicolor*) and Brown-rot (*Gloeophyllum trabeum*).

Leaves and flowers of *L. origanoides* and buds of *S. aromaticum* were collected in Brasilia, Brazil, and hydrodistillated in a Clevenger-type apparatus for 2 hours. The essential oils were analyzed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems, both with HP-5MS fused silica capillary columns (30 m x 0.25 mm x 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL/minute. Oven temperature was raised from 60 to 240°C at 3°C/minute. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (3,4). Biological assays were performed by a modified well-diffusion method (5). 20ul of a solution of both essential oils (100, 50, 25 and 12.5% v/v in ethanol) were added to the center of a culture medium in a petri dish. Two mycelia of fungi (*Gloeophyllum trabeum* and *Trametes versicolor*) were incubated (5 and 10 days respectively) at 27°C and 70% of relative humidity. Inhibition index was measured through photo and image processing (Image J software).

The main compounds of *L. origanoides* oil were thymol (71.1%), *p*-cymene (9.6%) and γ-terpinene (5.9%), while in *S. aromaticum* eugenol (81.6%), eugenol acetate (8.1%) and β-caryophyllene (7.3%) were the major components. The essential oil of *L. origanoides* inhibited the growth of *G. trabeum* in all concentrations, while *T. versicolor* was inhibited in concentrations of up to 25%. *S. aromaticum* essential oil completely inhibited *G. trabeum* up to a concentration of 50%. The inhibition against *T. versicolor* was less intense, but still above 50% at a concentration of 12.5%.

The oil of *L. origanoides* has demonstrated a higher activity, and commercial potential since the oil was obtained in high yield (3%). To the best of our knowledge, this oil has not been tested against wood decay fungi yet.

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PP-174. Effect of estragole on acute inflammatory response

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Estragole, a monoterpene, is a chemical constituent of the essential oils of many aromatic plants. It is used as flavoring in beverage and food industries. *In vivo* and *in vitro* experimental assays have shown that estragole has sedative, anticonvulsant, myorelaxant, vasoactive, antioxidant, antimicrobial and anesthetic activity. In this work, we evaluate the effect of estragole on leukocyte behavior and phagocytic activity of macrophages. Specifically it has measured the carrageenan-induced peritonitis in mice, *in vivo* leukocyte migration in rats, in vitro chemotaxis assay, cytotoxicity and macrophages phagocytes assay. In the peritonitis model, estragole (500 and 750 mg/kg), decreased the infiltration of peritoneal exudate leukocytes. In vitro chemotaxis showed that estragole (3, 10, 30 and 60 µg/mL) inhibited neutrophil migration toward fMLP. In the in vivo microcirculation assay, estragole at doses of 250, 500 and 750 mg/kg significantly reduced the number of rolling and adherents leukocytes and at doses of 250 and 500 mg/kg diminish leukocyte migrated number to perivascular tissue. Recently, it was demonstrated by our research group that anethole, a position isomer of estragole, decreased the acute inflammatory process by inhibiting some mediators such as NO, PGE2, TNF, IL-1 β e IL-17 and it was also able to inhibit leukocyte migration *in vitro* and *in vivo*. Considering the structural similarity, since estragol e anetol are chemically isomer, it is possible that estragole could be acting in the adhesion molecules production by inhibiting TNF and IL-1β production, with consequent reducing in the migration of leukocytes. Our results also demonstrated that estragole stimulated significantly the macrophages phagocytic ability in concentrations of 3, 10 and 30µg/mL, when compared with negative control group. A similar effect was observed when LPS was used as positive control. LPS, a component of the membrane of gram-negative bacteria, actives macrophages by binding the Toll-like receptors 4 (CD14/TLR4) complex, which promotes phagocytosis. Thus, the estragole can also have stimulated the phagocytosis via binding with Toll-like receptors 4, but it is still necessary additional experiments to confirm the mechanism. It is well known that NO produced by macrophages is a cytotoxic molecule acting by a killing mechanism against invading microbes. In this work, the addition of estragole significantly stimulated production of NO only at the concentration of 10 µg/ml. In conclusion estragole showed anti-inflammatory activity as demonstrated by the inhibition in the leukocyte recruitment and stimulation of phagocytic activity in macrophages. Thus this compound may be promising herbal medication in the inflammatory process with excessive leukocyte migration. This finding is relevant since estragole is a constituent of many essential oils used in aromatherapy and should be considered as nutraceutical. Further studies are needed to elucidate this possibility and the anti-inflammatory mechanism of this drug.

PP-175. Hepatoprotective effect of pretreatment with *Thymus vulgaris* essential oil in experimental model of acetaminophen-induced injury

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Acute liver damage caused by acetaminophen overdose is a significant clinical problem and could benefit from new therapeutic strategies. This study investigated the hepatoprotective effect of *Thymus vulgaris* L. essential oil (TEO), which is used popularly for various beneficial effects, such as its antiseptic, carminative, and antimicrobial effects. The hepatoprotective activity of TEO was determined by assessing serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in mice. Their livers were then used to determine myeloperoxidase (MPO) enzyme activity and subjected to histological analysis. *In vitro* antioxidant activity was evaluated by assessing the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•)-scavenging effects of TEO and TEO-induced lipid peroxidation.

Pretreatment with 250 and 500 mg/kg TEO but not 125 mg/kg TEO for 7 days prior to acetaminophen administration markedly reduced serum ALT, AST, and ALP levels compared with vehicle treated controls. The effect of TEO was also comparable to silymarin, a standard hepatoprotective agent. The activity of MPO in TEO-pretreated mice that received doses of 250 and 500mg/kg was significantly decreased (0.073 ± 0.008 and 0.069 ± 0.008 IU/L, resp.) compared with the group that received acetaminophen only (0.251 ± 0.149 IU/L). The histopathological analysis of control group (APAP vehicle) did not show hepatocellular damage. However, the acetaminophen-treated group showed severe injury characterized by hemorrhagic and necrotic areas, presence of inflammatory infiltrate and piknotic nucleus. Considering the sylimarin group (standard drug), although the hepatic parenchymal did not present homogenous, necrotic areas were not observed. Also, cellular nucleus was more basophilic than of that control mice and many cells showed cytoplasm vacuolization, showing minor injuries. The group of animals treated with TEO 125 mg/Kg showed interspersed necrotic areas with non-necrotic areas, with cytoplasm vacuolization and hemorrhagic points, characteristics of moderate injuries, differently to that observed after TEO 250 mg/kg treatment, where mild injuries were observed, characterized by basophilic nucleus almost piknotic with shrinkage level. Besides, the group treated with 500mg/kg of TEO showed the hepatic parenchyma with similar morphology to the control group. Therefore, TEO appeared to provide significant protection against hepatocyte injury. In the DPPH test, the ability of TEO to act as a donor for hydrogen atoms or electrons in the transformation of DPPH· in its reduced form (DPPH-H) was measured spectrophotometrically. The Free radical-scavenging capacity (RSC) of TEO at concentrations of 60–2500 µg/mL showed significant antioxidant activity in vitro (IC50 = 1377 ± 1.6970 µg/mL. The IC50 value of ascorbic acid (i.e., the positive control) was 4.40 ± 0.07928 µg/mL in the DPPH assay. Egg yolk lipids undergo rapid lipid peroxidation when incubated in the presence of ferrous sulfate. At concentrations of 5–5000 µg/mL, TEO significantly inhibited lipid peroxidation (IC_{50} = 8461 ± 7.778 µg/mL). The IC_{50} value of ascorbic acid (i.e., the positive control) was 63.00 ± 3.3870 µg/mL in the lipid peroxidation assay. Therefore, TEO was significantly correlated with total antioxidant activity (R > 0.99), demonstrating that TEO had antioxidant activity.

These results suggest that TEO has hepatoprotective effects on acetaminophen-induced hepatic damage in mice.

PP-176. Essential oil yield and composition of *Salvia officinalis* L. as a function of sustainable agronomical management

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The genus *Salvia* is employed in many applications including pharmaceutical, ornamental, food and cosmetics industries. For each purpose, many chemotypes and species should be evaluated for the vegetable yield and composition of essential oils. Among these, the essential oil (EO) of sage (*Salvia officinalis*) shows different biological activities for the antioxidant, anti-inflammatory, antispasmodic, antimicrobial and stimulant properties. Besides, several authors studied antiviral, antifungal and insecticidal activities.

To date, little attention has been given to the evaluation of the biological factors affecting biomass production and oil content and composition of sage. It is known the biosynthesis of EO is dependent on inorganic phosphorus content in the plant which is often poorly available in the soil. To overcome this limitation, plants developed several mechanisms of P acquisition, among them symbiotic associations with arbuscular mycorrhizal (AM) fungi. The symbiosis can improve the growth and development of plants by promoting the absorption of mineral elements and by increasing the stress resistance abilities. So AM fungi may be considered as biofertilisers for the sustainable management of agricultural ecosystems.

The aim of the present study was to evaluate the effects induced by P fertilization and AM fungi on growth and EO content and composition of *Salvia officinalis* seedlings grown in the greenhouse.

The experiment consisted of two levels of P-fertilization and three AM fungi inocula (*Glomus mosseae* Nicol. & Gerd, *Glomus viscosum* H.T. Nicolson or commercial mix of 6 AM fungi), applied separately and in combination, and the control without any treatments.

After 4 months the morphological parameters were estimated and air-dried leaves of plants submitted to water distillation in a Clevenger type for 4 hours. The chemical composition of the oils obtained by hydrodistillation was fully characterized by GC-MS.

All applied treatments increased root and shoot biomass of sage as compared to the control even if the morphological responses of the total biomass (leaves, stems and roots) did not show any significant differences between treatments.

The EO content (ml 100g-1DW) varied among the treatments, higher content was obtained from the commercial formulate of fungi, while the lowest value was reached with *G. mosseae.*

Essential oil analysis by GC/MS found different 72 compounds, having the total area around (99%) in order of retention index with their percentage area.

In terms of EO composition, the treatments determined significant differences regarding the percentage of measured compounds and oil quality. In fact, the quantity of the above major constituents markedly varied in the oil: cis-thujone- from 7.83 to 28.69%, manool- from 13.74 to 39.33, camphor – from 5.44 to 13.61%, viridiflorol from 9.46 to 18.27%, α- humulene – from 5.64 to 8.56%, ß-thujone from 1.58 to 5.67, 1.8-cineol from 1.15 to 5.08%, trans- caryophyllene from 1.37 to 4.4%, borneol (1.37- 3.67%), α- humulene expoxide II (0.83- 1.94 %), camphene (0.34- 1.32%).

The studied treatments influenced the chemical compositions of the sage essential oils. Both phosphorous fertilization and mycorrhizal symbiosis produced an increase of the amount of diterpenes (manool) in comparison with the control, while hydrocarbons and oxygenated monoterpenes decreased.

PP-177. Chemical composition of the essential oil of the leaves of *Psidium oligospermum* (Myrtaceae) and its inotropic activity in cardiac muscle

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The discovery of the active components present in plants as well as their mechanisms of action, has been a major challenge for the pharmaceutical chemistry, biochemistry and pharmacology [1]. The Myrtaceae family is one of the largest families in the neotropical flora which has about 100-144 genera and variations between 4600-5800 species distributed around the planet. Among the genera of this family highlights the *Psidium* genus with 95 species, 63 are native to Brazil [2]. The literature reports the negative inotropic effect of essential oil of *Psidium guajava* (L.) Kunze on the heart muscle [3]. This study reports on the chemical composition of the essential oil obtained from fresh leaves of *Psidium oligospermum* Mart. ex DC. and its inotropic activity in heart muscle. This specie was collected in the forest of Rio Poxim, GPS (10°55´11.851”S; 37°6´10.129”W), near Federal University of Sergipe, Brazil. The GC-MS analysis revealed 43 components of the total, accounting for 100.00% of the oil composition, of which 35 (87.88%) were identified. The major constituents are α-pinene (11.51%), β-pinene (2.96%), 1,8-cineole (10.69), α-copaene (23.3%), *E*-caryophyllene (24.88%), δ-cadinene (6.05%) and cubenol (3.46%). This oil showed a negative inotropic effect of concentration-dependent and partially reversible manner, with efficiency of 73% and EC_{50} 1340 mg/ml when tested in isolated left atrium electrically stimulated guinea pig (*Cavia porcellus*). To the best of our knowledge, there are neither scientific studies related to chemical constitution of essential oil from fresh leaves of *P. oligospermum* nor its inotropic activity in cardiac muscle.

Acknowledgments

CNPq, CAPES, FAPITEC

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PP-178. Volatile profile and flavour of cold pressed *Citrus* essential oils

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The volatile fraction composition of commercially produced cold pressed essential oils from *Citrus* fruit peels was studied objective of this work by using GC-MS and GC-FID. More than fifty components were identified in the oils using their mass spectra and linear retention indices. The monoterpene limonene was the most abundant component even trough not in a quantity expected for a fresh orange essential oil. Aldehydes, followed by alcohols and esters, were the main components in the oxygenated fraction. Aldehydes were the major oxygenated components in the sweet orange oil, whereas alcohols and esters were present in higher amounts in the bitter orange oil. Among them, nonanal, decanal and linalool are the most important components for the flavor of sweet orange oil and carvone is the most important ketone for the flavor of bitter orange oil in combination with the other components. The amount of carvone gives a good indication about the freshness of the lemon and mandarin oils and the quantities of α-pinene and ß-pinene, sabinene and myrcene give an indication about the natural or artificially changed composition of the essential oils. Among the flavor compounds, the major volatile flavour compounds from different chemical classes namely monoterpene hydrocarbons (i.e. limonene, sabinene plus β-pinene, γ-terpinene, myrcene, α-pinene and α-terpinolene), esters (i.e. neryl acetate and geranyl acetate), alcohol (i.e. α-Terpineol) and aldehyde compounds (i.e. geranial and neral) were composed of > 90% of total volatile flavor compounds of target cold pressed lemon oils. α-pinen and camphene were the most abundant compounds after limonene responsible for the flavor of the mandarin oil. α-copaene, *trans*-α-bergamotene and β-farnesene were the most important sesquiterpene responsible for the sweet taste of the "murcott" mandarin oil. Octanal, nonanal, decanal from aliphatic aldehydes and geranial and α-sinensal from the class of monoterpene aldehyde were dominant in all samples of mandarin oils. Methyl antranylate was the main ester which contributes the most to the overall aroma of mandarin oil. Linalool was the major alcohol responsible for the sweet and floral smell of the oil. Significant quantity of flavons was detected around 250°C during chromatographic analysis. Polyphenolic compounds 4‟,5,6,7,8-pentamethoxy flavon (tangeretin), 3,3‟,4‟,4,5,5‟7,8-heptamethoxy flavon and 3‟,4‟,5,6,7,8 hexamethoxy flavon (nobiletin) in the role of natural antioxidants protected the oils from oxidation. Applied statistical analysis (PCA and Cluster analysis) explained classification of mandarin oils by origin and process of production.

PP-179. Extract from medicinal plants mixture as anticoccidial and antioxidant in broilers

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The mixture of *Artemisia absinthium, Thymus vulgaris, Menthae piperitae, Thymus serpyllum* was evaluated for blood and liver oxidative status (glutathione peroxidase-GSHPx, superoxide dismutase-SOD and concentration of malondialdehyde-MDA) and anticoccidial effects in broilers experimentally infected with mixture of oocists of *Eimeria* spp. (20,000 oocysts/bird), in comparison to coccidiostatic salinomycine. The *in vivo* investigation were carried out on 120 day-old Arbor acres broilers, of both sexes, separated into 4 equal groups with 3 replicates each. Group A was uninfected and untreated (negative control). Group B was infected and was kept untreated (positive control). Group C preventivly received coccidiostatic salinomycine in quantity of 60 mg/kg and inoculated with coccidia species at 21st day-of-age. Group D consumed a basal diet supplemented with extracts of herbs mixture in quantity of 2 g/kg and was infected with *Eimeria* oocysts at 21st day-of-age. Clinical signs, number of oocysts per gram faeces (OPG) and mortality were monitored daily for 42 days per teatment. The anticoccidial effects of chosen medicinal plants extract caused significant decrease in output number of oocysts per gram of faeces in broilers challenged with *Eimeria* spp.

The obtained results indicate a statistically significant ($P < 0.05$) increase of GSHPx activity in blood hemolysates. On the other hand, the catalytic activity of SOD showed a statistically very significant reduction in positive control group compared with the negative control group. The preventive doses of coccidiostatic salinomycin indicated a statistically significant ($P < 0.05$) decrease of MDA concentration and statistically significant ($P < 0.05$) increase of GSHPx activity and reduce of catalase-activity of SOD. The activity of GSHPx in liver homogenates of broilers group B showed a statistically significant increase ($P < 0.05$) in comparison with the negative control group, while the SOD activity decreased the level of statistical significance.

It is concluded that medical plants mixture reduces the severity of coccidial infection induced by *Eimeria* spp. and exibits a significant antioxidant activity in broilers fattening. Thus, it can be used as prophylactic feed additive and source of antioxidant in dietary supplement.

Acknowledgments

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PP-180. *Utilization of essential oils in food industry*

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Essential oils become increasingly popular as natural antimicrobial and antioxidant agents that may be used in food preservation. Antimicrobials are used in the food industry for two main reasons: to control natural spoilage processes (food preservation), and to prevent the growth of micro-organisms, including pathogenic micro-organisms (food safety).

Nowadays, different strategies are applied in order to control food-borne pathogens in food commodities, and particular interest has been focused on the application of natural antimicrobial agents,including the essential oils from various species of edible and medicinal plants, herbs, and spices that are relatively much safer to humans. A study from Portugal showed that the bush-basil essential oil showed the highest antimicrobial activity, with minimum inhibitory concentration ranging between 0.6 and 5 m L/ mL against bacteria and 0.04-2.5 m L/mL against yeasts (Silva et al., 2013).

Combination of N,O-carboxymethyl chitosan and oregano essential oil resulted to the complete inhibition of L. monocytogenes after 2 days in the low inoculum experiment and 4 days in the high inoculum experiment. The combination of both antimicrobial agents resulted to a 6 day shelf life extension of chicken fillets (Khanjari et al. 2013).

In general terms, essential oils are bioactive compounds formed of a mixture of many substances; they have a wide chemical composition and are obtained from plants secondary metabolism. Besides the antibacterial, antifungal, and anti-inflammatory activities, many essential oils also have been confirmed to possess the antioxidant activity. In addition, essential oils are gaining in interest for their potential as preservatives and as decontamination agents, since such substances have been recognized as safe (GRAS) and are widely accepted by consumers. Essential oils are classified as GRAS by the USFDA. In the EU essential oils are considered as safe food additives at concentrations < 2 mg/kg b.w./day (EFSA J, 2010).

Oregano essential oil and its fractions inhibit the lipid oxidation process in sunflower oil. Fractions of oregano essential oil rich in terpenes with low boiling point and without a functional group present high antioxidant property. These kind of fractions obtained from oregano essential oil by short path molecular distillation can be used as natural antioxidants for food products sensitive to lipid oxidation even in organic food (Ruben et al. 2014).

New technologies for food packaging have emerged and among them “active packaging” is one of the most promising innovation. It consists of incorporating into the packaging material active compounds useful for the food protection in terms of antioxidant and/or antimicrobial effect. Biologically active ingredients such as essential oils can be added to impart desired functional properties to the resulting packaging materials.

The results obtained from area tests run in Petri dishes indicated that citronella, oregano and rosemary at concentrations higher than 0.005 µL/cm^2 showed potential in terms of repellent activities against the red flour beetle, *Tribolium castaneum*. Assays performed with coated packages containing wheat semolina showed repellency results ranging from 53 to 87% for citronella and rosemary, respectively(Licciardello et al. 2013).

PP-181. Essential oil composition of *Acorus calamus* L. growing in Turkey

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Acorus calamus L. (Acoraceae), sweet flag, is a well-known medicinal plant that grows worldwide wildly along swamps, rivers and lakes [1,2]. Rhizomes of *A. calamus* have been used since the ancient times owing to their medical properties [3]. In Turkey the rhizomes are used for digestive problems such as bloating, wind, colic and poor digestive functions especially by children and some prostata complaintments [4]. Previous phytochemical investigations on *A. calamus* resulted with monoterpenes, sesquiterpenes, flavonoids and volatile phenylpropanoids, α-and β-asarone [5-8].

In this study, the rhizomes and leaves of *A. calamus* growing in Turkey were subjected to conventional hydrodistillation and rapid microdistillation methods with subsequent investigation of chemical composition of the essential oils by simultaneous GC-FID and GC/MS techniques. β-Asarone content in *A. calamus* oils was determined.

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PP-182. Evaluation of role of essential oil in hypnotic herbs based on Iranian Traditional Medicine (ITM) books

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Insomnia is a common sleep disorder and oral sedative drugs are the most common treatment. In ITM, different drugs are used for the treatment of insomnia. One of the most important characteristics of ITM is that it recommends the use of non-oral drugs to treat insomnia. In this method speed of efficacy of drug increases compared to oral method. One of the best methods of consumption of non-oral drug is smelling of aromatic herbs in ITM. The aim of this study is the investigation of the most important of inhaled hypnotic herbs and introduced of them as a treatment protocol. We collected some of inhaled hypnotic herbs which are used to treat insomnia from ITM documents. These plants are: Lemongrass, Oakmoss, Poppy, Feverfew, Chamomile, German Madwort, Sweet violet, Harmel, Amomon, Melon, Saffron, Basil, Safflower, Lettuce, Coriander, Belladonna, Myrrh and Cucumber. The essential oil of these plants are effective in increasing the sleep duration in comparison with pentobarbital and benzodiazepins. The anxiolytic-like effect of essential oil mediated by the $GABA_A$ receptor–benzodiazepine complex some of them inhibit nicotinic and muscarinic acetylcholine receptors. Linalool in the essential oil of these plants can be good reason of sleeping effect. Among these plants, we couldn't find any reason to hypnotic effects of Oakmoss, Myrrh and Safflower but in ITM these 3 plants has used in insomnia treatment, too. So we suggested more researches about them and these plants can be used as a protocol to cure insomnia.

PP-183. Thermogravimetric analysis of oregano (*Origanum vulgare* L.) essential oil encapsulated by spray drying

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Oregano (*Origanum vulgare* L.) essential oil is a complex mixture of volatile and chemically unstable substances when in contact with oxygen, moisture and heat. Due to their antioxidant power and the high volatility of these compounds oil microencapsulation processes have been studied in an attempt to minimize loss of these important components [1,2,3]. This study evaluated the thermal stability of oregano (*Origanum vulgare* L.) essential oil microparticles. The oil was encapsulated by a spray drying technique using a wall system consisting of 25% w/v of gum arabic solution and 75% w/v of modified starch. The microencapsulation was carried out in a spray dryer, LABMAQ Brazil, Model MSD 1.0, dual-fluid nozzle spraying system. The inlet and outlet air temperatures were 180 ± 2 °C and 105 ± 2 °C, respectively. The feed flow rate was adjusted to 2.97×10^{-7} m^3 s^{-1}, and the air flow inlet maintained at 5.8×10^{-4} m^3 s^{-1}. The powder was stored at different relative humidities from 11.3 to 84.34% using seven saturated salt solutions (LiCl, MgCl, K_2CO_3, $NaNO_3$, $Mg(NO_3)_2$, NaCl and KCl) [4]. The product was characterized by thermal gravimetric analysis in a Shimadzu TG-DTA 50 H, Kyoto, Japan, performed under an atmosphere of nitrogen at a flow rate of 50 mL min^{-1}. Samples were heated at 10 °C min-1 from 25 °C to 500 °C [5]. The Thermogravimetric analysis (TGA) showed three stages of mass decomposition for all the samples (Table 1). We observed that the first mass loss from the material occurred at temperatures close to 63.5 °C with an average loss of 8.78%, and the mass loss percentage was directly proportional to the equilibrium moisture content of the microparticles for all water activities evaluated. In this first decomposition step there may have been loss of water and volatiles. The successful loss of water in this stage possibly corresponds to the critical moisture content of the microparticles, which ranged from 4.74 to 22.37%, plus the adsorbed oil (unencapsulated) on the surfaces of the microparticles. It was found that samples with low equilibrium moisture content (4.74%), stored under a relative humidity of 11.3%, had the lowest mass loss percentage (5.96%) and higher initial degradation temperature (T_{onset}) compared to other samples. The second stage of mass decomposition occurred at the average temperature of 245 °C where the particles showed a marked mass loss, about 68.16% on average. Thus, samples stored at lower relative humidity showed lower mass loss at the first decomposition, and consequently, higher thermal stability.

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PP-184. Effect of the addition of rosemary essential oil microcapsules using whey protein-inulin matrix on shelf life extension of Minas cheese

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The rosemary essential oil presents aromatic, antioxidant and antimicrobial properties and the conversion of oily materials into powder, through microencapsulation processes, allows alternative uses in the food industry [1]. This study aimed to evaluate the encapsulation process of rosemary essential oil using whey protein isolate and inulin as wall materials and to verify the effects of the use of these particles on shelf life extension of Minas cheese. The following produced cheeses were evaluated in this study: control (without oil), added with pure essential oil (0.5%) and added with microencapsulated oil (0.5%). The encapsulation efficiency was determinate and the components profile of the pure and the microencapsulated essential oil were analyzed using GC-MS [2,3]. Analysis of mesophilic aerobic count and total coliform in the cheese over time of storage at 6° C were performed, as well as analysis of pH, moisture content and acidity [4,6]. The microcapsules presented 8% of rosemary essential oil and reached 40% of encapsulation efficiency. The main components found in the oil were 1,8-cineole (40.8% and 44.8%), camphor (28.8% and 20.2%), α-pinene (10.9% and 14.9%); β-pinene (8.1% and 12.3%); caryophyllene (5.7% and 3.4%), D-limonene (2.1% and 0.2%), β-linalool (1,6% e 1,7%) for the pure oil and the extracted oil from the microcapsules respectively. Mesophilic aerobic count in Minas cheese added with microcapsules was significantly lower ($p<0.05$), when compared with other treatments, evidencing the antimicrobial effect of the encapsulated oil. For this treatment, reduction of 1.36 log cycle at time 3 and 0.73 log cycle at time 15 were observed compared to control treatment. On the other hand, no significant difference ($p>0.05$) were observed between the treatments when regarded the growing of total coliforms. Moisture content showed no difference between the treatments. The samples presented a decrease of the pH value and an increase of the acidity over time. Control treatment showed the lowest pH (4.93 ± 0.05) e the highest acidity (0.52 ± 0.04%) after 15 days of storage. Microencapsulated rosemary essential oil could be promising on the growing control of mesophilic bacteria in Minas cheese and other similar types of cheese.

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PP-185. Volatile constituents, anioxidant and antimicrobial properties of *Salvia chudaei* Batt. Et trab. endemic medicinal plant from Algeria

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The essential oil composition and *in vitro* antioxidant and antimicrobial activity of the essential oil of *Salvia chudaei* Batt. Et Trab. were evaluated in this research for the first time. GC and GC/MS analysis of the plant essential oil resulted in the identification of 50 compounds representing 90.81% of the oil.The principal constituents were identified as bornyl acetate (20.54%), β-eudesmol (13.64%), β-caryophyllene (10.6%), Valencene (9.34%), τ-cadinol (9.31%), α-Pinene (6.85%) and γ-cadinene (5.78%). Antimicrobial activity of the essential oil was evaluated using disk diffusion and Agar Dilution methods [1]. A great potential antimicrobial activity was observed against *Staphylococcus epidermidis* and *Candida albicans* followed by *Bacillus subtilis*, *Staphylococcus aureus* and *Listeria monocytogenes* with their respective zones of inhibition of 13.33±1.52to 45.5 ±2.12 mm and MIC values of 0.062 to 1mg/ml. The plant essential oil was also subjected to screenings for the evaluation of their antioxidant activities using 2.2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene–linoleic acid tests [2,3], and weak activities were found for this oil.

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PP-186. Anti-asthma and anti-cough activity of the essential oils from the fresh and dry leaves of *Eucalyptus grandis* W. Hill ex Maiden.

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The leaves of *Eucalyptus grandis* are used by Zulu traditional healers in the management of respiratory tract infections and asthma. The traditional treatment is by means of steam inhalation; hence, the essential oils of the fresh and dry leaves of the plant were obtained by hydro-distillation and investigated for anti-asthma and anti-cough activities. The percentage yields of the essential oils from the fresh and dry leave were 0.19% and 0.40% respectively. The chemical compositions of the oils were analyzed by GC and GC-MS. The identified main components of the essential oil of the fresh leaves constituted 99.25% and the major constituents were α-Pinene (29.69%), *p*-cymene (19.89%), 1,8-Cineole (12.80%), α-Terpineol (6.48%), Borneol (3.48%) and D- Limonene (3.14%), while that of the dry leaves was 92.63% with the major constituents being 1,8-Cineole (47.44%), D- Limonene (13.34%), α-Pinene (7.49%), (-)-spathulenol (7.13%) and Benzene,1-methyl-4-(1-methylethyl)-(5.42%). The essential oils were able to delay wheezing and coughing in rats after exposure to irritants, histamine and acetylcholine for asthma and ammonia for cough. The anti-asthma and anti-cough activities of the essential oils were seen to be concentration dependent.

The antimicrobial activities of the essential oils were tested against respiratory tract infecting microorganisms, *Klebsiella pneumoniae, Staphylococcus aureus* and *Moraxella catarrhalis*. The minimum inhibitory concentration (MIC) ranged from 0.3125mg/ml to 1.25mg/ml, and the minimum bactericidal concentration (MBC) ranged from 0.625mg/ml to >5mg/ml. The cytosolic LDH release assay revealed bacterial membrane damage ranging from 1% to 11% in comparison with the standard TritonX-100. Accumulation of rhodamine 6G in bacterial cells was used to determine the activity of the essential oils as drug efflux pump inhibitors (EPIs). Results showed that the essential oils were effective as EPIs with inhibition range of 5% to 53%. The essential oils were also seen to inhibit the activity of COX 2 with no significant effect on COX1.

PP-187. Chemical composition and antimicrobial activities of essential oils of *Seseli varium* Trev.

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Seseli varium Trev. *(Seseli palasii)* essential oil obtained by hydrodistillation, was analyzed by GC and GC-MS, applying the liquid injection mode. These results were compared with the chemical composition of volatiles achieved by the "headspace" injection mode, followed by GC and GC-MS (HS-GC-MS). Total of 38, 100 and 49 components were identified in root, stem and fruit essential oils of *S. varium* (GC-MS analysis), respectively. The main constituents identified in *S. varium* stems and fruits essential oil were monoterpenes, with α-pinene as major constituent of both, stems and fruits essential oil (27.3% and 84.7 %, respectively). Saturated hydrocarbons comprise 58.5% of *S. varium* root essential oil, 34.5% are monoterpenes. The major constituents identified in *S. varium* root essential oil were *n*-nonane (45.2%), (*Z*)-β-ocimene (34.5%) and *n*-undecane (13.3%). HS/GC-MS volatiles analysis resulted in total of 15, 11 and 11 components identified in roots, stems and fruits, respectively. Main constituents of root volatiles were *n*-nonane and (*Z*)-β-ocimene (63.7% and 22.3%); α-pinene, limonene, δ-3-carene and myrcene (71%, 10.6%, 5.9% and 4.9%) were the main constituents of stem volatiles and α-pinene and β-pinene (93.7% and 2.1%) main constituents of fruit volatiles. Number of components identified by GC, GC-MS is considerably higher than components identified by HS/GC-MS. It is clear that only the most volatile components are identified by HS/GC-MS.

The microbial activity of the essential oils was screened against a panel of four bacterial and two fungal strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*). The oils were active against all strains tested with minimum inhibitory concentration (MIC) ranging from 21.9 to 416 µg/ml and minimum bactericidal concentration (MBC) ranging from 54.2 to 644.8 µg/ml. Root essential oil showed the best activity against bacterial strain *Bacillus cereus* (MIC = 21.9 µg/ml, MBC = 87.8 µg/ml). The present study indicates that *Seseli varium* Trev. essential oils have considerable antimicrobial activities and potentials for future investigations.

PP-188. Chemical diversity of Peucedanum *longicarpum* A.Duran & M.Öztürk volatiles

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In this study we investigated for volatile metabolites a new species recently added to *Peucedanum* genus, *Peucedanum longicarpum* A.Duran & M.Öztürk collected in Antalya: Gazipaşa, Turkey on October, 2011. The plant material (herba + fruit and stem) was hydrodistilled in a Clevenger type apparatus to yield essential oils (0.59 and 0.15%, resp.). Gas-chromatographic analysis with FID and MS detectors revealed 109 and 106 constituents in the two oils. Chemical profile of the oils was dominated by monoterpene hydrocarbons (71.9 and 72.9%, resp.) with α-pinene (12.9 and 22.7%, resp.), β-pinene (10.5% and 13.4 resp.), myrcene (23.3 and 13.1%, resp.), limonene (4.9 and 7.5%) and β-phellandrene (5.7 and 2.3%, resp.) as major constituents. The sesquiterpene hydrocarbons constituted 13.0% and 9.1% of the oils with germacrene D (7.5% and 3.4%, resp.) as major constituent. The oxygenated mono- and sesquiterpenes amounted to 4.0% and 4.5%, and to 6.0% and 10.6% in the herba + fruit and stem oils, respectively. The oils were subjected to preliminary antioxidant activity test with DPPH reagent.

PP-189. Chemical composition of volatile metabolites of *Arctium lappa L*. flower, root, leaf and stem

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Volatile metabolites from the flower, leaves, root and stem of *Arctium lappa* L. (Asteraceae) were obtained by hydrodistillation and then analyzed by gas chromatography (GC/FID) and gas chromatography – mass spectrometry (GC/MS) methods. The plant material was collected in Altai region along Valley of Bistruha River in 2013 year (Kazakhstan).

The oxygenated sesquiterpenes predominated in flower and stem volatiles of *A. lappa* with β-eudesmol (41.0% and 39.2%, respectively) as the main constituent. In the flowers, γ-eudesmol (6.4%), α-eudesmol (8.7%), elemol (6.3%), and hexahydrofarnesyl acetone (4.3%) followed the series of major sesquiterpenes. The root oil of *Arctium lappa* was characterized by high abundance of unsaturated hydrocarbon 1,8,11,14-heptadecatetraene, aplotaxene (30.2%). This compound has earlier been reported as major constituent for several *Asteraceae* species, *Inula helenium* (1), *Carduus* species (2), *Cirsium japonicum* (3) and *Centaurea incana* (4). Hexadecanoic acid (50.2%, 39.3% and 46.0%) was the major representative of fatty acids in stem, root and leaf oils, respectively. The present work is the first contribution into chemistry of volatile metabolites from different organs of *Arctium lappa* plant.

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NVEO Natural Volatiles & Essential Oils

PP-190. The key role of ^{13}C-NMR analysis in the identification of individual components of *Polyalthia longifolia* leaf oil

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Polyalthia longifolia Sonn. (Annonaceae) produces sesquiterpene-rich essential oils (EOs) whose compositions varied substantially from sample to sample depending of the origin of the plant (Nigeria, Vietnam). Nothing is known about the phytochemistry of Ivoirian *P. longifolia.*

The chemical composition of the leaf oil of Ivoirian *P. longifolia* was characterize using a strategy that allows the identification of minor oxygenated sesquiterpenes whose MS data are not compiled in commercial or laboratory-constructed MS libraries.

The EO was submitted to GC(RI), GC-MS and ^{13}C-NMR analysis. Then hydrocarbons and oxygenated components were separated and the oxygenated fraction was chromatographed on silica gel. The fractions were analyzed by GC(RI) and ^{13}C-NMR.

Seventy compounds accounting for 91.8% of the EO were identified. Sesquiterpene hydrocarbons, (*E*)-β-caryophyllene (27.8%), α-zingiberene (20.0%) and *allo*-aromadendrene (15.0%) were the major components. Various oxygenated sesquiterpenes whose MS data were not compiled in commercial and laboratory-made MS libraries were identified by comparison of their chemical shift values in the spectrum of the fraction of CC with those reported in the literature and compiled in a laboratory-constructed ^{13}C-NMR data library. The composition of the investigated Ivoirian *P. longifolia* oil sample presented similarities and differences with Nigerian and Vietnamese oils.

Combined analysis of Ivoirian *P. longifolia* EO by chromatographic and spectroscopic techniques including ^{13}C-NMR without isolation of the components, appeared particularly efficient to identify minor components of EOs, whose MS spectra are insufficiently differentiated or MS data are not compiled in commercial and lab-constructed MS libraries.

PP-191. Germacra-1(10),5-dien-4α-ol in *Fortunella* sp leaf oils

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Citrus genus belongs to the plant family Rutaceae which includes two other genera, Poncirus and Fortunella. The latter genus is represented by *Fortunella crassifolia*, *F. hindsii*, *F. japonica*, *F. margarita* and *F. obovata* and is wellkonwn because of its fruits called Kumquat. Fruits are smaller than those of Citrus, ovoid or oblong and when split; they present the typical segmented appearance of a Citrus fruit.

Chemical characterization of Essential oil (EO) from leaves of *Fortunella japonica* (Thunb.) Swing. is a complex task due to a tremendous diversity of sesquiterpenes bearing various skeleton. The essential oil isolated by water distillation using a Clevenger-type apparatus was analyzed by GC(RI), GC-MS and ^{13}C NMR. EO was also submitted to repeated CC and all the fractions of chromatography were analyzed by GC(RI) and ^{13}C NMR. Sixty -nine compounds representing 93.1% of the essential oil were identified. The major components are germacrene D (14.9%), β-elemol (9.1%) and limonene (7.1%). We report herein the identification of various tertiary alcohol sesquiterpenes whose identification was achieved using ^{13}C NMR and of germacra-1(10),5-dien-4α-ol, whose spectroscopic data, RI and further the chemical name reported in the literature are confusing. Structures were elucidated using the concept of "Extraction NMR". [1]

The chemical composition of leaf oils from four other species of *Fortunella* (kumquat) were also investigated, with a particular attention to the occurrence of germacra-1(10),5-dien-4α-ol. Chemical compositions are similar to those described in the literature from Greece and Colombia by the occurrence of germacrene D, β-elemol, γ- and β-eudesmols.[2,3] However such a high amount of sesquiterpenes (>80%) and moreover the occurrence, in a Citrus species, of α and β amorph-4-en-10-ols, trans-guai-6-en-10-ol and germacra-1(10),5-dien-4α-ol is reported for the first time.

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PP-192. Evaluation of composition of bitter orange leaf oil obtained by hydrodistillation methods and Antioxidant activity

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The bitter orange tree (*Citrus aurantium* L.) which comes from China, is very frequent on the Mediterranean periphery. Because of its high resistance to cold and humidity, this tree is used as a rootstock in many Mediterranean countries. Bitter orange oil is widely used in perfumery and for flavoring candies, soft drinks, and baked goods (1-3).

The bitter orange *(Citrus aurantium)* leaf oil was hydrodistillation methods. The bitter orange samples used in the present study were planted at Batı Akdeniz Tarımsal Araştırma Enstitüsü "BATEM" located at Antalya, South Turkey, in october 2011.

The chemical compositions of the leaf oil samples were analyzed by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). 48 components were identified by mass spectra (4), linear retention indices. Bitter orange leaf oil has high content of oxygenated components (92.48%). The major oxygenated components of the leaf oils were found as the following ester components (55.89%): linalyl acetate (48.23 %), geranyl acetate (4.82 %) and neryl acetate (2.59 %); alcohol components (35.73 %): linalool (28.07 %), α-terpineol (5.53 %) and geraniol (1.21 %); carbonyl components (0.86 %): geranial (0.50 %) and neral (0.29 %). The major monoterpene hydrocarbons (6.77 %) were myrcene (2.06 %), limonene (1.78 %), and *(E)*-β-ocimene (1.23 %). The major sesquiterpene hydrocarbons (0.64 %) were *(E)*-caryophyllene (0.43 %). The antioxidant activity of the bitter orange leaf oil samples were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay. Bitter orange leaf oil samples showed high DPPH scavenging activities.

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PP-193. Variation in essential oil of *Daucus sahariensis*

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The essential oils obtained by hydrodistillation from *Daucus sahariensis* Murb. harvested at three different growth stages were characterized by GC/MS analysis. In total, 88 compounds were identified, with myristicin (29.8–51.7%), myrcene (6.7–31.1%), α-pinene (11.6–14.8%), and limonene (5.3 –11.5%) as main constituents. Monoterpene hydrocarbons were the most represented compounds in the oils of the plant samples collected during the flower-budding and full-flowering periods. On the contrary, during the fruiting stage, the oils were dominated by phenylpropanoids. The essential oils were subject of considerable variation in their composition during the various developmental stages, particularly concerning the content of myrcene that decreased significantly passing from the vegetative to the fruiting stage. Conversely, for myristicin, the opposite trend was observed. Furthermore, the essential-oil yields were quite low during the flower-budding phase (0.27%), but rapidly increased during plant development (0.63 and 0.68% for the flowering and fruiting phases, resp.)

PP-194. Chemical diversity of essential oil and lipids of *Marrubium peregrinum* L. from Turkey

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Marrubium L. genus (Labiatae) is represented by 20 species and 11 of them are endemic in Turkey (1). *Marrubium peregrinum* L. was collected during the flowering time in B3: Eskişehir at an altitude of 950 m around Aşağı Kalabak Village on September 2013. Essential oil from herb of *M. peregrinum* was obtained by hydrodistillation in a Clevenger apparatus while lipids were extracted by Folch method. Fatty acids were methylated with Boron trifluoride-methanol complex reagent. The essential oil and fatty acid methyl esters were analyzed by GC/FID and GC/MS techniques. The essential oil yield was 0.1% (calculated on a moisture free basis).

Sesquiterpene hydrocarbons predominated (45.9%) in the oil with β-caryophyllene (24.9%) and germacrene D (6.6%) as major constituents. Oxygenated sesquiterpenes (31.9%) were the next high abundance group with caryophyllene oxide (21.6%) as major constituent. Among fatty acids of the essential oil, hexadecanoic acid (*syn.* palmitic acid) was detected in high percentage (9.1%). The essential oil was subjected to preliminary test for antioxidant activity with DPPH reagent using HP-TLC technique.

Lipids of *M. peregrinum* contained a wide range of fatty acids from C_{12} to C_{21}. Lipid fraction was mostly constituted with unsaturated fatty acids like linolenic (25.0%), linoleic (22.0%) and oleic (6.5%), while saturated ones were presented by palmitic acid (21.0%).

The present work is the first detailed contribution into the chemistry of the essential oil and lipids of *Marrubium peregrinum* from Turkey.

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PP-195. Antibacterial evaluation and chemical characterization of *Cupressus sempervirens* L. from Algeria

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The antibacterial activity of the essential oil of the leaves of (*Cupressus sempervirens* L.) on human pathogenic bacteria by disc diffusion method via average inhibition zone was studied. The chemical composition of the essential oil of the leaves of (*Cupressus sempervirens* L.) were obtained by hydrodistillation method and analyzed by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS).

Analyses find the presence of 18 constituents, major components being kaurene (37.6 %), the monoterpenes, mainly α-pinene (24.7 %), δ-3-carene (8.7%), the sesquiterpenes: germacrene D (3.1%), cadinene (0.2%), sesquiterpenol : cedrol (6.2 %) and Monoterpenols terpinen-4-ol (2.9 %).

We tested bacterial strains frequently isolated in hospitable circles. So, during our study we worked on Gram positive bacteria *Staphylococcus aureus , Enterococcus sp ,* and *Streptococcus D and* Gram negative bacteria: *Escherichia coli, Proteus mirabilis, Acinetobacter sp* .

For every bacterium, we used four concentrations: the raw oil, the oil diluted in 1/2, in 1/4, and in 1/8. The essential oil was evaluated by disc diffusion method and the minimum inhibition concentration (MIC) of the *C sempervirens* L extracts was 5 mg/mL for all the microorganisms tested. The results showed that the essential oil tested varied in their antibacterial activity

PP-196. Nematicidal activity of essential oils of *Lavandula hybrida*

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Phytoparasitic nematodes can be strongly destructive and extremely difficult to manage due to their poliphagy and worldwide spread. Plant-derived compounds can represent an environmentally sustainable alternative to chemicals for the control of these plant pests, due to the presence of nematicidal secondary metabolites in many botanical families (1). The essential oils from a large variety of plants have been already demonstrated for their high toxicity on phytonematodes, mainly root-knot species (2, 3), though the nematicidal potential of many other plant essential oils is still to be investigated and commercially exploited. Lavender hybrids or lavandins (*Lavandula hybrida* Rev.; Lamiaceae family) rank among the most economically important crops within the worldwide flavor and fragrance market. Their essential oils, extracted from flowering tips of a huge number of cultivars are widely used in soaps, cosmetics, perfumes, but recent developments in research have suggested a higher applicative potential of lavandin essential oil as a consequence of proven *in vivo* and *in vitro* analgesic, anti-inflammatory, sedative and antibacterial properties. The aim of this work was a preliminary assessment of the biocide activity of essential oils from lavandin varieties RC, Sumiens, Abrialis and Alba against two economically relevant phytonematode species, i.e. the root-knot nematode *Meloidogyne incognita* and the root-lesion nematode *Pratylenchus vulnus*. The four essential oils were obtained by a 2 h steam distillation of freshly collected apical flowering parts of each lavandin variety. The infective stages of *M. incognita* and *P. vulnus* were exposed to 100, 50, 25 and 12.5 µl ml^{-1} solutions of each essential oil for 4, 8 and 24 h, assessing their viability after each exposure time. All the tested oils resulted highly active on *P. vulnus*, as 45 – 62% mortality rates occurred even after a 4 h exposure to the lowest concentrations and up to 90% of nematode specimens died after a 24 h treatment with the 100 µl ml^{-1} oil solutions. Juveniles of *M. incognita* were found less sensitive than *P. vulnus*, mainly at the shorter times of exposure to the lower oil rates. Biocidal effect of *L. hybrida* oils was strictly dose and time related for both nematode species. Among the four lavandin cultivars, oil of Alba and RC were the most toxic to *P. vulnus* and *M. incognita*, respectively, whereas the oil of Abrialis was the less effective on both nematode species. Essential oils of *L. hybrida* demonstrated a high potential for the formulation of new environmentally safer nematicides, as highly and promptly active also at very low application rates.

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PP-197. Essential oil and absolute of *Elaeagnus angustifolia* L. from Turkey

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The genus *Elaeagnus* of Elaeagnaceae is represented by one specie, *Elaeagnus angustifolia* L. in Turkey (1). *Elaeagnus angustifolia* L. was collected from Eskişehir in Central Anatolia. Traditionally infusion prepared from the flowering tops and leaves is used as diuretic and antipyretic (2). The essential oil and absolute of the flowering tops were initially obtained by hydrodistillation and solvent extraction, respectively. Furthermore the essential oil and the absolute were analyzed by both GC and GC-MS, simultaneously. The major compounds were identified as ethyl cinnamate (35.7 %), hexahydrofarnesyl acetone (20.6 %), ethyl oleate (7.5 %) for the essential oil and hexadecanoic acid (27.9 %), hexahydrofarnesyl acetone (9.3 %), 1-docosene (8.7 %) for the absolute. To the best of our knowledge this is the first study on the essential oil and absolute of *Elaeagnus angustifolia* L. from Turkey.

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PP-198. Essential oil composition of *Centaurea aggregata* Fisch. & Mey. Ex. Dc. subsp. *Aggregata*

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The genus *Centaurea* is a large genus represented about 1000 species and is widespread all around the world. *Centaurea* genus is represented with 192 taxa in Turkey. Many species of the genus have been used in herbal medicine for their tonic, antipyretic, expectorant and antidiarrhoeal effects in Anatolian folk medicine. *Centaurea* species have been mostly studied for sesquiterpene lactones, flavonoids and alkaloids. Many *Centaurea* species have been investigated especially for their volatile constituents. The essential oil composition of some *Centaurea* species from Turkey have been investigated.

The present work reports on the essential oil of *Centaurea aggregata* subsp. *aggregata* collected Elazığ, Turkey. The oil was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) systems. A total of 26 components were identified in the essential oil. The main constituents of the oil have been revealed as follows: hexadecanoic acid (32.7%), germacrene D (14.8%), and phytol (12.3%).

PP-199. Antimicrobial activity and chemical composition of *Thymus praecox* subsp. *grossheimii* var. *grossheimii* volatiles by HD, MW and SPME

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Genus *Thymus* L., has more than 200 species, is one of the most important genera as regards number of species within the Lamiaceae family. *Thymus* belongs to tribe Mentheae, subfamily Nepetoideae. *Thymus* is distributed throughout the arid, temperate and cold regions of the world north of the Equator and on the coasts of Greenland.[1] *Thymus* genus is presented by 64 taxon and 27 of them endemic for Turkey[2,3]. *Thymus praecox* subsp. *grossheimii* var. *grossheimii* is used for cold, cough, and infectious disease.

The volatile constituents of the essential oils from *T. praecox* subsp. *grossheimii* var. *grossheimii* extracted by hydrodistillation (HD), microwave distillation (MW) and solid phase micro extraction (SPME) and analyzed with GC-FID/MS.[4,5] A total of 61 compounds were identified, constituting over 99.6%, 99.0% and 99.6%, respectively. The main components of the essential oils were carvacrol (41.6%), thymol (16.7%), *p*-cymene (13.3) and E-caryophyllene (3.1) in HD; thymoquinone (47.7%), γ-terpinene (10.7%), carvacrol (9.0%) and *p*-cymene (6.4%) in MW; carvacrol (33.3%), *p*-cymene (20.9%), thymol (18.6%) and thymoquinone (6.3%) in SPME. In addition, the antimicrobial activities of the essential oils of *T. praecox* subsp. *grossheimii* var. *grossheimii* extracted by HD and MW were investigated. The oils showed antimicrobial activity against Gram negative bacteria, Gram positive bacteria, *Mycobacterium* and fungi.

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PP-200. Antimicrobial activity and chemical composition of *Thymus pseudopulegioides* volatiles by HD and SPME

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The genus *Thymus* (Lamiaceae) consists of about 215 species of herbaceous perennials and subshrubs [1]. *Thymus* genus is presented by 64 taxon and 27 of them endemic for Turkey [2,3]. *Thymus pseudopulegioides* Klokov & Des.-Shost. grows in the Northeast Turkey [2]. The aerial parts of *T. pseudopulegioides* are used as a herbal tea.

The volatile constituents of the essential oils from the *T. pseudopulegioides* were extracted by hydrodistillation (HD) and solid phase micro extraction (SPME) and analyzed with GC-FID/MS [4,5]. A total of 58 compounds were identified, constituting over 99.4% and 99.6%, respectively. The main components of essential oils were *p*-cymene (39.1%), thymol (28.3%), carvacrol (5.7%) and thymoquinone (4.4) in HD; carvacrol (22.2%), α-pinene (16.6%), caryophyllene (16.2%) and thymol (11.6%) in SPME. In addition, antimicrobial activities of the essential oil *T. pseudopulegioides* extracted by HD was investigated. The essential oil showed activity against Gram negative and Gram positive bacteria, Mycobacterium and fungi.

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PP-201. Biological activities and essential oil composition of *Salvia veneris* hedge.

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Salvia veneris Hedge is an endemic plant of Cyprus which is reported to occur in a very limited region around Kythrea (Değirmenlik) village. In our field studies we have observed this species in a wide area occurring in small but continuous populations between Taşkent to Buffavento in the sides of the mountain road unlike the previous reports. This plant is confused with *Salvia officinalis* and used as a herbal tea in the region. Aerial parts (200 g) of the plant was collected from the Taşkent location without disturbing the roots or the population in the region. Water-distilled essential oil with a 1% yield was obtained from aerial parts of *Salvia veneris* Hedge from Cyprus. The essential oil was investigated for acetylcholinesterase, butyrylcholinestearase inhibition and contact toxicity against *Spodoptera exigua* (Hübner). The essential oil produced 63.03% contact toxicity against the *S. exigua* (beet armyworm) larvae at 100 µL/mL concentration. The essential oil was also analyzed by gas chromatography and gas chromatography/mass spectrometry. The oil obtained from the aerial parts of the *Salvia veneris* was characterized by 1,8 cineole 30.4%, camphor 18.4%, camphene 12.9%., α-pinene 8.2%, borneol 5.8% and β-pinene 5.0 %.

PP-202. Chemical composition, antibacterial activity of a new essential oil chemotype of Algerian *Artemisia arborescens* L.

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The aim of the present study is to investigate the chemical composition, antibacterial activity of three essential oils extracted by hydrodistillation from the aerial parts of *Artemisia arborescens* L., which was collected from three different regions near Tlemcen city in the West Northern of Algeria: Beni Snous, Bidar and Chetouane. The chemical composition was investigated using both capillary gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) techniques. Fifty-two compounds were detected, a total of fifty compounds, representing 81.8 to 90.2% of the total oils were identified in the three samples of essential oils. The essential oils of *A. arborescens* were rich in camphor (Beni Snous: 72.2%, Bidar: 50.3% and Chetouane: 32.8%). The present composition of the essential oils, with camphor as the only most abundant component, was considered as a new chemotype of *A. arborescens* growing in West Northern of Algeria. Antibacterial activity of the essential oils against Gram-positive and Gram-negative bacteria, was tested using the diffusion method and by determining the inhibition zone. The results showed that the oils had a great potential antibacterial activity against some bacteria. The maximum zone of inhibition was obtained against *Enterococcus faecalis* (22 mm), whereas the oils were ineffective on the inactivation of *Lysteria monocytogenes* and *Escherichia coli*.

PP-203. Characterization and biological evaluation of Chia (*Salvia hispanica*) essential oil

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Salvia L. is the largest genus within the mint family (Lamiaceae), with nearly 1000 species of shrubs, herbaceous perennials, and annuals. *Salvia hispanica* L.also wellknown as "Chia" is native to southern Mexico and northern Guatemala, and has recently been re-introduced and marketed as a seed crop starting from its orijin South America to Europe. Generally, *Salvia* species are popularly used in folk medicine as antiseptics, astringents and spasmolytics, however, in modern times its preparations are used as antioxidant, antimicrobial and due the antiviral activities.

In this present study, Chia seeds were first propagated and then cultivated, which was later collected at flowerging stage from the experimental field in Konya, Turkey. Essential oil in good quantities (more than 1%) were isolated from the dried flowers of the plant was obtained by Clevenger hydrodistillation. For the chemical characterization the oil was analyzed by both GC-FID and GC-MS techniques, simultaneously. In addition the essential oil was evaluated for its antimicrobial activity against a wide spectrum of human pathological strains. Using an *in vitro* photometric quantitative radical scavening activity assay and acetylcholinesterase (AChE) and butyrylcholine esterase (BuChE) enzyme inhibition assays according the Elmann method were also performed.

As result the major essential oil compounds were identified as β-caryophyllene (43%), germacrene D (28.4%), α-humulene (8.6%), bicyclogermacrene (3.9%), caryophylleneoxide (3.4%), respectively. For the biological activity results of the Chia essential oil showed rather modest antibacterial activity against *Propionibacterium acnes* and *Candida glabrata* with 125 µg/mL. The antioxidant activity using the DPPH radical scavenging assay of sample showed 63.4% inhibition at 1% conc. Finally, AChE and BuChE activities were less than 1% when compared to the standard reference compounds, suggesting rather low inhibitions.

Acknowledgments

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PP-204. Chemical composition and antibacterial activity of four essential oils from *Ferula vesceritensis* Coss. & Dur. against clinical isolated and food-borne pathogens

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The genus *Ferula* (Apiaceae) is represented by more than 170 species distributed in Central Asia and the Mediterranean region[1]. The Algerian flora comprises 5 species of *Ferula* of which 2 are endemic[2,3]. Antimicrobial, antioxidant and antiepileptic activities have been reported for *Ferula* essential oils[4-7]. We report here the chemical composition and antibacterial activity of four hydrodistilled oils of *Ferula vesceritensis* Coss & Dur, grown in Algerian Sahara. Essential oils were obtained from the hydrodistillation of fresh flowers (FF), dry flowers (DF), fresh stems (FS) and dry stems (DS). The GC–MS analyses of the oils revealed the presence of forty two compounds for FF, thirty seven compounds for DF, forty eight compounds for FS and thirty six components for DS. The total yields of the volatile essential oils were respectively: 97.9% (FF), 88.6% (DF), 96.4% (FS) and 87.4% (DS) with the prevalence of α-pinene (FF 32%, DF 16.1%, FS 11.5%, DS 17.4%), β-pinene (FS 8.1%, DS 8.9%) α-phellandrene (FF 8.5%, DF 24.3%), fenchylacetate (FF 10.40%, FS 7.3%, DS 8.8%), elexene (DF 6.3%, FS 5.4%), aristolene (FF 5.4%, FS 7.2%, DS 6.8%), caryophyllene oxide (FS 7.6%) and carotol (FF 13.9%, DF 6.7% FS 18.8%, DS 10.8%). The essential oils were tested against 9 bacteria and showed a good antibacterial activity against almost food-borne pathogens and clinical isolated microorganisms at a concentration of 128 µg.ml^{-1} Minimum inhibitory concentration (MIC) values for all the bacteria were ranged between 16 µg.ml^{-1} and 80 µg.ml^{-1}.

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PP-205. Antioxidant activity of Chamomile essential oil

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The objective of this study was to characterize the antioxidant capacity of the chamomile (*Matricaria recutita* L) essential oil as well as its major constituents. For this purpose, Pharmacopoeia grade chamomile essential oil was purchased from commercial sources and used after the analytical quality evaluation, which was confirmed by GC/MS and GC/FID, respectively. The main components were identified as bisabolol oxide A (47.7%) and B (6.2%), (E)-β-farnesene (21.5%), bisabolon oxide A (5.7%), chamazulene (4.1%) and α-bisabolol (2.1%). Bisabolol oxide A, farnesene, chamazulene and α-bisabolol were isolated using successive preparative thin layer chromatography techniques from the chamomile essential oil and the identification and quantification of the constituents were through GC/MS and GC/FID analysis. The antioxidant activities were evaluated both qualitatively and quantitatively spectrophotometric methods by the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals using the common scavenging assay.

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PP-206. Phytochemical composition and biological activities of the essential oils and non-volatile extracts of six medicinal and aromatic plants of the Algerian flora and their uses in the food and pharmaceutical industries

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The vegetal extracts of aromatic and medicinal plants start to have much of interest like potential sources of natural bioactive molecules. Actually, it make the object of many study not only for their therapeutic activities as alternatives for the treatment of infectious diseases but also in agriculture as food preservatives additives in the formulations of functional foods and nutraceuticals. This functions, are in fact conferred by the nature of the chemical function of their major constituents, which play a preponderant role in the efficacy of their biological activity. This bio-potential led us to focus on the study of three main biological activities, the antioxidant, antibiotic and insecticidal activities of six Algerian aromatic plants belonging to the families of Lamiaceae, Rutaceae and Asteraceae.

Chromatographic analyses was carried out by GC and GC/MS. The contents of oxygenated monoterpenes represented the most prominent group of constituents in the majority of plants. **α**-Terpineol (28,3%), carvacrol (47,3%), pulegone (39,5%), chrysanthenone (27,4%), thymol (23,9%), **γ**-terpinene (23,9%) and 2-undecanone (94%) were the main components. The antioxidant activity of these essential oils was evaluated *in vitro* by inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity (ABTS•+), the thiobarbituric acid reactive substances (TBARS) assays and the reducing power. The measures of the IC_{50} of these six EOs and the three phenolic extracts revealed potent activity (between 254,64-462,76 mg/l), almost similar to that of BHT, BHA, Tocopherol and Ascorbic acid. The study on the insecticidal activity effect by contact, inhalation, fecundity and fertility of *Callosobruchus maculatus* and *Tribolium confusum* showed a strong potential biocide reaching 95-100% mortality only after 24 hours. The antibacterial and antifungal activity of our essential oils were evaluated by a qualitative study (aromatogramme) and quantitative (MIC, MBC and CML) on four bacteria (Gram+ and Gram-) and one strain of pathogenic yeast, the results of these tests showed very interesting action than that induced by the same reference antibiotics (Gentamycin, and Nystatin Ceftatidine) such that the inhibition diameters and MIC values for tested microorganisms were in the range of 23–58 mm and 0.015–0.25%(v/v) respectively.

PP-207. Efficacy of parsley and celery essential oils against the growth of *Bacillus cereus* in commercial chicken soup

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Parsley (*Petroselinum crispum* (Mill.) Fuss) and celery (*Apium graveolens* var. *dulce* (Mill.) Pers.) are well known spices from the Apiaceae plant family, used in almost all cousins of the world. The most frequent culinary use of these herbs is to improve or impart the flavor of/to soups and various sauces. On the other hand, as plant species with known antimicrobial properties [1, 2], they can be used for short-term conservation of these types of food. Up to date, efficacy of these spices in food models, which represent the only valid type of investigations related to food, was not investigated. *Bacillus cereus* is a foodborne pathogen, which grows at various temperatures (from 4 °C up to 50 °C, optimum at 30-37 °C) and produces several types of toxins [3]. In the case of ingestion of food heavily contaminated with this bacterium, severe diarrhoea and vomiting occurs as a consequence.

The goal of this study was set to investigate the potential of parsley and celery essential oils for prevention of soup spoilage caused by *B. cereus*. The essential oils were isolated from fresh plant material (leaves) and their chemical composition investigated by GC and GC-MS. The composition of the oils was in general agreement with those previously published for the two plant species [1, 4]. Minimal inhibitory concentrations (MIC) of both oils against two strains of *B. cereus* (an isolate from food and an ATCC strain) were primarily determined by a microdilution method. In order to find out whether the two oils exhibit the same effect on bacteria in an actual food system, commercial chicken soup was used as a model, and the effects of both essential oils in their MIC concentrations on the growth of *B. cereus* (food isolate) were evaluated. Influence of different storage temperatures (4, 18 and 37 °C), and different periods of cultivation with the essential oils was also investigated, where the bacterial number was monitored over a total cultivation period of 72 h. It was determined that the growth of the bacterium was significantly reduced at MIC concentrations of both oils in soup and that these oils can be used as very effective food spoilage preventing ingredients.

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PP-208. Antimicrobial activity of the essential oil of *Heracleum sphondylium* L. (Apiaceae): synergistic interactions of individual constituents, cell membrane damaging and antibiofilm activities

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Heracleum sphondylium L. is a perennial plant species belonging to the family Umbelliferae. Its roots and stems are used in folk medicine against diarrhoea, hypertension, menstrual disorders and dyspepsia [1, 2]. The results of previous investigations on the antimicrobial activity of the essential oil of this plant species were promising [2, 3]. However, these studies were mostly oriented towards screening for antimicrobial activity, without an attempt at revealing the mechanism of antimicrobial action of *H. spondylium* essential oil.

For this reason, we decided to investigate the antimicrobial effect of the essential oil of the fruits of *H. sphondylium* collected in Serbia. It was our intention to locate the active principle(s) by studying the antimicrobial effect of the major constituents of the essential oil on their own, and of their combinations (to possibly reveal a synergistic/antagonistic interaction between them). The oil was also assayed for membrane damaging effects in order to pin point its mechanism of action.

The essential oil was isolated by hydrodistillation from unripe fruits of *H. spondylium*, collected in the vicinity of the city of Niš, and analyzed by GC and GC-MS to determine its chemical composition. The essential oil and its three main components - octanol, octyl acetate and octyl butyrate were tested for antimicrobial efficacy against a total of 22 bacterial (ATCC and isolates) strains and one yeast species. In addition, the effect of the main compounds on the bacterial cell membrane was investigated by a spectrophotometric quantification of protein and nucleic acid material leakage that accompanies damage of the cell membrane. Further investigations were focused on a synergistic potential of the three individual components, where synergism was evaluated by checkerboard and time-kill methods against a representative set of the tested microorganism species (Gram-positive, Gram-negative and the yeast). Also, the antibiofilm potential of the oil and its components was evaluated against a strong biofilm producer strain of *Pseudomonas aeruginosa*.

The results pointed to the highest activity of the essential oil, while octanol was the most active among the tested pure components. Low levels of leakage of cell material showed that cell membrane damage is not the primary mechanism of action of this oil. The checkerboard method results revealed an existing synergy between all three compounds in the cases of *Candida albicans* and *Escherichia coli* and a synergistic interaction between octyl acetate and octyl butyrate against *Staphylococcus aureus*, while the other two combinations demonstrated only an additive effect against this strain. The time kill method results were in accordance with the active combinations (of a total of 21 combinations) noted in the checkerboard method. The antibiofilm activity testing revealed that the three essential oil components possess a significant effect on biofilm formation.

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PP-209. Effect of water supply on growth, physiological and essential oil properties of *Thymus vulgaris*

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Thyme (*Thymus vulgaris* L.) is a widely cultivated medicinal and aromatic plant originating from the West Mediterranean, where adapted to sunny and dry habitats. 'Varico 3' is a very productive Swiss F_1 hybrid variety of thyme accumulating high level of essential oil of thymol chemotype. Drought is one of the greatest limitation to crop production and will be increasingly important due to the recognized effects of the global climate change. In our studies we were aimed at evaluating the effects of water deficit on Thymus *vulgaris* 'Varico 3' plants, with special respect to its impact on plant growth, physiological and essential oil properties. With these data we were intended to assess certain environmental factors influencing yields and drug quality under cultivation.

The experiments were carried out among controlled conditions in phytotron chambers on 48 young plants potted into normal soil, divided into three groups according to different soil water levels (SWL) (Control: 70 %, S1: 50 % and S2: 30 %). The experiment was conducted from October until December 2013, including one month of acclimatization (October) and two months of drougth stress treatment.

The water deficit resulted in a decrease of growth rate (C: 23.56-30.69 cm; S1: 21.56-27.56 cm; S2: 18.75-26.00 cm) in a four week period of measurements. Concerning biomass production (fresh, dry and drug yields), there were not significant differences among treatments, only in the case of leaf % of the drug, where the highest value was found at the lowest SWL (S2: 52.19%). Relative water content (RWC%) of the leaves were evaluted twice, where significantly lower average were detected at S2 (58.62 %) if compared to those of C (78.54%) or S1 (74.55 %) at the end of the stress treatment.

Concening chlorophyll content expressed in SPAD units, significantly higher values were detected in the case of decreased SWL (C: 52.10, S1: 64.30, S2: 72.63). Leaf water potential (LWP) showed similar tendencies and the means were statistically different (C: -10.33 bar, S1: -11.33 bar, S2: -14.50 bar). However, the amount of essential oil has not been affected considerably by SWL (C: 5.34, S1: 5.04, S2: 5.25 ml/100 g DW). Concerning volatile compounds, thymol% has decreased significantly with lower SWL, while the percentages of those with lower molecular weight (incl. *p*-cymene) had opposite tendencies.

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PP-210. Essential oil composition and aroma components of *Sideritis scardica* Griseb. from R. Macedonia

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Sideritis scardica (Mountain tea) is an endemic species for the Balkan Peninsula and its fertile steams are commonly used as medicinal and refreshment tea [1]. Up to present, there is a lack of data concerning the essential oil composition and aroma components relationship of Sideritis scardica, thus the main objectives was to investigate these components in dried rosette leaves (RL) and fertile steams (FS) in samples collected from R. Macedonia. Essential oil isolation was made by steam distillation in Clevenger apparatus for four hours and ranged from 0.07 to 0.20 % for RS and from 0.06% to 1.40 % for FS. Aroma components were obtained by headspace (HS). Essential oils' GC/FID/MS analysis revealed total of 90 components encompassing α-pinene (0.74-8.96 %; 0.51-2.16 %), β-pinene (0.05-16.17 %; 3.90-4.58 %), trans (E)-caryophyllene (5.91-15.11 %; 7.98-21.58 %), germacrene D (2.64-8.74 %; 4.62-7.92 %), caryophyllene oxide (6.02-10.02 %; 11.44-16.68 %), and hexadecanoic acid (3.13-16.07 %; 7.45-7.76 %), as a major constituents for FS and for RL, respectively. Among twenty two identified HS aroma components, predominant were α-pinene (20.38-29.60%; 10.04-27.36 %), camphene (0.05-1.88 %; 1.60-12.76 %), β-pinene (3.02-36.34 %; 3.23-40.85 %) and limonene (4.13-10.60 %; 4.95-39.52 %), for FS and for RL, respectively. Rosette leaves have comparable aroma compounds and essential oil composition and yield to those from the fertile steams and can be recommended for use as optional herbal material form *Sideritis scardica*.

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PP-211. Comparative analysis of the chemical composition of berries and leaves essential oils from *Juniperus excelsa* Bieb. (Cupressaceae) from R. Macedonia

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Juniperus excelsa is a mountain forest element in the East Mediterranean Basin used in the folk medicine to treat dysmenorrhe, cough, bronchitis, jaundice and tuberculosis and to induce menses and expel fetus [1]. Up to present no chemical investigations are done on the samples from territory of R. Macedonia (RM), thus the main objective was to determine the yield and the chemical composition of essential oils of berries (EOB) and leaves (EOL) of this Juniperus species. Essential oil isolation was made by steam distillation in Clevenger apparatus for 4 h and the oil yield ranged from 1.6 to 9.4 ml/kg and from 8.9 to 13.9 ml/kg for EOB and EOL, respectively. Essential oils' GC/FID/MS analysis revealed total of 74 components, representing 82.69% to 98.13% of the oil. The monoterpene constituents were dominant fraction which was present with more than 80% in EOB compared to the 40% in EOL. On the other hand the sesquiterpene fraction although present in lower amounts, represent min 7% of the EOL and max 6.5% of the EOB. Two different chemotypes of EOB and EOL were distinct: pinene-type (70.81% α-pinene in EOB and 33.83% in EOL), and sabinene-type (58.85-62.58% sabinene in EOB and 28.52-29.49% in EOL), originated from south-eastern part of RM (Lake Dojran) and from south-western part of the country (Lake Ohrid), respectively. Therefore collections sites can be considered as important factor for the variability of the berries and leaves essential oil composition of J. excelsa.

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PP-212. Microbial transformation of α-Ionol

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Flavour and fragrance compounds have a wide range of use in food, beverages, cosmetics, pharmaceuticals and create a worldwide market [1-2]. Their productions are mostly based on synthetic routes, however, consumers prefer natural compounds. Biotechnological processes are a natural way of producing flavours and fragrances. Especially microbiological transformation reactions have gained importance in recent years for the production of industrially important aroma compounds. Terpenes are a wide class of compounds showing aroma properties and are used as starting material for biotechnological production of new flavour and fragrance compounds [3-4].

In this present study, we aimed at obtaining new compounds starting from α-ionol through microbial biotransformation. For this purpose, pre-biotransformation of the selected substrates were carried out with 19 different microorganisms. We found that from the biotransformation of α-ionol with A. niger NRRL 326, *F. culmorum* interesting oxygenated metabolites. Upon scale up the formed metabolites were purified by column chromatography and their structures were elucidated by NMR spectroscopy. For α-ionol, both biotransformation reactions produced the same metabolite with % 6,6 (*A. niger* NRRL 326) and % 15,3 (*F. culmorum* -isolate) yields, respectively.

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PP-213. Creation of a vapour phase fourier-transform infrared spectroscopy database as a tool for the identification of components in complex mixtures

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Fourier-transform infrared (FT-IR) spectroscopy is a useful detection technique in gas chromatography and it can be used as a universal, selective and specific detection method, even if a major drawback is its lack of sensitivity.

The power of GC/FT-IR is the unique structural information that can be extracted from the spectral data and cannot be obtained from any other analytical method. In fact sometimes mass spectrometry fails in the discrimination of isomers having the same basic molecular structure and thus the same fragmentation pattern.

In this concern, such information is an additional valuable support to mass spectral and retention data, for a correct identification of targeted and untargeted compounds. Furthermore, the non-destructive nature of IR detection makes the analyte available for other purposes such as fraction collection or other detection techniques.

The present research describes the construction of a novel vapour phase FT-IR spectral database, containing more than 1000 pure spectra, belonging to the flavour and fragrance field. The database was evaluated by using a multidimensional gas chromatography system, an approach that enabled the injection of higher sample amounts, thus avoiding sensitivity issues.

Acknowledgments

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ADDENDUM

PP-214. Comparison of chemical composition and yield of *Thymelaea hirsuta* volatile oils obtained by supercritical carbon dioxide extraction and hydrodistillation methods

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Thymelaea hirsuta (L.) Endl., commonly known as "Methnane", is a shrub of Thymeleaceae. This plant is known for its powerful antiseptic, anti-inflammatory properties and for the treatment of hypertension. Besides, it has been used in folk medicine as antimelanogenesis (1), antioxidant (2), and antidiabetic (3) drugs.

In the present work, volatile oils from the aerial parts of *T. hirsuta*, cultivated in Tunisia, were obtained by two different extraction methods: hydrodistillation (HD) and Supercritical fluid extraction using carbon dioxide (SCE). The volatile oils obtained by both methods were quantified by gas chromatography-flame ionization detection (GC-FID) and identified by gas chromatography-mass spectrometry (GC-MS). The compounds were identified according to their retention indices and mass spectra. The major compounds of *T. hirsuta* present in the extracts obtained using SCE were hexadecanoic acid (15.4%), 4, 8-dimethylhecosane (12.9%) and 13-methylhexacosane(5%). For the oil obtained by HD stylopsal (15.5%), hexadecanoic acid (14.4%) and (Z,Z)-5,7-dodecadienal (12.2%) were the main compounds. Finally, the SCE was compared with the hydrodistillation methods in terms of selectivity, extraction duration and chemical composition. The results showed that the oil extracted from SCE was richer in organic compounds and leading to a higher yield.

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PP-215. Two-Dimensional low-temperature thin layer chromatography tests for detection of free radical scavengers and acetyl-cholinesterase inhibitors in volatile samples

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Neurodegenerative diseases such as Alzheimer's disease have become major health problems globally. Numerous plants' essential oils have been investigated for their potential use in neurodegenerative diseases as well as to prevent oxidative stress. There is a need to develop a simple, high throughput analytical procedure in order to point out volatile samples possessing free radical scavenging and acetyl-cholinesterase (AChE) inhibitory activity. Two procedures, TLC-DPPH● and TLC-AChE tests were used for assessing both free radical scavenging and acetyl-cholinesterase inhibitory activity of different essential oils (1). *Lavandula stoechas* L., *Lavandula multifida* L., *Origanum majorana* L. and *Salvia officinalis* L were the studied plants. The application of low temperature thin layer chromatography is necessary because of the volatility of the samples.

In the first step, "dot blot" tests were performed to pinpoint the volatile samples with the aforementioned activities. As all the studied essential oils were found to contain active compounds, they were all passed to further analysis. In a subsequent step, the volatile samples components were separated on TLC silica gel by means of two dimensional development. 2D-TLC is performed by spotting the sample in the corner of a square chromatographic plate and developing in the first direction with the first eluent (15% ethyl acetate in n-heptane). After the development is completed, the plate is then removed from the developing chamber and the solvent is allowed to evaporate from the layer. The plate is rotated through 90° and then developed with another eluent (80% dichloromethane in n-heptane) in the second direction. Upon completion of the 2D-development and plates' drying step, the plates were stained with DPPH● chloroformic solution to generate free radical scavenging fingerprints. Bleaching of the purple DPPH● colour was observed and recorded using a scanner. AChE inhibitory activity for partially 2D separated compounds was detected with diazotization method originally proposed by Marston et al. (2). The plates were immersed in freshly prepared acetylcholine solution. After incubation 20 min at 37°C, the plates were dipped for 5s in derivatizing agent prepared from equal volumes of two solutions: 1-naphtyl acetate methanolic solution and aqueous Fast Blue Salt solution. Finally an improved image processing protocol, with the use of Sorbfil TLC Videodensitometer, was applied to quantitatively measure the terpens' activity and screen complex samples for the presence of free radical scavengers and acetyl-cholinesterase inhibitors.

The application of these procedures revealed that essential oils obtained from *Lavandula stoechas* L., *Lavandula multifida* L., *Origanum majorana* L. and *Salvia officinalis* L. contain substances with both direct antioxidant and AChE inhibitory activities.

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PP-216. Essential oils composition variability of *Daucus gracilis* growing in Algeria

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The aerial parts of *Daucus gracilis* Steinh. (1) was collected from twenty different locations in West Northern of Algeria. Essential oils were prepared by the hydrodistillation of fresh plants and analyzed by GC and GC-MS methods (2-5). The dominant constituents were 2-methylbutyl 2-methylbutyrate (9.2-20.2%), linalool (12.5-22.6%), 2-methylpropyl 3-methylbutyrate (2-5.8%), E-β-ocimene(0.2-12.8%), 2-methylbutyl 2-methylpropionate (4.2-12.2%), 3-methylbutyl 2-methylbutyrate (2.1-5.7%), 3-methylbutyl 3-methylbutyrate (3.3-9.6%) and 2-methylbutyl 3-methylbutyrate (2.8-8.3%). The amounts of individual constituents in the oils varied from 2 to 20 times, while the quantities of oxygenated terpenes were close. The *D. gracilis* essential oils included higher amounts of the oxygenated terpenes (16.6-36.4%) compared to hydrocarbons terpenes (11.1–16.4%). The fifty one identified compounds comprised 95.0–99.1% of the oils. To identify possible relationships between volatile compound abundances and geographical origins, principal component analysis (PCA) and cluster analysis (CA; dendrograms) were applied to a matrix linking essential oil compositions to sample locations.

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PP-217. Antioxidant activity and chemical composition of the essential oil from aerial parts of *Mentha rotundifolia* from Algeria

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In this present study, it was aimed to evaluate the in vitro antioxidant activity and essential oil of *Mentha rotundifolia* (L.) Huds. from Algeria. Chemical composition of the essential oil from the aerial parts of *M. rotundifolia* obtained by hydrodistillation were analyzed by both GC and GC-MS. The oil yield of dried plant was 0.21%. The essential oil mainly consisted of monoterpene hydrocarbons (0.4%), oxygenated monoterpenes (22.5%) sesquiterpene hydrocarbons (6.3%) and oxygenated sesquiterpenes (15%), respectively. Also the extract sample was subjected to screening by using DPPH assay. Methanol extract was tested against antioxidant activity by using DPPH assay. It was found that methanol extract *M. officinalis* exhibited strong *in vitro* inhibitory antioxidant activity.

PP-218. A comparative study for the variation of essential oils composition of Jordanian *Achillea fragrantissima* obtained by different extraction methods

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Three extraction methods were used for a comparative study for the determination of the chemical constituents of the volatile principles of Jordanian *Achillea fragrantissima* (Forssk.) Sch. Bip. essential oils, including the conventional Hydro-Distillation (HD), the Super Critical Fluid and the Solid Phase Micro-Extraction Methods followed by GC and GC/MS analysis. The identified chemical constituents for the three extraction methods amounted to 96.61 %, 99.72 % and 98.59 % of the total composition, respectively. Oxygenated monoterpenes dominated all analyzed oils (83.48, 69.02 % and 78.11 %, respectively). SCF-essential oil was richer in sesquiterpenoids (hydrocarbons 20.32 %, oxygenated 8.31 %) as compared to the HD (3.55 %, 0.26 %) and SPME (0.50 %, 0.98). The SPME was richer in monoterpenoids (17.52 %) as compared to the conventional HD (6.22 %) and SCF (1.16 %) methods. The HD and SCF essential oils were dominated by thujone (α, β) isomers (22.87 %, 19.51 %., respectively), artemisia ketone (19.51 %, 13.27 %) and santolina alcohol (15.40, 11.62 %). The major constituents detected in the SPME method were α-thujone (33.60 %) and thujol (17.39 %), the last was undetected in the oils of the HD and SCF methods.

PP-219. The effects of essential oils of selected medicinal and aromatic plants against the phytopathogenic fungi

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The biocontrol of plant pathogens, in order to decrease the hazardous impacts of chemical pesticide application including problems of environmental pollution, public health, toxic effect on non-target organisms and causing resistance disease agents is a priority. On the other hand, interest in essential oils as an alternative to synthetic fungicides has recently gained momentum. In this present study, the antifungal properties of four standart essential oils (*Thymus vulgaris*, *Eucalyptus camaldulensis*, *Lavandula stoechas*, *Cuminum cyminum*) were evaluated *in vitro* on a medium of potato dextrose agar in three concentrations (150, 200, 250 ppm) by addition to the fungal growth medium of three pathogens (*Botrytis cinerea*, *Fusarium solani* and *Fusarium oxysporum*). The tukey test results showed the highest inhibition in mycelia growth was observed at highest concentration (250 ppm) of *Cuminum cyminum* against *Fusarium solani*. In all four samples with increasing concentration of essential oils fungal growth reduced and all of essential oils were active against of three fungi. The results showed that the essential oils could be suitable alternative as pesticides and fungicides in future.

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