ISEO 2023

53rd INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 13-16, 2023 – Milazzo, Messina, Italy

BOOK OF ABSTRACTS

"Jasmine fields" by Rosa Elisa La Rosa, acrylic on canvas, Milazzo 2022

ISEO 2023

53rd INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 13-16, 2023 – Milazzo, Messina, Italy

ORAL PRESENTATIONS

Some Thoughts on the Future of Essential Oils

K. Hüsnü Can Başer*

Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, N. CYPRUS

* E-mail: khcbaser@gmail.com; kemalhusnucan.baser@neu.edu.tr

Keywords: Essential oils, aromatherapy

Essential oils have been used for centuries in various cultures for their aromatic and therapeutic properties. These “low volume-high value” products are commercially important commodities traded and used in food, perfumery, personal care, pharmaceutical and household chemicals industries, and aromatherapy.

The global essential oil market was valued at USD 21.79 billion in 2022 and is expected to grow to USD 40.12 billion at a CAGR of 7.9% by 2030. This growth is being driven by a number of factors, including the increasing demand for natural remedies, the growing popularity of aromatherapy, and the increasing availability of essential oils.

Although, there are important issues to consider in the field of essential oils such as paucity of industrial standards, regulatory matters, environmental concerns, sustainable supplies, etc. applications in aromatherapy, veterinary and agricultural areas are promising. Advancements of essential oil extraction and analysis techniques continue. Their integration with modern medicine is considered. Evidence-based research to validate and expand the applications of essential oils has been carried out world-wide. While more comprehensive research is needed, preliminary findings highlight the potential of essential oils as a valuable addition to conventional treatment modalities.

Innovations in essential oil products and applications like micro-encapsulations and nano-emulsions are promising techniques for novel delivery systems, and to improve the bioavailability, stability, and targeted delivery of essential oils.

As the popularity of essential oils continues to rise, ensuring consumer education and safety becomes paramount.

The future of essential oils looks promising, with numerous factors contributing to the growth and development of the industry. Technological advancements in extraction and production, expanding research, product innovations, and increased sustainability efforts will all play a role in shaping the essential oils market in the coming years. With potential applications in healthcare, agriculture, and personal care, essential oils have the potential to become an increasingly important part of modern life. As the industry continues to evolve and adapt to the demands of the 21st century, it will be essential to prioritize research, innovation, and sustainability to ensure the long-term success and viability of essential oils.

The Sensomics approach, important tool for the characterisation of the key odorants in five species of the mint family (*Lamiaceae*): Aroma quality assessment of commercial spices and odorant receptor research

Peter Schieberle*

Faculty of Chemistry, Technische Universitaet München, Lichtenbergstrasse 4, 85748 GARCHING; Germany

* Email: peter.schieberle@ch.tum.de

Keywords: Key odorants, Sensomics, *Lamiaceae*, receptor studies

The rising demand for ready-to-eat meals as well as for health, and wellness foods can be expected to boost market growth for natural food flavours. Thus, the shift towards natural and healthy food ingredients is also fueling the demand for essential oils. Terpenes and terpenoids are the most important constituents of essential oils, which are generated as secondary metabolites in plants. Today, the number of known terpenoid molecules are estimated to be at least over 30000. The mint family (*Lamiaceae* or *Labiates*) is among the largest groups of aromatic plants consisting of 230 genera and more than 7000 species. Most of them contain aromatic essential oils, and due to their characteristic smell, for example the leaves of sage (*Salvia officinalis L.*), rosemary (*Rosmarinus officinalis L.*), thyme (*Thymus vulgaris L.*) oregano (*Origanum vulgare L.*) or marjoram (*Origanum majorana L.*), are either used as commercial dry powders in Mediterranean dishes or fresh from garden grown plants. For industrial purposes, essential oils are isolated by hydrodistillation from the plant material, and these oils are used for their flavour, fragrant and medicinal properties. Up to now, numerous investigations on the components present in the essential oils of the five above-mentioned plants have been published. However, there is only a very limited information on those chemical compounds generating their typical smell. The reason is that most analytical approaches applied, such as metabolomics in combination with sophisticated mass spectral measurements do lack one important issue: they are not able to address the interplay between the key aroma compounds at the bunch of more than 400 odorant receptors present in the human olfactory system. But since the characterization of odorant receptors by Buck and Axel in 1991, aroma compounds are classified as bio-active ingredients of foods. To address this issue, we have developed in the past 40 years the so-called sensomics approach to decode the chemical blueprint of a given food needed to cause the characteristic aroma perception in the human brain. Consequently, the "sensome" thus characterized is the "…complete set of small molecules present in a food able to interact with the human odorant receptors" [1]. In the first part of the lecture, results obtained by application of the sensomics approach on fresh or freeze-dried leaves of the five plants given above are discussed. The identification experiments, odorant quantitations by stable isotope dilution assays and finally aroma recombinations showed that only 14 (thyme) to 23 odorants (rosemary) are needed to mimick the overall aroma impression. Since human aroma perception is an important point in the commercial production of spices, the results obtained for the key aroma compounds in the self-prepared plant material were then compared to those present in commercial powders of sage and thyme. Detailed studies on the drying procedure indicated that drying is not a crucial step for the lower acceptance of most of the commercial products. More than 15 years ago, it has been reported that structurally the same odorant receptors (ORs) are also located in non-olfactory tissue and organs, and such ORs located in non-chemosensory organs were categorized as "ectopic" because they are expressed in unexpected regions [2]. Because certain essential oils are known as active ingredients in medicinal applications, the idea was born that probably key odorants interacting with ORs in the olfactory system might additionally show postprandial effect in the human body. The second part of the lecture will thus be focused on receptor/agonist interactions in cell studies using a receptor database containing nearly 400 different receptors. The results clearly indicated that the broadly tuned receptor OR1A1 showed interaction with thymol and carvacrol as well as with carvon suggesting a postprandial activity.

REFERENCES

[1] The sensomics approach: A useful tool to unravel the genuine aroma blueprint of foods and aroma changes during food processing. (2022) Granvogl. M., Schieberle, P. In: COMPREHENSIVE ANALYTICAL CHEMISTRY Characterization of Odorant Patterns by Comprehensive Two-Dimensional Gas Chromatography . (Cordero, C.E.I. ed)., Elsevier, Volume 96, pp.41-66.

[2] Olfactory receptors in non-chemosensory organs: The nervous system in health and disease. (2016) Ferrer, I. Esparcia1, P.G., Carmona, M., Carro, E., Aronica, E., Kovacs, G.G., Grison, A., Gustincich, S. (2016) *Frontiers in Aging Neuroscience*, Volume 8, 1-17

Chemometric strategies for the verification of authenticity claims and fraud detection in essential oils

Paolo Oliveri[1,*]

[1] Department of Pharmacy (DIFAR), University of Genova, Genova, ITALY

* Email: paolo.oliveri@unige.it

Keywords: chemometrics, authenticity verification, fraud detection, multivariate classification, class modelling

Objective

Verification of authenticity claims is a challenging analytical problem of interest to many fields, including the trade of essential oils and derived products. The question to be addressed – namely compliance of a given product with the declared claims – is qualitative and closely related to the one of quality control. In the scientific literature, most of the published papers answer such a question by means of discriminant classification methods, but it can be easily demonstrated that discriminant strategies are not appropriate and, in many practical situations, they may lead to incorrect predictions. In fact, all discriminant methods look for a delimiter between two – or more – classes, determined using a contribution from all of the classes considered. This means that all of the classes must be correctly defined and the samples included must be thoroughly representative of each class since they have a crucial influence on the decision rule to be derived. This is extremely important when the focus is on a single class like, for example, cases involving verification of an authenticity claim. In fact, in such a case, the discriminant approach would require the collection of two sets of training samples: one representative of the product to be characterised and a second representative of the entire production of the same product that does not comply with the given claim. Such a condition is rarely realisable in practice, and collected sets of non-compliant samples are often under-representative of the non-compliance possibilities. This inevitably leads to biased decision rules, the outcomes of which are heavily dependent on those samples included in the non-compliant set [1].

Methods

The most appropriate family of chemometric methods for addressing this type of problem goes by the name of class modelling [2]. Such methods perform verification of compliance with a specification by defining a multivariate enclosed class space, at a predetermined confidence level, for authentic samples of the class under investigation, enabling what is referred to as one-class classification. The first class modelling methods introduced into chemometrics were SIMCA (soft independent modelling of class analogy) [3] and UNEQ (unequal dispersed classes) [4]. Recently, strategies based on partial least squares (PLS) regression have been introduced, such as the partial least squares density modelling method (PLS-DM) [5].

Conclusions

Models built by class modelling strategies have the advantages of describing perfectly the compliant samples and being free from the distribution of non-compliant samples in the training set. Issues related to development, optimisation and validation of suitable class models for authenticity verification and fraud detection will be critically analysed and discussed, with several examples from the literature related to essential oils.

ACKNOWLEDGMENTS

Financial support provided by Università degli Studi di Genova (Research Project Curiosity Driven 2020, CUP: D34G20000100005) is gratefully acknowledged.

REFERENCES

[1] P. Oliveri, Anal. Chim. Acta. 982 (2017) 9–19.
[2] P. Oliveri, G. Downey, TrAC - Trends Anal. Chem. 35 (2012) 74–86.
[3] M. Wold, Svante; Sjöström, in: B.R. Kowalski (Ed.), Chemom. Theory Appl., Am. Chem. Soc., Washington, D.C., 1977: pp. 243–282.
[4] M.P. Derde, D.L. Massart, Anal. Chim. Acta. 184 (1986) 33–51.
[5] P. Oliveri, M.I. López, M.C. Casolino, I. Ruisánchez, M.P. Callao, L. Medini, S. Lanteri, Anal. Chim. Acta. 851 (2014) 30–6.

Laboratories facing environmental challenges: shifting the paradigm

Christophe Peres[1,*], Bastien Raccary[1,2]

[1]LE LABO DURABLE, 6 rue fontaine de geste, F-43100, Brioude, France
[2]Univ. Bordeaux, CNRS, Bordeaux INP, ISM, UMR 5255, F-33400, Talence, France

* Email: christophe.peres@lelabodurable.com

In October 2020, the European Commission published the EU's Sustainable Development Strategy for Chemicals, stating that 90% of Europeans are concerned about the impact of chemicals on the environment.
Public mistrust, growing concerns about decreasing supplies of raw materials and energy resources, and increasing environmental pollution have led the chemical community to redefine its thinking from a performance-based approach to a broader vision of sustainable development.
While the chemical synthesis community is very advanced in this direction thanks to the principles of green chemistry, analytical chemistry has received less attention on this topic. The proliferation of norms and quality standards has increased the need for accurate measurements, regardless of the nature of the samples and analytes studied. Analytical chemistry is present at every stage of a product's development, and potentially throughout the life of a product.
Today, the quantified elements of each analysis in terms of solvent, electricity, gas, etc., are mostly ignored and the environmental impact of these materials and processes is often considerable. In addition, analytical chemistry produces intangible information but requires several intensive processes (sample extraction and preparation, analysis, data management).
While there is a growing interest in analytical chemistry communications to improve the sustainability of strategies, instruments and methods, there are many projects to move from observation to action.

A first part of the work was therefore devoted to identifying the methodologies and tools missing from the analytical science community to know and reduce the environmental impact of laboratories.

In a second step, work on measurement strategies and tools was carried out.

Finally, a discussion was conducted to improve the dissemination of these methodologies allowing laboratories to be consistent with the challenges posed by climate change

Organic synthesis: a valuable tool to explore the chemistry and the properties of essential oils

Nicolas Baldovini*

Institut de Chimie de Nice, UMR 7272, Université Côte d'Azur, Parc Valrose 06108 Nice, FRANCE.

* Email: baldovin@unice.fr

Keywords: Organic synthesis, chemical transformations, combinatorial synthesis, sandalwood, agarwood.

If we consider their chemical composition, essential oils are fascinating materials: Despite their ease of preparation, the number and the molecular complexity of their constituents can be extremely high. Consequently, many chemical reactions have been discovered by exploring the reactivity of essential oil constituents, fairly accessible from natural sources and easily purified by distillation or crystallization. For example, Baeyer-Villiger oxidation and Wagner-Meerwein rearrangement were observed for the first time on classical constituents of essential oils such as camphor, menthone, alpha pinene, borneol etc. [1-3]. Furthermore, sesquiterpenic components of essential oils have often been chosen by chemists as targets for total synthesis, and the efforts to synthesize their challenging molecular structure has thus contributed to the refinement of the art of organic synthesis.

In return, practical organic chemistry can also provide very valuable tools for researchers trying to better understand the chemical composition and the properties of an essential oil. It is indeed complementary to the classical analytical techniques such as GC-MS, as it can help the fractionation of essential oils for the structural analysis of their constituents, and make possible the preparation of reference samples to confirm their presence and validate their pharmacological and olfactory properties. In addition, combinatorial synthesis represents a particularly powerful approach to discover new molecules in essential oils. Furthermore, the valorization of essential oils often rely on the chemical transformation of its constituents, which remain very valuable building blocks for organic synthesis.

In this presentation, the input of organic chemistry to the science of essential oils will be detailed by recalling some historical breakthroughs and by presenting some contributions of the author on the knowledge of various essential oils, assisted by chemical transformations and total syntheses. Some recent findings on the chemistry of the essential oils of precious woods (sandalwood and agarwood) will also be presented to show how implementing organic chemistry in analytical procedures can help to push the boundaries of the exploration of essential oils.

REFERENCES

[1] Baeyer A., Villiger, V., (1899). The effect of Caro's reagent on ketones. *Ber. Dtsch. Chem. Ges.* 32, 3625–3633.
[2] Wagner G., Brickner W. (1899). The conversion of pinene halohydrates to the haloanhydrides of borneol. *Ber. Dtsch. Chem. Ges.* 32, 2302–2325.
[3] Meerwein H. (1914). Pinacolin rearrangement. III. Mechanism of the transformation of borneol into camphene. *Ann.* 405, 129–175.

Study of aromatic plants cultivated in Colombia and development of Colombian essential oil and natural ingredient industry.

Elena E. Stashenko[1, *]

[1]National Research Center of Excellence for Aromatic and Medicinal Tropical Species Study (CENIVAM), Industrial University of Santander, Bucaramanga, Colombia, South America.

* Email: elena@tucan.uis.edu.co

Keywords: Steam distillation, hydrodistillation, circular economy, natural ingredients, tropical plants biological activity

Objective

Colombia together with Brazil and Costa Rica is one of the most biodiverse country in the| world, possessing more than 50,000 higher plants growing in a great variety of climates and habitats. The objective of the CENIVAM national research center is to study both introduced and native aromatic and medicinal plants to establish their potential and economical viability as a essential oil (EO) and natural ingredient source. This study, which begins in the field (botanical expeditions and plant collection) and continues at the Laboratory, returns back to to the field for domestication of crops and industrial production of EOs. The main purpose is to establish the EO industry in the country and to generate new economical opportunities for small farmers and their families and to find some alternatives for coca plantation substitution.

Methods

Essential oils distillations were performed at laboratory by microwave-assisted hydrodistillation and in field by steam distillation or hydrodistillation employing steam generator and 0,4 m^3 or 1 m^3 stainless steel stills. GC/FID/MS and GCxGC-HRMS analyses of EOs were carried out using both polar (DB-WAX) and nonpolar (5%-Ph-PDMS) GC columns. Linear retention indices and mass spectra (EI, 70 eV) were obtained for EO constituent identification, and more than 80 standard compounds were used for EO quantification by GC/FID. More than 60 different EOs were selected for biological activity studies (antioxidant, antifungal, antibacterial, photoprotective, cytotoxic, etc.) in more than a thousand assays. Residual biomass obtained after EO distillation was subjected to SFE, matrix solid-phase dispersion and solvent extraction of flavonoids, phenolic acids, and other biologically active compounds.

Results

During botanical outings (2019-2022) more than 160 accessions were gathered of 28 botanical families (mostly Asteraceae, Labiatae, Piperaceae, Verbenaceae, and Myrtaceae) and more than 65 genera. Approximately one hundred different EOs were obtained and chemically characterized. More than 1000 biological assays (antioxidant, cytotoxic, anti-quorum sensing, antimicrobial, antiviral, photoprotective and insect repellence properties) were carried out. Several EOs (citrus, geranium, palmarrosa, citronella EOs) were fractionated, and their fractions were studied separately for biological activity, mostly for insect repellence. The integrated or full use of mountain oregano (*Lippia origanoides*, Verbenaceae family) was studied, *i.e.*, EO distillation and fractionation, thymol, carvacrol, *p*-cymene and other individual EO component isolation, use of the residual biomass for bio composting, or as biofuel, as well as a source of bioactive compounds (mostly, flavonoids and phenolic acids).

Conclusions

The EOs distilled from Colombian aromatic and medicinal plants as well as extracts obtained from residual biomass (after distillation) were important ingredients in different final products designed in CENIVAM (insect repellent, disinfectants, cosmeceuticals, and phytotherapeutical products). The EO field production showed an interesting economic viability for small farmer associations and a good complement to the traditional crops.

ACKNOWLEDGMENTS

Funding from the Ministry of Science, Technology and Innovation (grant RC-FP44842-212-2018), the General Royalties System (contract BPIN-2018000100044), and the Vice rectory of Research and Extension at UIS (Gran 3736) are gratefully acknowledged.

Volatiles from liverworts – chemical diversity and bioactivity

Agnieszka Ludwiczuk[1,*], Yoshinori Asakawa[2]

[1] Department of Pharmacognosy with the Medicinal Plant Garden, Faculty of Pharmacy, Medical University of Lublin, Lublin, POLAND
[2] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, JAPAN

* Email: aludwiczuk@pharmacognosy.org

Keywords: first land plants, bryendophytes, sesquiterpenoids, diterpenoids, diketopiperazines

Liverworts are considered to be the oldest terrestrial plants. As such, they are the first land plants to synthesize volatiles and other metabolites. As the first inhabitants of terrestrial habitats they were frequently exposed to adverse environmental conditions. This group of spore-forming plants display a low morphological complexity, but a high degree of chemical diversification. They produce a wide array of specialized metabolites, and among them terpenoids are most abundant and structurally diverse. These compounds are accumulated in the oil bodies, which are a prominent and highly distinctive organelle uniquely found in liverworts. Over the last 40 years, more than 3000 compounds have been reported, and among them about 1600 compounds are terpenoids [1-3].

The mentioned high degree of chemical diversification in liverworts suggesting that secondary metabolites, and especially terpenoids, may pay an important role in bryophyte-environment interactions [2, 3]. It should also be mentioned that endophytes associated with liverwort species are able to synthesize bioactive compounds, and consequently contribute, in part, to the control of microbial or herbivore attack [4]. Volatiles present in liverworts as well as their endophytes were shown to be phytotoxic, inhibiting germination and growth of vascular plants in standard lab tests. This toxicity inspired the search of other valuable compounds with antibacterial, antifungal, anti-inflammatory, cytotoxic, insect repellent activities, among others [1-6].

Conclusions

An enormous chemical diversity of liverworts is associated with the evolution and environmental changes. Unique metabolite blends observed in liverwort species is interesting from the chemistry point of view but also help in the identification process and taxonomic work. The biologically active compounds present in liverworts are also important for human health.

REFERENCES

[1] Asakawa, Y., Ludwiczuk, A., Nagashima, F., (2013) Chemical constituents of bryophytes: Bio- and chemical diversity, biological activity, and chemosystematics, in: Kinghorn, A.D., Falk, H., Kobayashi, J. (Eds.), *Progress in the Chemistry of Organic Natural Products*. Springer-Verlag, Vienna, vol. 95, pp. 1-796.
[2] Chen, F., Ludwiczuk, A., Wei, G., Chen, X., Crandall-Stotler, B., Bowman, J.L., (2018) Terpenoid secondary metabolites in bryophytes: chemical diversity, biosynthesis and biological functions. *Crit. Rev. Plant Sci*., *37*, 210-231.
[3] Ludwiczuk, A., Asakawa, Y., (2019). Bryophytes as a source of bioactive volatile terpenoids – A review. *Food Chem. Tox.*, *132*, 110649.
[4] Stelmasiewicz M., Świątek Ł., Gibbons S., Ludwiczuk A., (2023) Bioactive compounds produced by endophytic microorganisms associated with bryophytes - The "Bryendophytes". *Molecules*, 28, 3246; https://doi.org/10.3390/molecules28073246
[5] Asakawa, Y., Nagashima F., Ludwiczuk, A., (2020) Distribution of bibenzyls, prenyl bibenzyls, bis-bibenzyls, and terpenoids in the liverwort genus *Radula*. *J. Nat. Prod.*, 83,756-769.
[6] Asakawa, Y., Ludwiczuk, A., Novakovic, M., Bukvicki, D., Anchang, K.Y., (2022) Bis-bibenzyls, bibenzyls, and terpenoids in 33 genera of the Marchantiophyta (liverworts): structures, synthesis, and bioactivity. *J. Nat. Prod.*, *85*, 729-762.

Essential oils bearing specialized metabolites with potential hypoglycemic activity: a bio-guided fractionation approach driven by *in vitro* (porcine, human and fungal) α-amylase inhibition assays

Barbara Sgorbini[1,*], Marta Pavarino[1], Salvatore Adinolfi[1], Francesca Spyrakis[1], Cecilia Cagliero[1], Arianna Marengo[1], Carlo Bicchi[1], Patrizia Rubiolo[1]

[1] Department of Drug Science and Technology, University of Turin, Turin, ITALY

* Email: barbara.sgorbini@unito.it

Keywords: α-amylase inhibition, bioguided fractionation, *Juniperus communis* L., *Piper nigrum* L., *Zingiber officinal*e Roscoe

Objective

Essential oils (EOs) are extensively studied for their biological properties due to their complex composition and use in various fields (e.g., cosmetics, food, pharmaceuticals) [1]. Among the biological activities, the inhibition of enzymes is studied in depth, especially enzymes involved in important human diseases. Type 2 diabetes mellitus (T2DM), a metabolic disorder characterized by uneven maintenance of blood glucose levels, can be treated by inhibition of α-amylase to reduce postprandial hyperglycemia. Acarbose and voglibose are inhibitors actually used in clinical practice. However, these drugs are associated with unpleasant gastrointestinal side effects. The aims of this study were to 1) investigate the use of homologous enzymes as models for human α-amylase by comparing human salivary α-amylase, *Aspergillus oryzae* α-amylase, and porcine pancreatic α-amylase, and 2) explore potential new α-amylase inhibitors derived from EOs and their constituents.

Methods

Human salivary α-amylase, *Aspergillus oryzae* α-amylase, and porcine pancreatic α-amylase were compared using a combination of *in vitro* and *in silico* approaches. Enzyme sequences were aligned and structures superimposed, while kinetics were studied spectroscopically. Sixty EOs obtained by steam distillation or hydrodistillation from different plant species and botanical families were subjected to α-amylase *in vitro* enzyme assay and chemically characterized by gas chromatography coupled with mass spectrometry. Acarbose was used as a positive control. A bio-guided fractionation approach was adopted to isolate and identify the active fractions/compounds of the most active EOs.

Results

The three α-amylase enzymes show strikingly different activities mediated specifically by different ions, despite relevant structural homology [2]. These differences must be carefully considered when using α-amylases from different organisms as models for the human enzymes, which requires appropriate experimental conditions.

The most active EOs for both human and porcine α-amylase were juniper (*Juniperus communis* L.), black pepper (*Piper nigrum* L.), and ginger (*Zingiber officinale* Roscoe), whereas different results were obtained for the fungal enzyme, against which each of the above EOs was inactive. The bio-guided fractionation approach showed that the α-amylase inhibitory activity of the EOs is the result of various interactions (antagonistic, additive, or synergistic) between their components and led to the identification of some bioactive compounds.

Conclusions

This study confirmed that porcine pancreatic α-amylase can be used as a cheaper substitute for the human enzyme because they show similar activity behaviour, which is clearly related to greater structural similarity. In contrast, α-amylase from *Aspergillus oryzae* markedly differs from the human enzyme, making it a poorer choice.

The above screening showed that some EOs are active towards α-amylase. These results are very promising and deserve further in-depth studies in view of developing a complementary treatment to conventional therapy.

REFERENCES

[1] Baser K. H. C., Buchbauer G. (Eds), (2010). Handbooks of essential oils: Science, Technology and Application, CRC Press, Boca Raton

[2] Marengo M., Pezzilli D., Gianquinto E., Fissore A., Oliaro-Bosso S., Sgorbini B., Spyrakis F., Adinolfi S., (2022). Evaluation of Porcine and Aspergillus oryzae -Amylases as Possible Model for the Human Enzyme, *Processes*, 10, 780.

Chemical words: microbial volatiles (mVOCs) interact with plants and promote growth by gene modulation and oxidative stress

Massimo E. Maffei[1,*]

[1] Plant Physiology Unit, Department of Life Sciences and Systems Biology, University of Turin, Turin, ITALY

* Email: massimo.maffei@unito.it

Keywords: biological activity, plant growth, *Erwinia amylovora*, *Pseudomonas syringae*, systems biology

Objective

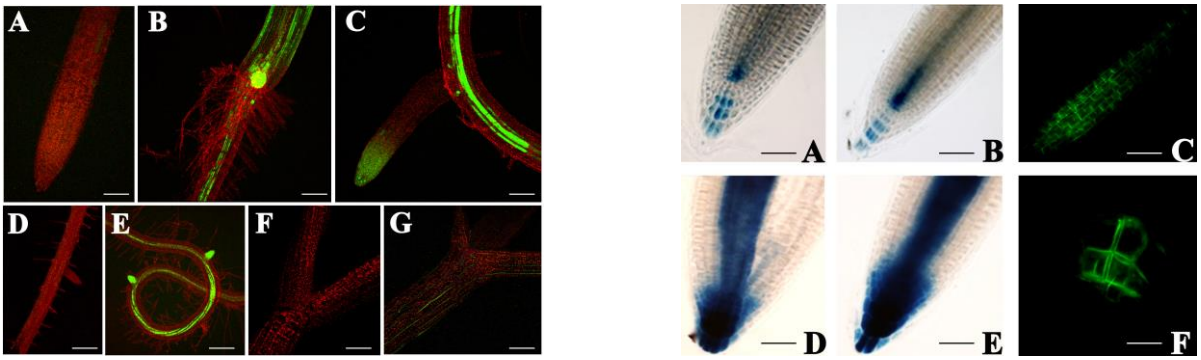
In the past decade considerable progress has been made in understanding the role that microbial volatile organic compounds (mVOCs) play in below- and above-ground multitrophic interactions and mVOCs functions in modulating the growth, nutrition, and health of interacting partners [**1,2**]. The objective of this presentation is to report on the biological activity of mVOCs produced by two phytopathogens (*Erwinia amylovora* and *Pseudomonas syringae* pv *tomato*) on the model plant *Arabidopsis thaliana*.

Methods

Experiments were done *in vitro* by bipartite squared Petri dish with in the upper part a medium for plant growth and on the lower a medium for bacterial growth. mVOCs are analyzed by Stir Bar Sorptive Extraction, desorpted with a CIS-TDU and analyzed by GC-MS. Plant intracellular calcium variations, voltage-gated and ligand-gated potassium channels, and the localization of ROS and NO was done by confocal laser scanning microscopy. Full transcriptomics was done by gene microarray analysis validated by quantitative RT-PCR. PIN1 and PIN3 mutants bearing both GUS and GFP gene reporters were used. Synthetic mVOCs were used to evaluate expression of the main genes involved in ROS production. Several Arabidopsis mutans were also used to explore the biological effects of mVOCs.

Results

mVOCs triggered early signalling events including Vm depolarization, cytosolic Ca^{2+} fluctuation, K^+-gated channel activity, reactive oxygen species (ROS) and nitric oxide (NO) burst from few minutes to 16 h upon exposure. These early events were followed by the modulation of genes involved in plant growth and defence responses and auxin (including the efflux carriers PIN1 and PIN3). When tested, synthetic mVOCs induced root growth and modulated genes coding for ROS [**3**].



Left: Localization of ROS (A-C) and NO (D-G); Right: GUS staining and GFP localization of auxin efflux PIN1 (A-C) and PIN3 (D-E) in control plants (A and D) and after exposure to mVOCs (B and E, respectively), GFP localization of PIN1 in the elongation zone (C) and PIN3 in the columella (F). Metric bars: 200 µm. [From **3**].

Conclusions

Our results show that mVOCs emitted by bacterial phytopathogens affect *A. thaliana* growth through a cascade of early and late signalling events that involve the gene modulation of phytohormones and ROS.

REFERENCES

[1] Kanchiswamy C.N,. MalnoyM., Maffei M.E., (2015) Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci. 20: 206-211.

[2] Kanchiswamy C.N., Malnoy M., Maffei, M.E. (2015) Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front. Plant Sci. 6: 00151.

[3] Parmagnani et al. (2023) Bacterial volatiles (mVOC) emitted by the phytopathogen Erwinia amylovora promote Arabidopsis thaliana growth and oxidative stress. Antioxidants, 12, 600.

Agarwood (*Aquilaria malaccensis* L.) a quality fragrant and medicinally significant plant based essential oil with pharmacological potentials and genotoxicity

Mohan Lal[1,*], Roktim Gogoi[1,2], NeelavSarma[1,2], Twahira Begum[2]

[1]Academy of Scientific and Innovative Research, Ghaziabad, UP-201002, INDIA

[2]CSIR-North East Institute of Science and Technology, Jorhat, Assam -785006, INDIA

*E-mail: drmohanlal80@gmail.com

Keywords: agarospirol, anti-diabetic, anti-urolithic, *Aquilaria malaccensis*, genotoxicity

Objective

Aquilaria malaccensis (agarwood essential oil), is a highly priced medicinal and most expensive aromatic oil in the industry. The purpose of the current study was to assess the chemical makeup of a naturally insect infested agarwood essential oil and its potential therapeutic uses.

Methods

Essential oil of infected agarwood was extracted using Clevenger apparatus and the chemical analysis of essential oil was carried out using Gas chromatography(GC) andGas chromatography /mass spectroscopy (GC/MS) analysis. Pharmacology evaluation includes – tyrosinase inhibitory, anti-cholinesterase (AChE), anti-urolithic, anti-inflammatory, anti-diabetic, and antioxidant assays. Standard methodologies such as tyrosinase inhibitor, α-amylase inhibitor, AChE inhibitory, aggregation, protease inhibitor, DPPH, ABTS, and metal chelating assays were used for evaluation of pharmacological potential.*Allium cepa* assay was used for genotoxicity analysis of the essential oil.

Results

A. malaccensis essential oil GC, GC/MS analysis revealed cubenol (22.26%), agarospirol (14.35%) and aristolene (13.22%) as major compounds. Radical scavenging activity of agarwood essential oil showed 50% inhibition (IC_{50} using XLSTAT software) at the concentration of 40.14±0.0192 µL/mL. Furthermore, the antioxidant capacity from ABTS assay, α-amylase inhibitory potential, tyrosinase inhibitory activity, AChE inhibitory assay, anti-urolithic activity and *in-vitro* anti-inflammatory activity protein denaturation assay were confirmed with IC_{50} values of 76.95±0.0090µL/mL, 30.78±0.0018µL/mL, 38.06±0.0016µL/mL, 13.41±0.0374 µL/mL, 34.14±0.0202 µL/mL and 22.42±0.0560 µL/mL respectively. At 1 µL/mL concentration *Allium cepa* genotoxicity assay resulted agarwood essential oil Mitotic Index (MI) value of 14.26%, with a chromosomal aberration of 9.30%. Genotoxicity results for *A. malaccensis* essential oil showed negative toxic effect at 1 µL/mL concentration. The essential oil did not exhibit any anti-microbial activity against all the tested microbial strains.

Conclusions

From the study it is concluded that agarwood essential oil could be used for antioxidant, anti-diabetic, skin whitening, and anti-inflammatory drug formulations in near future after multiple validation as well as clinical trials.Apart from all of this, agar is one of the most expensive goods in the world, and can be develop this industry and increase exports, it may become a major source of foreign exchange.

ACKNOWLEDGMENTS

We appreciate that the director of CSIR-NEIST Jorhat, Assam, India gave us access to the research laboratoriesas well as experimental farm. The research work was funded by Government of India, in the form of project CSIR-Aroma Mission, HCP-0007.

Tridimensional gas chromatographic system coupled to an olfactometric port and FID/MS detection for the quali-quantitative and odour evaluation of trace components

Danilo Sciarrone[1*], Gemma De Grazia[2], Lorenzo Cucinotta[1], Luigi Mondello[1,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Capua 1880 Srl, Reggio Calabria, ITALY
[3] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: dsciarrone@unime.it

Keywords: GC-O, MDGC, trace odour components

Objective

This study describes the effectiveness of a multidimensional gas chromatographic system obtained by the hyphenation of three gas chromatographic systems coupled to an olfactometric port and to a simultaneous FID/MS system (MDGC-O-FID/MS) for the identification, quantification and dour evaluation of trace odor components in essential oils.

Methods

To compare the capabilities of conventional and multidimensional GC-O approaches, a sulphur compound with a low-odour threshold was used, namely, p-mentha-8-thiol-3-one, characterised by a tenacious sulphurous odour type, which can be described as catty and black currant.

In a first step, the odour treshold of the selected component was determined exploiting monodimensional GC-O, equipped with a 5% diphenyl micro-bore column. Following, a wide-bore column with same stationary phase was used, first in monodimensional condition and then in multidimensional condition exploiting the TDGC system. The latter was a prototype consisting of three GC systems, each equipped with a Deans switch transfer device, and a set of three mega-bore columns of different selectivity, i.e., 5% diphenyl-polyethylene glycol-ionic liquid stationary phases.

Results

The odour threshold determined exploiting monodimensional GC-O employing a micro-bore column was around 10ppm. The same experiment employing a wide-bore column was not successful since the reduced efficiency of the column did not provide the separation of the target compound. On the contrary, the TDGC approach exploiting the set of three wide-bore columns provided an improved sensitivity of 10 ppb.

Conclusions

The use of micro-bore column in monodimensional GC-O hindered the odour evaluation of the target component below the 10ppm level. In fact, to achieve a high chromatographic efficiency it is necessary to accept a compromise injecting very low sample amount. On the contrary, wide-bore columns allow higher sample capacity but with lower efficiency level. Only the use of the TDGC-O system, combining the heart cut method and the use of wide-bore columns, allowed to reach the goal of a high sample capacity coupled to high efficiency levels. This approach represents an effective tool to guarantee an enhanced odour evaluation at ppb level. Moreover, the possibility to switch between the olfactometric port and the simultaneous FID/MS detections allowed the quaali-quantitative evaluation of the target component.

Biocidal properties of essential oils and aromatic extracts

Lucyna Balcerzak, Alicja Surowiak, Kinga Baberowska, Zuzanna Bacińska, Daniel Jan Strub*

Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, POLAND

* Email: daniel.strub@pwr.edu.pl

Keywords: biocides, essential oils, aromatic extracts, antibacterials, antifungals

Objective

Natural flavor and fragrance materials, including essential oils, resinoids, absolutes, concretes, oleoresins, and raw exudates, play a crucial role in society. They are widely used in various areas of human interest, have established toxicological profiles, and are considered relatively safe. While sensory properties of these natural products are important, their diverse components offer novel biological activities and practical properties that are continuously being explored. However, their mainstream application is restricted due to various legislative requirements and the need for research studies to prove their effectiveness and obtain necessary registrations.

This presentation aims to provide a brief overview of the proven properties and applications of natural volatile materials. It will also discuss our current work in the matter, as well as the potential use of hundreds of essential oils and aromatic extracts as supporting preservatives or active antimicrobial agents. Furthermore, mid- to long-term perspectives on the subject will be presented. [1-3]

Methods

The screening of essential oils and aromatic extracts and the determination of their minimal inhibitory concentration (MIC) parameter were conducted using the Alamar blue assay [4]. Thirteen bacteria and four fungi were tested in these experiments. Furthermore, the biocidal properties of a model formulation consisting of surfactants and a mixture of antimicrobial essential oils were evaluated according to EN 13697:2001, EN 1276:2009, and EN 1650:2008 standards.

Results

The antimicrobial properties of over 500 essential oils and aromatic extracts were evaluated. The results showed that Hiba (*Thujopsis dolabrata*) and blue cypress (*Callitris intratropica*) essential oils exhibited the strongest and broadest antimicrobial effects in the alamar blue assay.

Conclusions

Although none of the essential oils studied showed sufficient antimicrobial potential to be used alone as active agents in functional products, we have demonstrated that a mixture of essential oils with a broad spectrum of activity has the potential to be used as active antimicrobial agents in various industries.

ACKNOWLEDGMENTS

We would like to thank representatives of the essential oils industry and International Federation of Essential Oils and Aroma Trades (IFEAT) Members: A.Fakhry & Co., Albert Vieille, Amigo & Arditi, Ashna Samai, Berje Inc., Bordas S.A., BoreA Canada, Brüder Unterweger, Christodoulou Bros S.A., Citrus and Allied Essences Ltd, D.V. Deo Industries, Dutjahn Sandalwood Oils, Essential Oils and Herbs, Essential Oils of Tasmania, Eucaforest, Hierbas Patagónicas, S.R.L, Kallin ltd, Jacarandas International, Mane Kancor Ingredients Private Limited, Lebermuth, Les Arômes du Maroc, Lluch Essence, Metainy, Nelixia, New Zealand Mānuka Group, Payan Bertrand, Plant's Power, Van Aroma, Robertet S.A., Ultra International, and Vessel Essential Oils for the donation of most of the samples of natural flavor and fragrance materials for this study.

REFERENCES

[1] Strub, D.J. *et al.* Evaluation of the anti-SARS-CoV-2 properties of essential oils and aromatic extracts. *Scientific Reports*, 2022, 12, 14230

[2] Strub, D.J. *et al.* Evaluation of biocidal properties of essential oils and aromatic extracts, manuscript in preparation

[3] Strub, D.J. *et al.* Evaluation of preservative efficacy of essential oils and aromatic extracts in cosmetic products, manuscript in preparation

[4] Baker, C. N. *et al.* Evaluation of Alamar colorimetric MIC method for antimicrobial susceptibility testing of gram-negative bacteria. *Journal of Clinical Microbiology*, 1994, 32, 1261-1267.

Exploring the Stereochemical Influence of Essential Oil Compounds on Antimicrobial Activity: Uncovering the Power of Enantiomers in Combination Studies

Sandy van Vuuren[1,*], Nazihah Hoosen[1], Alvaro Viljoen[2, 3]

[1] Department of Pharmacy and Pharmacology, Faculty of Health Science, University of the Witwatersrand, Johannesburg, South Africa

[2] Department of Pharmaceutical Sciences, Faculty of Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

[3] Department of Pharmaceutical Sciences, SAMRC Herbal Drugs Research Unit, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

*Email: sandy.vanvuuren@wits.ac.za

Keywords: Stereochemistry, compounds, antimicrobial, toxicity, combination, synergy

---

Objective

Essential oil compounds are often investigated for their biological properties, including antimicrobial activity, however, what is often overlooked is the influence of the specific stereochemical configuration. The aim of this study was to investigate whether a selection of optical enantiomers differed in antimicrobial and toxicity activity when tested independently and in combination with a selection of essential oil compounds.

Methods

Each enantiomer (Borneol, Camphor, Citronellal, Limonene, Menthone and Pinene) were comparitively investigated. The broth micro-dilution minimum inhibition concentration (MIC) assay was undertaken against a selection of test micro-organisms. Anti-quorum sensing (QS) was investigated with *Chromobacterium violaceum* as the monitor strain, The toxicity was screened using the brine shrimp lethality assay (BSLA). Enantiomers were studied independently and in combination where equal ratios of each stereochemical configuration was combined with essential oil compounds (Camphene, β-Caryophyllene, *p*-Cymene, Estragole, Eucalyptol, Eugenol, Geraniol, Isoeugenol, Linalyl acetate, Menthol, Ocimene, Sabinene hydrate, γ-Terpinene and
α-Terpineol) and results interpreted using the fractional inhibitory concentration (FICI) index.

Results

Overall, (+)-β-Pinene, (–)-Borneol, (–)-α-Pinene and (–)-Limonene often displayed better antimicrobial activity over their enantiomeric counterparts. The results of the MIC 1:1 combination study revealed that the most prevalent interaction observed was additivity (56.46%), followed by non-interactive (37.93%) interactions. A total of 5.61% of the combinations were found to be synergistic, most of which was seen against *Cryptococcus neoformans* (ATCC 14116) and *Candida albicans* (ATCC 10231). No antagonism was observed in any of the combinations tested. For the QS studies, all enantiomers and selected essential oil compounds had strong anti-QS activity. The combination studies were evaluated and the fractional quorum sensing inhibitory concentration index (FQSICI) demonstrated that the majority of the combinations were non-interactive (44.90%), followed by additive (20.41%), synergistic (8.16%), and antagonistic (0.51%) interactions. (+)-Limonene and (+)-Citronellal often (dependent on the compounds in combination) displayed better interactive activity than their enantiomeric counterparts. The only toxicity variation observed when investigated independently was between the enantiomers of β-Pinene after 48 hrs, where (+)-β-Pinene had a percentage mortality (PM) of 30.75% and (–)-β-Pinene had a PM of 93.82%. The results of the toxicity combination studies demonstrated antagonistic (34.69 - 40.82%), followed by non-interactive (17.35 - 30.61%), synergistic (18.37 - 23.47) and additive (9.69 - 15.51%) interactions.

Conclusions

This study demonstrated that an often-neglected evaluation of the enantiomeric configuration of the essential oil compounds is in fact an important consideration, with the potential to identify therapeutically active combinations with safe toxicological profiles.

Influence of the selected essential oils on invasive *Solidago canadensis* L.

Mária Pľuchtová[1], Beata Baranová[1], Daniela Gruľová[1,*]

[1] Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, SLOVAKIA

* Email: daniela.grulova@unipo.sk

Keywords: canadian goldenrod, seed germination, essential oils, phytotoxic effect, bioherbicide

Objective

Solidago canadensis belongs to the invasive plant species which was introducted to Europe for decorative purposes from North America. Plants produce thousands seeds annually which help them to spread easily. Expanding areas of invasive plants threat biodiversity of native flora. Thousands of plants belonging to different families are known to produce volatile oils serving as pollinator attractants, determinants of vegetation patterning or regulator of community structure via allelopathy. Many of these substances have been used for their herbicidal effect or for their ability to regulate plant growth. The identification of new and suitable inhibitors is important for the development of new herbicidal substances with higher agronomic capacity, lower environmental impact, and fewer resistance problems. The use of volatile substances in the suppression of unwanted plants could be a good opportunity to reduce the employment of industrial agrochemicals. The main aim of the research was to evaluate possible influence of the selected essential oils (*Salvia officinalis* L., *Mentha×piperita* L., *Origanum vulgare* L., *Foeniculum vulgare* Mill., *Anethum graveolens* L., *Pimpinella anisum* L.) on seed germination of *S. canadensis*.

Methods

M. piperita and *S. officinalis* were harvested in the university field, and for 2 hours hydrodistillation was used Clevenger type of apparatus, other EOs were commercial obtained from the local store. GC-MS was used for determination main components in each EO. Essential oils were applied in six different concentrations in the range from 0.0625 – 2.5 µg /mL into the Petri dish, where on filtrate paper were placed hundred of *S. canadensis* seeds. For the treatment were used seeds from different collection years. Seed germination was evaluated after 8 and 16 days.

Results

The dominant components in *M. piperita* were identified menthol (49.3 %) and menthon (22.4%), *in S. officinalis* were thujone (34.2 %) and camphor (19.8 %), in *O. vulgare* were thymol/carvacrol (78.2 %), in *F. vulgare* were estragol (40.1 %) and anethol (38.1 %), in *P. anisum* were anisol (88.6 %) and in *A. graveolans* were carvone (58.4 %) and limonene (35.8 %). Seed germination of older seeds was 40 % lower than younger seeds. Thestrongest imhibitory activioty on seed germination was evaluated in *O. vulgare* in each concentration and seeds age. The inhibitory effect on germination was evaluated in olders seeds and stimulatory effect in younger seeds by using EO from *S. officinalis*. Both effect was evaluated in other EO in different pattern.

Conclusions

Generally, all used EOs influence seed germination of invasive species *S. canadensis*. EOs presented inhibitory as well as stimulatory effect based on the dosis and the age of the seeds. As was already mentioned in previous publication *O. vulgare* and its main components were identified as a natural product with high and promising potential with the herbicidal activity.

ACKNOWLEDGMENTS

Presented research was supported by the grants VEGA 1/0087/20 and VEGA 2/0018/20.

Biological Properties of fractions obtained from winter lemon and grapefruit essential oils

Marzia Pucci[1,*], Stefania Raimondo[1], Roberta Gasparro[1], Vincenza Tinnirello[1], Valeria Corleone[2], Gioacchino Aiello[2], Simona Fontana[1], Riccardo Alessandro[1]

[1]Department of Biomedicine, Neuroscience and Advanced Diagnostics (Bi.N.D), Section of Biology and Genetics, University of Palermo, 90133 Palermo, Italy.

[2]Agrumaria Corleone s.p.a., Via S. Corleone, 12-Zona Ind. Brancaccio, 90124 Palermo, Italy.

* Email: marzia.pucci@unipa.it

Keywords: essential oil, fractions, inflammation, oxidative stress, fibrosis

Objective

Essential oils (EOs) are known for their anti-inflammatory properties, while less consideration is given to the biological properties of their fractions. Chronic inflammation is correlated to the development of numerous diseases and is accompanied by increased cytokine secretion associated to ROS production and fibrosis. This study aims to evaluate the protective activities against pro-inflammatory stimuli of citral-enriched fractions of winter lemon EO (Cfr-LEO) and of aldehydes-enriched fraction of grapefruit EO (Fr-GEO) by using in vitro models of lipopolysaccharide (LPS)-injured macrophages and healthy human hepatocytes.

Methods

C. limon essential oil (LEO) was recovered by cold-pressed extraction mechanical process from the peels of winter fruits at the company Agrumaria Corleone S.P.A. (Palermo, Italy).

After cold dewaxing at −20 °C for 48 h and subsequent filtration through a paper filter with 10-micron pores, LEO was fractionated by a newly developed adsorption column chromatography. Some volumes of essential oil flowed through the chromatographic column filled with a particular type of stationary phase under the following operating conditions: pressure: atmospheric; temperature: 25 °C; flow: 3 mL/min. This newly developed method allowed the collections of fractions enriched with the main aromatic compounds present in *C. limon* essential oil, based on their affinity with the stationary phase. GEO was obtained by cold extraction from fresh fruit (Star Ruby variety) and fractions enriched in alde- hydes were obtained by a new column chromatography method that is currently covered by trade secret. Other technical details cannot here be reported and described since to date are covered by trade secrets.

Results

We reported that the pre-treatment with Cfr-LEO and Fr-GEO exerts protective effects against LPS-induced inflammation in murine and human macrophages, by decreasing gene expression and protein levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Similar anti-inflammatory effects of Cfr-LEO as well as their anti-oxidant effects, and the ability to inhibit the epithelial to mesenchymal were also observed in LPS-stimulated healthy human hepatocytes, The mechanisms through which the Cfr-LEO and Fr-GEO can exert their protective effects will be further investigated.

Conclusions

The results reported here encourage the application of selected essential oil fractions not only for their organoleptic properties but also in the nutraceutical industry for the development of new food supplements and/or functional foods and beverages.

ACKNOWLEDGMENTS

This study was funded by a grant to Riccardo Alessandro and to Agrumaria Corleone s.p.a.: “Salute in tazza: innovative soluzioni per l’isolamento e l’applicazione industriale, anche in capsule, di composti biofunzionali ottenuti da agrumi di Sicilia” – PON I&C—MISE F/050039/01-03/X32.

Use of liquid and supercritical fluid chromatography for determination of coumarins, furocoumarins, and polymethoxyflavones

Marina Russo[1,*], Maria Rita Testa Camillo[1], Giovanna Cafeo[1], Antonella Satira[2], Patrik Appelblad[3], Romana Rigger[4], Paola Dugo[1], Luigi Mondello[1,2]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[3] Merck LifeScience AS, Drammensveien 123, 0277 Oslo, Norge
[4] Merck Chemicals and Life Science GesmbH, Zimbagasse 5, 1140 Vienna, Austria

* *Email:* marina.russo@unime.it

Keywords: supercritical fluid chromatography, coumarins, furocoumarins, *Citrus*, essential oil

Objective

Citrus essential oils, thanks to their pleasant aroma, are certainly the most used ingredients in the formulation of hydroalcoholic fragrances. The non-volatile fraction of *Citrus* essential oil is composed for 10-20% of coumarins, furocoumarins and polymethoxyflavones. It is well known that furocoumarins induce photosensitization and have potential carcinogenic, and mutagenic effects. It follows that furocoumarins levels in cosmetics product are constantly monitored by opinions and regulations issued by the International Fragrance Association. An innovative analytical technique for the analysis these molecules is represented by supercritical fluid chromatography (SFC). The use of a supercritical fluid, mainly CO_2, as a mobile phase, at pressure and temperature above their critical point, take on intermediate characteristics between a liquid and a gas, with density and solvating power similar to liquids while viscosity and diffusion coefficient similar to gases. For these reasons, SFC is establishing itself as a greener technique in alternative to conventional liquid chromatography, thanks to the faster analysis time and the low solvent consumption. The present research is focused on the development of rapid analytical methods with a low impact on the environment, applying the latest generation HPLC columns. The aim was to validate two analytical methods for routine analysis of OHCs to save time and solvent.

Methods

The first method allowed the determination of OHCs through the use of SFC coupled both to photodiode array detector (PDA) and triple quadrupole mass spectrometry detector (QqQ-MS). A fast separation has been achieved with a low consumption of solvent in less than 8 min. The second method has been developed with the purpose of performing very rapid screening of OHCs , concluding the entire analysis in about 4 minutes and with the use of less tha 3 mL of ethanol per analysis. At this purpose, a RP-HPLC-QqQ-MS method was validated.

Results

This research was carried out in order to validate a more environmentally friendly analytical strategy aimed at the separation and quantification of coumarins, furocoumarins and polymethoxyflavones in cold-pressed *Citrus* essential oils. Calibration curves were constructed on distilled lemon essential oils in order to quantify these molecules in real samples. Different linearity ranges and good determination coefficients were obtained for each compound. The validation parameters resulted satisfactory, with low LoQs, that could allow the quantification of these compounds even when they are contained at trace level in finished products.

Conclusions

The developed methods showed to be a valid and environmentally friendly analytical approach for the analysis of coumarins, furocoumarins and polymethoxyflavones in *Citrus* cold pressed essential oils. The SFC-PDA approach is greener than the liquid chromatography approach previously developed. The SFC method is then clearly suitable to be applied for the quality control of coumarins, furocoumarins, and polymethoxyflavones in *Citrus* essential oils and finished products.

ACKNOWLEDGMENTS

The authors thank Shimadzu and Merck Life Science corporations for the continuous support.

Seduced by our noses: Odors in marketing †

Nina Berg[1], Iris Stappen[2,*]

[1] Marienapotheke Baden, Baden, AUSTRIA
[2] Department of Pharmaceutical Sciences, Faculty of Life Science, University of Vienna, Vienna, AUSTRIA

* Email: iris.stappen@univie.ac.at

† In memory of Juergen K.R. Wanner (1955-2023)

Keywords: consumption, emotion, essential oils, ambient odor

Objective

Besides their healing properties, essential oils have a long tradition of being used to influence human mood by olfactory stimuli. The scent of lavender, basil, cinnamon and citrus flavour are said to relax humans, peppermint, thyme and rosemary are known as invigorates. Ginger, cardamom, chocolate and liquorice are believed to create a romantic spirit and rose counteracts depressive moods.[1] Over the last decades, odors have become increasingly popular as marketing tools. In the beginnings, ambient scents have been used mainly to mask unpleasant odors. Nowadays natural as well as synthesised substances are deliberately released to channel customers towards enhanced purchase intentions, higher spending, heightened brand memory or increased food consumption.
Nature is the guide for the fragrance industry as a source of inspiration. All perfumes and scents produced in factories are modelled to a large extent after naturally occurring volatile molecules. The field of application is broad and ranges from scented products to fragrances released into public spaces like hotels, public transportations, health care institutions, shopping malls, supermarkets and retail stores.
This presentation focuses on the effects of ambient scents on customer behaviour in different surroundings like supermarkets, cafeteria, casinos, hotels or stores.

Methods

For this review, various databases such as Scifinder, Scopus, Google Scholar, and PubMed were used.

Results

Prerequisites for achieving positive effects are the congruency between product and fragrance as well as flavour intensity: The more intense the scent experience is, the more negative the judgement of the odor.

Conclusions

Marketers use ambient scents to differentiate their products from the rising competition because the sense of smell is evidentially related to emotional reactions. Odors have a high potential to influence human mood, not only in terms of well-being in a therapeutic setting but also in our shopping and eating behavior.

REFERENCES

[1] Chebat J.C., Michon R.., (2003). Impact of ambient odors and mall shopper's emotions, cognition and spending: A test of competitive causal theories, Journal of Business Research, 56, 529-539

About the use of alternative carrier gasses in gas chromatography for analysis of *Citrus* essential oils through the use of flame ionization detector and mass spectrometry

Antonio Ferracane[1,*], Micalizzi Giuseppe[1], Filippo Alibrando[1], Leonard M Sidisky[2], Luigi Mondello[1,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] MilliporeSigma, 595 N. Harrison Road, 16823, Bellefonte, PA, USA
[3] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: aferracane@unime.it

Keywords: alternative carrier gas, gas chromatography, *Citrus* essential oils.

Objective

The purpose of this research is to explore the performance of alternative carrier gasses such as nitrogen and hydrogen instead of the most common helium for routine analysis of *Citrus* essential oils in gas chromatography (GC). Helium is conventionally used as carrier gas in GC-FID and GC-MS analyses due to its chemical properties such as inertia that yields optimal chromatography while minimizing undesirable reactions. Currently, there is no intrinsic shortage of helium gas, but the price of raw material will increase in the next years. Thus, if and when the helium supply is disrupted, and the need to reduce the cost per analysis has led to investigations of nitrogen or hydrogen as alternative carrier gasses for GC analysis. A performances comparison, between helium *vs* nitrogen when FID and helium *vs* hydrogen when MS are used, will be showed in this research.

Methods

The optimal linear velocities of helium, hydrogen, and nitrogen carrier gasses were calculated by Golay curve approach. To compare the performace during routine analyses, *Citrus* essential oils were analysed in GC-FID and GC-MS methods using nitrogen and hydrogen carrier gases, respectively. The identification of volatile fraction in *Citrus* essential oils including monoterpenes, sesquiterpens, and oxygenated derivatives, was carried out using a mass spectral database containing LRIs used conventionally in He-based GC-MS analysis.

Results

The developed GC-FID method allowed a satisfactory separation of all components in about 47 min, in accordance with analysis times (ca. 45 min) obtained using helium as carrier gas. The GC-MS optimized method using H_2 as carrier gas allowed the separation and identification of 55 volatile compounds in comparable He-based analysis times. Also, most compounds showed a spectral similarity of more than 90%. Absolute correspondence was also registered between experimental and reference LRI.

Conclusions

Finally, carrier gas switching to H_2 for analysis carried out by GC-MS did not necessitate to adjust or to modify mass spectral database containing MS spectra and LRI values. In fact, absolute correspondence between experimental and reference data were obtained.

Furthermore, with regard to the GC-FID analysis the use of N_2 as carrier gas allowing to obtain comparable helium-based GC-FID analysis time.

Both approaches allowed to obtain separations with a very similar separation times without loss of resolution and sensitivity. Finally, the two approaches proposed here are more eco-friendly and reduce analysis costs as both carrier gases can be produced in the laboratory through the use of generators, thus reducing both transport/storage costs and any problems related to supply.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corporation and Merck Life Science for their continuous support.

Comprehensive study of photo- and thermal degradation of chamazulene contained in *Matricaria* and *Achillea* essential oils and setup of protection strategies.

Simone Gabbanini[1,*] Jerome Ngwa Neba,[2] Riccardo Matera,[1] Luca Valgimigli*,[2]

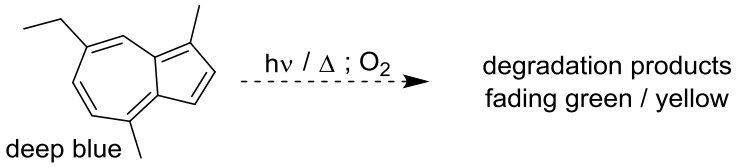
[1] R&D Department, BeC s.r.l., Forlì, Italy
[2] Department of Chemistry "Ciamician", University of Bologna, Bologna, Italy

* Email: laboratorio@bec-natura.com

Keywords: chamazulene, cosmetics, antioxidants, chamomile, yarrow.

Objective

Blue chamomile (*Matricaria recutita* L.) and Achillea (*A. millefolium* L.) essential oils are widely used in cosmetics due to their skin soothing effect attributable to particularly to sesquiterpene components such as Bisabolol and Chamazulene [1]. Chamazulene is a very interesting molecule that originates from matricine during the distillation process, endowed with a wealth of biological properties [2], and characterized by a distinctive intense blue color: in fact, in some cases, the presence of Chamazulene is exploited to give a natural blue color to the formulations. This molecule is highly unstable and tends to spontaneous degradation, losing its natural blue color along with the beneficial properties [3].



This process is accelerated by exposure to light and high temperatures.

Methods

Chamazulene was isolated by flash chromatography from *Artemisia arborescens* (L.) essential oil, obtained by hydrodistillation from wild specimens from Sicily (Italy). Thermal and light stability were investigated using different techniques such gas chromatography-mass spectrometry (GC-MS), and ultra-high-performance liquid chromatography combined with photo-diode-array detector and with electron spray ionization mass spectrometry (U-HPLC-PDA-ESI-MS/MS), and by direct infusion in ESI-MS^n, to evaluate the progress of reactions under different photo- and thermal stress conditions, and to identify the main products of degradation. The photodegradation was induced by irradiating chamazulene solutions with a combined mercury/tungsten light source with emission in the UV (from 290 to 400 nm)and visible emission (Solar Ultravitalux) at various times, while thermal degradation was induced by exposing chamazulene solutions at a temperature of 40 to 50°C in the dark for a period of 2 months. Data were used as the basis to design different antioxidant strategies alone and combined with photoprotection, which were comparatively evaluated to inhibit degradation and prevent the discoloring process. Most promising combinations were further tested in real cosmetic formulas.

Results

The prevalence of C-C dimeric species and oxygenated species among degradation products allowed to envisage a radical-mediated degradation sequence which was evaluated in the presence of different phenolic and non-phenolic antioxidants. Non-conventional antioxidant approaches based on persistent nitroxides and pro-aromatic essential oil terpene components were also comparatively tested, along with combination with sunscreens. The most promising combination were the transferred into real cosmetic products formulated with *M. recutita* (L.) or *A. millefolium* (L.) EOs.

Conclusions

Results allow the onsetting of strategies to stabilize cosmetic formulations containing chamomile and yarrow EOs.

REFERENCES

[1] Qasem, A.; et al. (2022), *Molecules*, *27*, 5850. DOI: 10.3390/molecules27185850
[2] Bakun, P.; et al. (2021) *Med. Chem. Res.*, *30*, 834–846. DOI: 10.1007/s00044-021-02701-0
[3] Fiori, J.; et al. (2008) *J. Pharm. Biomed. Anal.*, *47*, 710–715 DOI:10.1016/j.jpba.2008.02.020

In vitro evaluation of essential oils as antioxidant and sunscreen agents for cosmetic formulations

Lucia Montenegro[1,2,*], Edy Angela Siciliano[1], Valeria Consoli[1,2], Luca Vanella[1,2], Valeria Sorrenti[1,2], Annamaria Panico[1,2]

[1] Department of Drug and Health Sciences, University of Catania, Catania, ITALY
[2] CERNUT (Center for Research on Nutraceuticals and Health Products), University of Catania, Catania, ITALY

* Email: lmontene@unict.it

Keywords: sun protection factor; antioxidant activity; ginger essential oil; hazard assessment

Objective

The widespread knowledge of the harmful effects of UV radiation has increased the use of sunscreen products to protect the skin from short- and long-term damages. Such formulations generally contain mixtures of high percentages of organic and inorganic UV-filters whose safety has been considered as a major concern for both human and environmental health. Due to the great number of antioxidant and UV-absorbing constituents, essential oils could be used to replace organic UV-filters, thus providing effective and safer sunscreen formulations. Therefore, this work was aimed to investigate the feasibility of using essential oils as sunscreen agents by determining their in vitro antioxidant activity and sun protection factor (SPF) values. Ginger (*Zingiber Officinale* – SD - from roots), palmarosa (*Cymbopogon Martinii* – SD - from aerial parts), rosemary (*Rosmarinus Officinalis* – SD - from fresh flowering tops), tea tree (*Melaleuca Alternifolia* – SD – from fresh leaves), and basil (*Ocimum Basilicum* – SD – from flowering plant) essential oils were selected for this study and were obtained from Glamour Cosmetics (Italy).

Methods

The antioxidant activity of the investigated essential oils was evaluated using: a) the Oxygen Radical Absorbance Capacity (ORAC) assay; b) the NO-scavenging assay, which assays were performed as previously reported [1]. In vitro SPF values were determined by recording the UV absorbance of the sample from 290 to 320 nm, every 5 nm. The Mansur equation was applied to obtain SPF values. Octyl methoxycinnamate (OMC) was used as reference. The total phenolic content of ginger essential oil was estimated using the Folin Ciocalteau reagent as described in literature [2]. Authoritative lists and literature reviews were used to obtain information about human and environmental health endpoints to perform a hazard assessment for each essential oil in comparison to the commercial UV-B filter OMC, using the GreenScreen for the Safer Chemicals method [3].

Results

The hazard assessment highlighted the greater safety of all investigated essential oils compared to the commercial UV-filter OMC. Ginger essential oil showed the highest antioxidant activity (ORAC value 2,52; % of inhibition of NO = 45,7) and an in vitro SPF value close to that of OMC. However, the total phenolic content of this essential oil was quite low (0,53 expressed as mg of gallic acid equivalent/g) suggesting that its high antioxidant activity could be attributed to non-phenolic components such as monoterpenoids and sesquiterpenoids whose concentrations are quite high in ginger essential oil. Rosemary, tea tree, palmarosa and basil essential oils showed poor antioxidant activity using the ORAC and NO-scavenging assays, and low SPF values, apart from basil essential oil whose SPF value was not negligible.

Conclusions

The results of this study suggest that ginger essential oil could be a promising candidate to develop sunscreen formulations using lower concentrations of chemical (organic) UV-filters, thus improving human and environmental safety of the resulting cosmetic products.

REFERENCES

[1] Ronsisvalle S, Panarello F, Longhitano G, Siciliano EA, Montenegro L, Panico A., (2020) Natural Flavones and Flavonols: Relationships among Antioxidant Activity, Glycation, and Metalloproteinase Inhibition. Cosmetics. 7, 71.
[2] Siddiqui N, Rauf A, Latif A, Mahmood Z., (2017) Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sc 12, 360-363.
[3] GreenScreen Method: GreenScreen for Safer Chemicals. https://www.greenscreenchemicals.org/learn/full-greenscreenmethod (accessed May 16, 2023).

www.ai4essoil.com: A Comprehensive Essential Oil Database Empowered by Machine Learning: Unveiling Valuable Tools for Enhanced Exploration and Application

Rino Ragno[1,*], Filippo Sapienza[1], Roberta Astolfi[1], Alessio Ragno[2], Roberto Capobianco[2], Rossi Caterina[3] and Stefano Manfredini[3]

[1] Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University of Rome, Rome, Italy;
[2] Department of Computer, Control and Management Engineering "Antonio Ruberti", Sapienza University of Rome Rome, Italy[2]
[3] Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy;

* Email: rino.ragno@uniroma1.it

Keywords: Machine Learning, QCAR, Essential Oil Composition, Biological Activity

Objective

The rapid growth of the essential oil (EO) industry, coupled with the increasing demand for natural remedies and wellness products, necessitates the development of a comprehensive essential oil database equipped with machine learning capabilities. EOs databases are available [1-3], but none of them lists compositions associated to the biological activity. This abstract highlights the objective and key features of a novel essential oil database, the www.ai4essoil.com database, designed to serve as a valuable tool for researchers, practitioners, and enthusiasts in the field. It contains a list of useful information and among them the biological evaluation. The objective of the research is not only the compilation of a EOs' database, but to develop quantitative composition-activity relationships models (QCARs) through the application of machine learning (ML) algorithms [4,5].

Methods

The database was setup by means of the Django, a high-level Python web framework. Several other libraries were also used to display 2D molecular structures (RDKit) and to import PubChem data. ML binary classification models development and validation were carried out by an in-house python script based on the scikit-learn machine learning library. The binary classification models were evaluated by accuracy, Matthews correlation coefficient, receiver operating characteristic and precision-recall curves.

Results

At the time of writing, the www.ai4essoil.com database contained 2685 extracts compositions associated to 1091 plants, 1204 scientific articles and 20693 biological activities. Built ML model will be embedded and presented.

Conclusions

www.ai4essoil.com is a unique EO database that provides an extensive repository of information, encompassing a wide range of EOs. Leveraging the power of machine learning, the database offers an intelligent and user-friendly interface, that enhances the exploration and utilization of EOss.

REFERENCES

[1] Kumari S, Pundhir S, Priya P, Jeena G, Punetha A, Chawla K, Jafaree Z, Mondal S and Yadav G (2014). EssOilDB: A database of essential oils reflecting terpene composition and variability in the plant kingdom. Database, 2014, 1–12

[2] Maia, O. G. S., & Andrade, L. H. A. (2009). Database of the amazon aromatic plants and their essential oils. [Database of the amazon aromatic plants and their essential oils] Quimica Nova, 32(3), 595-622

[3] Libbey, L. M. (1991). A paradox database for GC/MS data on components of essential oils and other volatiles. Journal of Essential Oil Research, 3(3), 193-194

[4] Artini, M.; Patsilinakos, A.; Papa, R.; Božović, M.; Sabatino, M.; Garzoli, S.; Vrenna, G.; Tilotta, M.; Pepi, F.; Ragno, R.; Selan, L. Antimicrobial and Antibiofilm Activity and Machine Learning Classification Analysis of Essential Oils from Different Mediterranean Plants against Pseudomonas aeruginosa. Molecules 2018, 23, 482

[5] Patsilinakos A, Artini M, Papa R, Sabatino M, Božović M, Garzoli S, Vrenna G, Buzzi R, Manfredini S, Selan L, Ragno R. Machine Learning Analyses on Data including Essential Oil Chemical Composition and In Vitro Experimental Antibiofilm Activities against Staphylococcus Species. Molecules. 2019 Mar 3;24(5):890

Chemical and enantioselective analyses of new essential oils in Ecuador: the genus *Gynoxys* Cass. (*Asteraceae*)

Gianluca Gilardoni[1,*], Omar Malagón[1], Nixon Cumbicus[2]

[1] Department of Chemistry, Universidad Técnica Particular de Loja, Loja, ECUADOR
[2] Department of Biological and Agricultural Sciences, Universidad Técnica Particular de Loja, Loja, ECUADOR

* Email: ggilardoni@utpl.edu.ec; gianluca.gilardoni@gmail.com

Keywords: gas chromatography, mass spectrometry, enantiomers, β-cyclodextrins, NMR

Objective

As one of the seventeen Megadiverse Countries, Ecuador is an incredible reservoir of unprecedented botanical species. In fact, most of the Ecuadorian flora is still unstudied from the chemical point of view, constituting a great chance for the discovery of new natural products. The objective of the present communication is to resume the investigation carried out on the EOs from the genus *Gynoxys* Cass. in Southern Ecuador. The genus *Gynoxys*, belonging to the family *Asteraceae*, is a native taxon of the Andean region, distributed from Bolivia to Venezuela and including 116 accepted species. Ecuador is the center of the native area, and it hosts 33 species, of which 23 endemics, growing between 2000-4500 m above the sea level. In the present research, the essential oils of the following species are currently under investigation: *Gynoxys miniphylla* Cuatrec., *Gynoxys rugulosa* Muschl., *Gynoxys reinaldii* Cuatrec., *Gynoxys buxifolia* Cass., *Gynoxys cuicochensis* Cuatrec., *Gynoxys sancti-antonii* Cuatrec., *Gynoxys szyszylowiczii* Hieron., *Gynoxys laurifolia* Cass., *Gynoxys hallii* Hieron.; *Gynoxys pulchella* Cass., *Gynoxys azuayensis* Cuatrec., and *Gynoxys calyculisolvens* Hieron. [1-3].

Methods

The dry leaves are analytically steam-distilled in a Marcusson-type apparatus, obtaining an EO in solution of cyclohexane with *n*-nonane as internal standard [2]. The analytical samples are directly submitted to qualitative and quantitative analyses (GC-MS and GC-FID respectively), with two orthogonal stationary phases. The qualitative analyses are based on the linear retention index and mass spectrum of each compound, whereas in the quantitative analyses the components are quantified by external calibration, calculating the relative response factor of each constituent according to its combustion enthalpy. Furthermore, the most common chiral terpenes are submitted to enantioselective analysis on two β-cyclodextrin-based chiral selectors.

Results

All the species analyzed so far afforded unprecedented EOs, with about 0.02% (*w/w*) yield in most cases. The EOs were usually dominated by the sesquiterpene fraction, where germacrene D and β-(*E*)-caryophyllene were the main components. In the case of *G. buxifolia*, the major constituents were furanoeremophilane and bakkenolide A, whose identification was carried out through NMR spectroscopy and mass spectrometry. So far, only *G. miniphylla* EO appeared dominated by monoterpenes, being α-phellandrene the main compound. About the enantioselective analyses, a quite different enantiomeric composition was determined for each species.

Conclusions

The enantioselective analyses confirmed the existence, in all these species, of different biosynthetic pathways, devoted to the production of different enantiomers. Despite the chemical composition is often quite similar, a common chiral pattern could not be determined so far. This project is still in progress however, once all the chemical and enantioselective analyses will be available, a PCA-based statistical analysis will be conducted, to determine the possible existence of metabolically similar groups inside the *Gynoxys* population of Southern Ecuador.

REFERENCES

[1] Malagón O., Cartuche P., Montaño A., Cumbicus N., Gilardoni G., (2022). A New Essential Oil from the Leaves of the Endemic Andean Species Gynoxys miniphylla Cuatrec. (Asteraceae): Chemical and Enantioselective Analyses, Plants, 11, 398.
[2] Maldonado Y.E., Malagón O., Cumbicus N., Gilardoni G., (2023). A New Essential Oil from the Leaves of Gynoxys rugulosa Muschl. (Asteraceae) Growing in Southern Ecuador: Chemical and Enantioselective Analyses, Plants, 12, 849.
[3] Cumbicus C., Malagón O., Cumbicus N., Gilardoni G., (2023). The Leaf Essential Oil of Gynoxys buxifolia (Kunth) Cass. (Asteraceae): A Good Source of Furanoeremophilane and Bakkenolide A, Plants, 12, 1323.

Ohmic heating to valorize pineapple waste: GC-MS and headspace-SPME analysis of the extracted essential oils

Mohsen Gavahian[1,*]

[1] Department of Food Science, National Pingtung University of Science and Technology, Neipu, Pingtung, 91201, TAIWAN

* Emails: mohsengavahian@yahoo.com; mg@mail.npust.edu.tw

Keywords: pineapple essential oil, ohmic extraction, GC-MS; headspace-SPME; energy consumption; waste valorization

Objective

Ohmic heating is an emerging food processing technology that can extract essential oils. In a previous study, this technology was used to extract bioactive compounds from pineapple, and a preliminary design of an ohmic-assisted hydro-distillation system was proposed to collect the volatiles of pineapple peel as a considerable food waste/processing by-product in Taiwan. This study aims to analyze the chemical composition of essential oils/hydrosol obtained from pineapple waste through ohmic heating and to elaborate on the role of such a process in achieving sustainability.

Methods

The industrial waste of Tainung 17 pineapple (*Ananas comosus*) was collected from Pingtung Country of Taiwan. A new ohmic-assisted hydro-distillation system was designed and developed at the Emerging Food Processing Technology Laboratory of the National Pingtung University of Science and Technology, Taiwan. The raw materials were extracted using salted water as the extraction solvent. The extracts were collected using a Clevenger-type apparatus. The extraction time and consumed energy were analyzed and compared to that of a traditional extraction system. The essential oils were then analyzed by GC-MS and headspace-SPME/GC-MS methodologies. The detected peaks were then compared with the NIST database.

Results

The newly developed essential oil extraction system successfully isolated the volatiles from pineapple waste. Data demonstrated that the ohmic system saved a substantial amount of extraction time and reduced energy consumption in comparison with the traditional extraction system. Besides, various components were identified by both GC-MS and headspace-SPME-GC/MS, and major compounds' relative concentrations were calculated based on their relative peak area.

Conclusions

This study proposed an energy- and time-saving valorization platform for pineapple waste valorization. After further investigations, the extracted essential oil and hydrosol might be considered in the food and pharmaceutical industries. This can open new insight into future studies on essential oil extraction, analysis, and application. Considering the valorization of waste, and significant reduction of energy consumption, the proposed methodology could contribute significantly to achieving sustainability.

ACKNOWLEDGMENTS

This research was supported by the National Science and Technology Council (NSTC), project number NSTC 112-2221-E-020 -001-MY3, Taiwan, ROC. The technical help and scientific comments of Dr. Chih-Chun Jean Huang for chromatographic analysis are acknowledged and highly appreciated.

Fast GC(×GC)-TOFMS using Hydrogen carrier gas applied to differential analysis of Essential oils

Sebastiano Pantò[1,*], Lena M. Dubois[1], Dmitrii Rakov[1], Nick Jones[1]

[1] LECO European Application & Technology Center, Berlin, Germany

* Email: sebastiano_panto@leco.com

The qualitative characterization of the volatile fraction of essential oils (EOs) is generally attained by gas chromatography-mass spectrometry (GC-MS). The methods developed for this purpose typically use long GC capillary columns (30 to 60 m) and apply slow oven temperature ramp rates for optimal chromatographic resolution of crucial yet closely eluting analytes. In most cases, this translates into analysis times that are greater than one hour. In addition, many of these methods use helium (He) as carrier gas due to i) historical reasons, ii) manufacturer requirements and/or iii) scarce acceptance of hydrogen (H_2) as a GC-MS carrier gas. All these factors contribute to high economical costs and often limit laboratories' throughput.

To respond to today's laboratory needs, we developed a H_2-supplied GC method coupled to time-of-flight mass spectrometry (TOFMS) to facilitate full mass range spectra to be collected at fast acquisition rates, producing high quality data which allows automated deconvolution to be applied efficiently. This led to richer, faster, and more detailed results. GC×GC-TOFMS data are presented as well for comparative purposes and as proof-of-principle on the application of H_2 as carrier gas in multidimensional chromatography. Additionally, GC×GC-TOFMS data was generated using both He and $H_2$and results were compared in terms of chromatographic separation and spectral quality. Different EO grades were investigated to assess the method quality and robustness.

Furthermore, we show how laboratory workflows and insights can be significantly improved by processing the data using a new software tool where multiple EO samples can be easily processed and compared, allowing a fast and reliable route to determine analyte similarities and differences.

Unravelling the chemical complexity of essential oils: in-depth characterization and profiling by comprehensive two-dimensional chromatography and mass spectrometry (GC×GC-MS)

Daniela Peroni[1,*], Raul Franceschini[1]

[1] SRA Instruments SpA, Cernusco sul Naviglio (MI), ITALY

* Email: peroni@srainstruments.com

Keywords: essential oils analysis, GC×GC-MS, characterization, classification.

Objective

The aim of this work was to determine the chemical composition of essential oils for in-depth characterization and classification. The samples under study were leaf essential oils of lavender (*Lavandula Officinalis L., Lavandula Angustifolia Miller*) and rosemary (*Rosmarinus Officinalis L. Camphoriferum/Verbenoniferum/Cienoliferum*) plants harvested in Italy produced by steam extraction. Analyses were performed by comprehensive two-dimensional gas chromatography coupled to Mass Spectrometry (GC×GC-MS) to tackle high sample complexity and achieve insightful profiling. Detailed composition information was exploited to classify the essential oils based on plant strains. Commercial essential oils produced by solvent extraction were also analysed as reference material to evaluate the impact of isolation process.

Methods

Essential oils were obtained by steam distillation, this methodology was selected because it allowed simple production on a small scale and extraction does not involve the use of chemical solvents, removing the need for purification steps to make the products suitable for food purpose. In this process water was brought to a boil to generate a steam current that was directed through the plant material (leaves) to release the oil. Condensation to liquid phase by contact with a coil resulted in a biphasic solution of organic layer water. The aqueous phase was discharged by gravity, the organic phase was collected as the final product.

Analyses were performed with different GC×GC-MS platforms to weigh the impact of configurations on performance and applicability. We investigated approaches that favoured stability and ruggedness for routine use as well as more performing *high-end* solutions, respectively, for both modulation (differential reverse flow modulator, cryogen-free thermal modulator) and detection (single quadrupole MS, TOF-MS with low-energy Tandem Ionization). Data processing was performed with commercial software and included statistical tools such as Principal Component Analysis (PCA) for sample classification.

Results

GC×GC-MS allowed effectively separating and detecting a significantly higher number of components compared to conventional GC-MS. Individual compounds and chemical groups (e.g. aldehydes, ketones, alcohols, terpenes, etc.) were distributed across a 2D space, reducing co-elutions with beneficial effect on identification. The enhanced two-dimensional separation, combined with the third dimension added by MS data, allowed to profile the composition for all leaf essential oils with elevated degree of detail.

Highly informative 2D chromatograms confirmed excellent for untargeted screening aimed at samples comparison. Differences in composition between lavender (2 trains, different extraction batches) and rosemary (3 trains, different extraction batches) oils from different strains were observed. Similarly, it was possible to highlight key differences between essential oils produced by steam extraction and the commercial oils gaining useful information on extraction impact on chemical composition and thus properties. The streamlined workflows offered by the data processing software allowed to minimize laborious, challenging procedures to enable advanced yet (semi-) automated user-friendly operation.

Conclusions

GC×GC-MS is a powerful tool for detailed profiling of complex mixtures such as essential oils. Chemical composition of lavender and rosemary oils was characterized with much more insight compared to conventional separation techniques. Furthermore, characteristic 2D profiles demonstrated very effective to highlight insightful differences useful to classify origin in terms of plant strains. The multivariate analysis tools employed proved useful to extend the applicability of advanced data processing in an effective yet accessible manner.

Synergy between *Cinnamomum zeylanicum* essential oil and Fluconazole to bypass the resistance of *Candida auris*.

M. Di Vito[1*], S. Garzoli[2], R. Rosato[1], M. Mariotti[1], J. Gervasoni[3], L. Santucci[3], E. Ovidi[4], M. Cacaci[1], G. Lombarini[1], R. Torelli[5], A. Urbani[3], M. Sanguinetti[1,5], F. Bugli[1,5]

[1]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, ITALY
[2]Dipartimento di Chimica e Tecnologie del Farmaco, Università di Roma Sapienza, Piazzale Aldo Moro 5, Rome, ITALY
[3]UOC Chimica, Biochimica e Biologia Molecolare Clinica, Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, ITALY.
[4]Department for Innovation in Biological, Agro-food and Forest systems DIBAF - University of Tuscia, Viterbo, ITALY
[5]Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, ITALY

* Email: maura.divito@unicatt.it

Keywords: Essential oils, fungal infections, nosocomial infections, fungal resistance, antifungal drugs

Objective

Candida auris is a multidrug-resistant fungus known to be a global public health problem. The skin-based transmission, together with the marked resistance to drugs, resulted in its rapid spread to all continents. The aim of this study was to identify an essential oil (EO) able to reactivate the sensitivity to fluconazole in the fight against *C. auris* resistance.

Methods

According to EUCAST guidlines, the broth micro-dilution test was used to evaluate the efficacy of 15 EOs (*Anethum graveolens* L., *Cinnamomum camphora* L., *Cinnamomum zeylanicum* Blume (bark, CZ-EO), *Coriandrum sativum* L., *Cuminum cyminum* L., *Cymbopogon martinii* Roxb, *Cymbopogon citratus* Stapf, *Elettaria cardamomum* (L.) Maton, *Helichrysum italicum* (Roth) G. Don., *Lavandula angustifolia* Mill., *Melaleuca alternifolia* (Maiden & Betche) Cheel., *Mentha x piperita*, *Pelargonium graveolens* L'Hér., *Rosmarinus officinalis* L., and *Thymus vulgaris* L. thymol chemotype), against 10 clinical strains of *C. auris*. To identify the most active compound of the best EO, three fractions obtained from CZ-EO were microbiologically tested in comparison with its major chemical compound, the cinnamaldehyde (CIN). GC-MS analysis combined with headspace sampling was used to develop the quality analysis of both CZ-EO and the three fractions. Subsequently, the synergy between the fluconazole and CZ-EO, or its active fraction (FR2), or CIN were tested using checkerboard tests. Finally, to study the mechanism of action of CZ-EO and fluconazole synergistic concentrations, biochemical tests were done. *In vivo* toxicity studies were conducted on *Galleria mellonella* larvae by testing scalar dilution of CZ-EO ranging from 32% v/v to 0.5% v/v.

Results

Cinnamomum zeylanicum Blume EO (CZ-EO, CIN=66.1%) obtained from bark was the most effective EO with MIC90 and MFC90 values equal to 0.06% v/v. Microbiological analyses showed that the fraction containing CIN (FR2, CIN=85.7%) is the active fraction of CZ-EO with anti-fungal activity. Checkerboard test showed that CZ-EO and FR2, but not CIN, synergized with fluconazole at therapeutic concentrations of the drug (0.45 ± 0.32 mg/mL and 0.64 ± 0.67 mg/mL, respectively), while CIN only shows additive activity. Biochemical tests show that in the presence of synergistic cytocidal concentrations of both fluconazole and CZ-EO, the activity of fungal ATPases decreases and, at the same time, the amount of intracellular drug increases. *In vivo* studies show the absence of toxicity of CZ-EO up to concentrations of 16% v/v.

Conclusions

This study highlights how small doses of CZ-EO are able to inhibit the cellular secretion of fluconazole promoting its accumulation in the fungal cells. In this manner, the drug is able to exert its pharmacological effects bypassing the resistance of the yeast. If further studies will confirm this synergy, it will be possible to develop new therapeutic formulations active in the fight against *C. auris* resistances [1].

REFERENCES

[1] Di Vito M., Garzoli S., Rosato R., Mariotti M., Gervasoni J., Santucci L., Ovidi E., Cacaci M., Lombarini G., Torelli R., Urbani A., Sanguinetti M., Bugli F., (2023). A New Potential Resource in the Fight against *Candida auris*: the *Cinnamomum zeylanicum* Essential Oil in Synergy with Antifungal Drug. *Microbiol Spectr*. 11(2):e0438522.

Caper leaf essential oil as antimicrobial and antioxidant agent for food applications

Fabrizio Cincotta[1], Maria Merlino[1], Luca Nalbone[1], Anthea Miller[1], Martina Buda[1], Graziella Ziino[1], Antonella Verzera[1] and Concetta Condurso[1,*].

[1] Department of Veterinary Sciences, University of Messina, Messina, ITALY

* Email: ccondurso@unime.it

Keywords: Capparis spinosa L., leaf essential oil, chemical composition, antimicrobial activity, antioxidant activity.

Objective

The caper (Capparis spinosa L. Capparaceae) is a common aromatic plant that is widespread in the Mediterranean area and recognized as healthy for human consumption [1], mainly due to the amount of polyphenols and the presence of glycosinolates compounds.
Since different essential oils have been proposed as potential microbiological agents in meat and meat products [2], the objective of this work was to study the composition and the antimicrobial and antioxidant activity of the essential oil obtained from the capper leaves, coming from the agronomic practices, thus proposing the essential oil for food applications, such as the meat preservation.

Methods

To reach the objectives, the leaves have been collected from plants cultivated in the Eolian Island, the oil has been extracted by a hydrodistillation method and analysed by GC-MS for the volatile constituents, and UV-Vis for the polyphenols amount and antioxidant activity by Folin-Ciocalteu and DPPH assay respectively.
The antimicrobial properties against Gram-positive and Gram-negative bacteria were assayed measuring inhibition halons, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

Results

More than one hundred volatile compounds were identified in the essential oil, belonging to the class of aldehydes, alcohols, esters, ketones, terpenes, acids, sulfur compounds, pyrazines, and furanoic and aromatic compounds. The antioxidant activity of the oil has been determined and oil showed a significant inhibitory effect.
The results demonstrated the possibility of using the leaf caper oil to preserve the meat from microbial spoilage and lipid oxidation prolonging its shelf-life. The use of the leaves coming from the agronomic practices results of great interest in the context of a circular economy that aims to increase waste **reuse and recycling.**

REFERENCES

[1] Olas, B., (2023). The current state of knowledge about the biological activity of different parts of capers, Nutrients 15 (3), 623.
[2] Ji, J., Shankar, S., Royon, F., Salmieri, S., & Lacroix, M., (2023). Essential oils as natural antimicrobials applied in meat and meat products A review. Critical Reviews in Food Science and Nutrition, 63(8), 993-1009.

Boosting the confidence in identification in the Flavour and Fragrance field via a Gas Chromatography – Mass Spectrometry – Fourier Transform Infrared Spectroscopy instrument

Tania M. G. Salerno[1*], Carmelo Coppolino[1], Lorenzo Cucinotta[1], Paola Donato[1], Luigi Mondello[1,2]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: tsalerno@unime.it

Keywords: hyphenated instrument, gas chromatography, infrared spectroscopy, solid deposition

Objective

This study describes the development of a gas chromatography - mass spectrometry - solid deposition fourier transform infrared spectroscopy instrument (GC-MS-*sd*-FTIR). Hyphenation of GC-MS to FTIR simultaneously provide mass and vibrational spectra of the chromatographically separated compounds along with information about their retention behaviour. İn detail, a solid deposition FTIR interface allows to overcome the limitations related to the poor detection limits of light pipe GC-FTIR.[1] Furthermore, the use of a liquid nitrogen cooled disc allows for high volatile component to be trapped and detected.

Methods

The two detectors were coupled in parallel, by diverting the exit column flow; hence, MS and IR data were obtained in a single injection. Flavour and fragrance standard mixtures and a parfume sample were analyzed; components were identified by comparison of the MS spectra in a commercial spectral database (FFNSC 4.0, Chromaleont S.r.l.) using Linear Retention Index (LRI) filter and through library search in a solid-phase IR spectral library.

Results

Dealing with multiple instrument hyphenation in a single setup can be challenging in terms of parameters optimization (i.e. flow split) and data handling/processing. Typical parallel detection can be achieved by performing conventional GC followed by post-column splitting in a suitable ratio, to meet the sensitivity requirements of the two detectors (e.g. 90% *v/v* of the effluent to FTIR and the rest to MS). In detail, for this study the two instruments were connected by means of an external heated transfer-line developed in our laboratory. The exiting GC column flow was diverted to the two detectors by using capillaries of different length and size, to adjust the flow ratio. The FTIR interface parameter were optimized in terms of disk temperature and speed to obtain optimal deposit.

Conclusions

This approach demonstrates the capability of the instrument to increase the confidence in compound identification, offering complementary benefits from each technique. Compound identification was attained by comparison with libraries of GC retention indices, mass spectra, and infrared spectra. MS provided molecular and fragment ion *m/z* mass values and their relative abundances that, through database searching, offered a set of possible compounds; false-positive compounds were screened out based on their GC retention indices or on their infrared spectra.

ACKNOWLEDGMENTS

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

REFERENCES

[1] Salerno T. M. G., Donato P., Frison G., Zamengo L., Mondello L., (2020). Gas Chromatography-Fourier Transform Infrared Spectroscopy for Unambiguous Determination of Illicit Drugs: A Proof of Concept, *Frontiers in Chemistry*, 8, 624

Assessment and challenges of mineral oil contamination in *Citrus* essential oils

Alessia Arena[1*], Mariosimone Zoccali[2], Peter Q. Tranchida[2], Luigi Mondello[1,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Department of Mathematical and Computer Science, Physical Sciences and Earth Sciences, University of Messina, Messina, ITALY
[3] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: alessia.arena@unime.it

Keywords: mineral oil saturated hydrocarbons, mineral oil aromatic hydrocarbons, multidimensional liquid-gas chromatography, *Citrus* essential oils.

Objective

Citrus essential oils (EOs) are industrially extracted from the peels of *Citrus* fruits using either steam distillation, dry distillation, or by an appropriate mechanical process without heating. *Citrus* EOs are increasingly consumed as food supplements and, when marketed as foods in the European Union (EU), they fall within the scope of Directive 2002/46/EC. Over recent years, there has been significant attention towards the presence of mineral oil hydrocarbons (MOHs) in foods and their potential health risk. MOHs consist of three classes of compounds: paraffins, naphthenes, and aromatics. According to the best of the present authors' knowledge, no investigation has been published regarding the occurence of MOHs in *Citrus* EOs. Given the wide use of *Citrus* EOs in the food sector, the purpose of this research was to evaluate the feasibility of analyzing MOHs in such samples following the EU guidance. For such a scope, an heart-cutting multidimensional liquid-gas chromatography system (LC-GC) was used, highlighting the challenges posed by such samples, due to their specific chemical compositions.

Methods

The analysis of MOHs consists in the isolation and separation of the MOSH and MOAH fractions, which can be carried out on an LC silica gel column using an n-hexane/dichloromethane gradient. Then, the two fractions are transferred to a GC-FID system for quantification.

Results

Eighteen *Citrus* EOs were analysed; specifically, cold-pressed (CP) bergamot (two), lemon (two), mandarin (green, yellow, red), sweet orange, bitter orange, grapefruit, key lime B, and distilled (D) bergamot (two), lemon, mandarin (green), sweet orange, grapefruit, key lime B. Only the CP samples were found to be MOSH-contaminated (some at rather high levels), while the D ones, as expected, were not. With regard to the MOAH, none were detected.

Conclusions

Challenges and limitations will be discussed. In such a respect, the composition of such samples, rich especially in hydrocarbon monoterpenes, poses several difficulties. Presumably, the development of a sample treatment method will be necessary to achieve the proposed LoQ of 0.5 mg kg^{-1} for the "dry, low-fat content" category fixed by the EU guidance.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corporation and Merck Life Science for their continuous support.

Salvia apiana In Vitro Shoot System as a source of biologically active volatile fraction

Agata Król[1,*], Adam Kokotkiewicz[1], Bożena Zabiegała[2], Klaudia Ciesielska-Figlon[3], Jacek M. Witkowski[3], Maria Łuczkiewicz[1]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, POLAND
[2] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, POLAND
[3] Department of Department of Pathophysiology, Faculty of Medicine, Medical University of Gdańsk, Gdańsk, POLAND

* Email: agata.krol@gumed.edu.pl

Keywords: Essential oil, Lamiaceae, Abiotic and biotic elicitation, Lymphocytes, Flow cytometry

Objective

White sage (*Salvia apiana* Jepson) grows in mild, Mediterranean-type climate areas of the North America. This endemic species is a medicinal and ritual plant used as a calmative agent and to treat inflammation by Native North American people. Research into its chemical composition and biological activity is limited due to endemic nature and lack of effective cultivation methods [1]. *In vitro* cultures of *S. apiana* could potentially provide a alternative source of high-quality plant material. In view of the above, white sage was used to obtain *in vitro* shoot system capable of constant production of volatile oil. Essential oil (EO) from *in vitro* shoots was evaluated for its pro-apoptotic activity toward CD4 and CD8 T cells from peripheral blood samples from healthy volunteers. Biological tests were also performed on an EO obtained from a ground plant, as well as the dominant compound of this fraction, 1,8-cineol. This study also aimed to evaluate the influence of elicitation techniques on the EO production of *S. apiana* microshoots.

Methods

The microshoot culture was initiated from shoot tips of aseptically germinated seedlings of *S. apiana*. They were grown on Schenk – Hildebrandt (SH) agar medium supplemented with 6-benzylaminopurine (BAP) 2 mgl^{-1} and thidiazuron (TDZ) 0,22 mgl^{-1}. *S. apiana* microshoots were cultured in the commercial available temporary immersion bioreactor RITA and treated for 3 and 7 days with chitosan, ergosterol and yeast extract (YE) to stimulate the production of EO. The phytochemical investigations included qualitative and quantitative GC/MS and GC/FID analyses of volatile oils obtained by hydrodistillation (Clevenger apparatus) from the *in vitro* biomasses and from the ground plant material of white sage. Phenotyping and analysis of effects of EOs on proliferation rate of the perpiheral blood mononuclear cells were performed using flow cytometry.

Results

The EO of *S. apiana* microshoots was rich in 1,8-cineole, *α*-pinene, *β*-pinene, camphor and did not contain thujone. EOs from the microshoots and leaves form field-grown plant were demonstrated to have dose-dependent anti-proliferative and pro-apoptotic activity toward CD4 and CD8T cells. Elicitation with 100 mg/l YE improve the production of EO by 9,37%.

Conclusions

In vitro culture experiments and phytochemical research confirmed the feasibility of establishing microshoot cultures of *S. apiana* which are capable of accumulating essential oil with pro-apoptotic activity toward CD4 and CD8 T cells. *S. apiana* is rather resistant to elicitation strategies, both abiotic and biotic.

ACKNOWLEDGMENTS

The study was supported by the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Programme Knowledge Education Development 2014–2020

REFERENCES

[1] Krol, A., Kokotkiewicz, A., & Luczkiewicz, M. (2022). White Sage (Salvia apiana)–a Ritual and Medicinal Plant of the Chaparral: Plant Characteristics in Comparison with Other Salvia Species. Planta Medica 88(08), 604–627. doi: 10.1055/a-1453-0964

Hydrodistillation versus microwave-assisted hydrodistillation of dill (*Anethum graveolens*) seeds: Kinetics, chemical profile and bioactivity

Branimir Pavlić[1,*], Nevena Gladikostić[1], Bojana Ikonić[1], Aleksandra Sknepnek[2], Dunja Miletić[2], Zoran Zeković[1], Nemanja Teslić[3],

[1] University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2] University of Belgrade, Faculty of Agriculture, Institute of Food Technology and Biochemistry, 11000 Belgrade, Serbia
[2] Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

* Email: bpavlic@uns.ac.rs

Keywords: dill, microwave-assisted hydrodistillation, kinetics, essential oil, bioactivity

Objective
Essential oils are gaining interest from the academic and industrial communities due to numerous biological activities and broad spectrum of applications. With the arrival of the "green era", there was a need to reduce the production of waste materials and to make better use of natural resources. Due to ever-increasing market of functional foods, the search for new natural bioactive components is a hot topic on which a lot of research effort is being focused currently and biorefinery has recently emerged as promising approach which could lead towards sustainable concept of production. Therefore, the aim of this work was valorization of dill (*Anethum graveolens*) seeds for production of essential oils with potent antioxidant and antimicrobial activity.

Methods
Conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MWHD) were applied for essential oil recovery. Influence of heating (205 and 410 W) and microwave irradiation (90, 180, 360, 600 and 800 W) power on distillation kinetics and chemical profile of essential oil was evaluated. Empirical mathematical models were used for modeling of process kinetics. Chemical profile was evaluated by GC-MS and monoterpenes. Extracts were characterized in terms of *in vitro* antioxidant activity and antimicrobial activity towards selected food-borne pathogens.

Results
MWHD provided improvement in yield of essential oil comparing to conventional HD. Major terpenoids present in all samples were limonene and carvone. All utilized mathematical models provided excellent fit with the experimental data. Obtained samples provided moderate antioxidant and antimicrobial potential which highlighted them as candidates for further application in food products.

Conclusions
It could be concluded that dill essential oils had particularly high content of essential oil. These essential oils were already recognized with broad spectra of application as potent antioxidants in food, nutraceuticals and cosmetics. Based on this, our further research of application of dill essential oils as natural additives in various meat products.

ACKNOWLEDGMENTS

This research was supported by the Science Fund of the Republic of Serbia, 7750168, Novel extracts and bioactive compounds from under-utilized resources for high-value applications – BioUtilize.

Comparative in vitro and *in silico* enzyme inhibitory screening of *Rosa x damascena* and *Pelargonium graveolens* essential oils and geraniol

Ayşe Esra Karadağ[1*], Sevde Nur Biltekin[2,3], Betül Demirci[4], Fatih Demirci[4,5], Usman Ghani[6]

[1] Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Beykoz, Istanbul, TÜRKİYE
[2]Department of Pharmaceutical Microbiology, School of Pharmacy, Istanbul Medipol University, Istanbul, TÜRKİYE
[3]Institute of Sciences, Istanbul University, Istanbul, TÜRKİYE
[4] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, TÜRKİYE
[5] Department of Pharmacognosy, Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, N. Cyprus, Mersin 10, TÜRKİYE
[6] Clinical Biochemistry Unit, Department of Pathology, College of Medicine, King Saud University, Riyadh, 12372, SAUDI ARABIA

* *Email:* ayseesraguler@gmail.com

Keywords: Geraniol, *in vitro* and *in silico* enzyme inhibition, anticancer

Objective

Rosa x damascena Herrm. is reported against inflammatory conditions and menthal disorders. It is known that *Pelargonioum graveolens* essential oils (P) are used or adultared instead of *R. damascena* oil (R) due to the similarity of its chemical profile. This present study aimed to evaluate *R. damascena* and *P. graveolens* essential oils and their major constituent geraniol for their *in vitro* and *in silico* anti-inflammatory, anticholinesterase, potential antiviral and cytotoxic activities.

Methods

The commercially available R, P essential oils and geraniol were analysed and confirmed by GC/FID and GC/MS, respectively. MTT was used for *in vitro* cytotoxic/anticancer effects by using the HEK293/A549, MCF7, PC3 cell lines. 96 well microplate based *in vitro* COX-1, COX-2, and ACE2 commercial test kits were used, along with inhouse 5-LOX, AChE and BChE enzyme inhibition assays. Also, *in silico* based analyses were performed.

Results

The major component of *R* and *P* essential oils was identified as 27.2% and 38.7% geraniol, respectively. The ACE2 enzyme inhibitions of 20 µg/mL *R and P* essential oils were calculated as 56%, and 67%, respectively. ACE2 enzyme inhibition of the pure geraniol at 5 µg/mL was 86%. The IC_{50} value of AChE enzyme activity was calculated as 69.4, 47.7 and 16.7 µg/mL for *R, P.* and geraniol, respectively; The IC_{50} value for BChE was determined as 55.1, 50 and 9.1 µg/mL, respectively. *R., P.* essential oils and geraniol showed IC_{50} values of 24.4, 11.1 and 11 µg/mL against COX-1 enzyme; It was determined as 39.1, 50 and 8.1 µg/mL for the COX-2 enzyme, respectively. For the 5-LOX 19.1, 23.1 and 7 µg/mL IC_{50} values were calculated in the same order. The IC_{50} for *R.* essential oil on A549, MCF7, and PC3 cells were 473.5, 105.6, 412.1, 638.79±1.15 µg/mL, respectively. No cytotoxic effects on healthy HEK293 cells were observed for *the R.* essential oil. Whereas the IC_{50} values of *P.* essential oil on A549, MCF7, PC3, and HEK 293 cells were 382.4, 89.5, 112.1, and 611.8 µg/mL respectively. However, the tested essential oils increased apoptosis which were statistically significant.

As initial results, *in vitro* cholinesterase inhibitions were in correlation considering their traditional use. Both oils showed selective potential for COX-1 inhibition and apoptosis for the selected cancer cell lines, for the first time to the best of our knowledge. Molecular docking studies of geraniol with the target enzymes revealed that its interactions, especially with AChE, BChE and COX-1 and 2, are similar to that of known drugs for the enzymes. This shows that geraniol carries drug-like potential for the treatment of various diseases.

Conclusions

In vitro and *in silico* methods are an important stage of biological activity evaluation, standard essential oils may help for the repeatability. However, further *in vivo* assays are needed to confirm the experimental results.

ACKNOWLEDGMENTS

This research project was supported by both TUBITAK (The Scientific and Technological Research Council of Turkey) Project No: 2210437 and Anadolu BAP Project No:2005S058.

Hemisynthesis of limonaketone a barely available monoterpene in *Cedrus atlantica* essential oil: Viable route and MEDT study

Ayoub Abdelkader Mekkaoui[1*], Hicham Ben El Ayouchia[2], Hafid Anane[2], Rachid Chahboun[3], Larbi El Firdoussi[1], and Soufiane El Houssame[4]

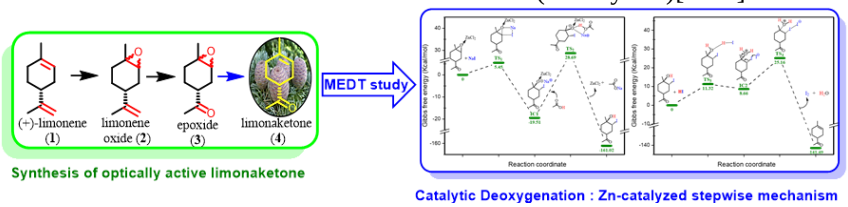
[1] Laboratoire de Chimie Moléculaire, Equipe de Chimie de Coordination et Catalyse, Département de Chimie, Université Cadi Ayyad, Faculté des Sciences Semlalia, BP 2390, 40001 Marrakech, Morocco.
[2] Laboratoire de Chimie Analytique et Moléculaire, LCAM, Faculté Polydisciplinaire de Safi, Université Cadi Ayyad, Safi 46030, Morocco.
[3] Departamento de Química Orgánica, Facultad de Ciencias, Instituto de Biotecnología, Universidad de Granada, 18071 Granada, Spain.
[4] Laboratoire des Sciences des Matériaux, Mathématiques et Environnement, Université Sultan Moulay Slimane, Faculté Polydisciplinaire de Khouribga, BP 145, 25000 Khouribga, Morocco.

* Emails: mekk.ayoub@gmail.com ; a.mekkaoui@uca.ac.ma

Keywords: *Cedrus atlantica*, limonaketone, limonene, deoxygenation, MEDT study.

Objective

Intense interest was given to derived carbonyls from monoterpenes, a class of naturally occurring compounds in plants which possess a range of pharmacological properties. In contrast, only few studies are reported in literature for the isolation, synthesis, and reactivity of limonaketone [1]. Herein, we report a novel, easy and efficient pathway as well as density functional theory (DFT) mechanistic study for the synthesis of optically active limonaketone (*Graphical abstract*), a high added value monoterpene, starting from limonene, natural and low-cost feedstock [2]. The strategy was developed in an excellent 3-step synthesis of limonaketone, potentially important and barely available in the Morrocan *Cedrus atlantica* essential oil (0.1% yield)[1b-2].



Graphical abstract: Synthesis and MEDT study of optically active limonaketone

Methods

The hemisynthesis of limonaketone (**4**) was carried out as follow: (i) Aerobic epoxidation of limonene (**1**) catalyzed by a ruthenium complex; (ii) Ozonolysis of limonene oxide (**2**) to obtain the characteristic ketone function; (iii) Deoxygenation of epoxide (**3**) catalyzed by Zn powder. All the prepared compounds were characterized by NMR and GC–MS analyses. The Molecular Electron Density Theory (MEDT) study of the Zn-deoxygenation step was performed using DFT calculations at the M06–2X/6–311G(d,p) (LANL2DZ for Zn) level.

Results

The optically active limonaketone was prepared in 3 steps with a total yield of 84%. Moreover, the yield of each step was (i) 91%, (ii) 92%, and (iii) 100% (after an optimisation study of Zn-deoxygenation). The observed chemoselectivity in the Zn-deoxygenation step as well as its corresponding mechanistic pathway were explained by MEDT study. Furthermore, the mechanistic aspect of each reagent or additive in the Zn-deoxygenation was investigated. Accordingly, the deoxygenation reaction of epoxide (**3**) follows a stepwise mechanism with an easy reaction in terms of energetic, which is in good agreement with the experimental observations.

Conclusions

Novel, easy, and efficient hemisynthesis of optically active limonaketone was carried out starting from limonene. The reactions afford an optically pure limonaketone with an overall yield of 84%. The obtained chemoselectivity was explained by means of MEDT study as well as local reactivity indexes obtained from the Parr functions.

References

[1] (a) N.M. Donahue, J.E. Tischuk, B.J. Marquis, K.E. Huff Hartz, (2007) Phys. Chem. Chem. Phys., 9, 2991–2998; (b) I. Ait Lahcen, S. Oubaassine, B. Boualy, S. El Houssame, L. El Firdoussi, A. Karim, (2018). Lett. Org. Chem., 15, 698–704; (c) E. Grimal, (1902). Compt. Rend. Hebd. Séances Acad. Sci., T135, 582–583; (d) V.V. Veselovsky, A.S. Gybin, A.V. Lozanova, A.M. Moiseenkov, W.A. Smit, R. Caple, (1988)., Tetrahedron. Lett., 29, 175–178; (e) A.A. Mekkaoui, A. Aberkouks, L. Fkhar, M. Ait Ali, L. El Firdoussi, S. El Houssame, (2020), Appl. Organomet. Chem., 34, e5917; (f) A.A. Mekkaoui, M. Laayati, H. Orfi, L. El Firdoussi, S. El Houssame, (2020). J. Chem., 2020, 1–8.
[2] A.A. Mekkaoui, H. Ben El Ayouchia, H. Anane, R. Chahboun, L. El Firdoussi, S. El Houssame, (2021). Journal of Molecular Structure, 1235, 130221.

Raw fragrance materials as potential antimicrobial agents

Alicja K. Surowiak[1,*], Lucyna Balcerzak[1], Daniel J. Strub[1,2]

[1] Department of Chemical Biology and Bioimaging, Wroclaw University of Science and Technology, Wrocław, POLAND
[2] Liquid Technologies sp. z o. o., Wrocław, POLAND

* Email: alicja.surowiak@pwr.edu.pl

Keywords: antimicrobial effect, essential oils, plant extracts

Objective

More than 600 of the available commercially available natural fragrance materials have been tested in terms of their antimicrobial activity against opportunistic pathogenes related to nosomical infections. Maintaining microbiological purity in hospitals, public spaces, and production spaces is still a challenge. In the face of growing drug resistance, other effective therapeutics and alternative disinfectants are still being sought. Although it is difficult to expect that natural products will be as effective as antibiotics, their supportive effect may have a positive effect on slowing the development of the disease and, in the early stages of its development, even on its cure. On the other hand, disinfecting cleaning agents based on natural raw materials with proven effectiveness will provide a better safety profile. Both in terms of contact with people and with the environment.

Methods

The antimicrobial potential was evaluated by determining the MIC parameter (Minimal Inhibitory Concentration) towards: *Pseudomonas aeruginosa* (ATCC 15442), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 12883), *Pseudomonas putida* (ATCC 49128), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231) and *Aspergillus brasiliensis* (ATCC 16404). The research was carried out in two stages, in the first place, a screening test was carried out for one concentration using the Alamar blue® dye. Substnaces that showed activity in the screening test were implemented in the appropriate tests to determine the MIC parameters by the serial microdilution method on a 96-well plates using the same dye.

Results

Commercially available raw fragrance materials proved their significant antimicrobial activity. The broadest spectrum of action possessed: Hiba (Vessel) and Hiba essential oil, *Thujopsis dolabrata* (Bristol botanicals Ltd); Turmeric oleoresin, *Curcuma longa* L. (Bordas S.A.); Ginger fresh, *Zingiber officinale* (Jacarandas International); Poplar bud absolute, *Populus balsamifera* (Hermitage oils). The lowest MIC of 12,5 mg/L towards *P. aeruginosa* possessed Vetiver oil Brazil, *Vetiveria zizanioides* and Mimosa dealbata absolute *Acacia dealbata* (Hermitage oils), against *E. aerogenes* the lowest MIC of 25 mg/L showed *Tasmania lanceolata* extract (Essential oils of Tasmania), Coriander herb essential oil, *Corriandrum sativum* L. (Ultra International), Turmeric oleoresin, *Curcuma longa* L. (Bordas S.A.) and Fir balsam absolute Canada, *Abies balsamea* (Berje Inc.). Against *K. pneumoniae* the most active was Sandalwood Kupang essential oil, *Sandalwood album* (Hermitage oils) MIC = 25 mg/L, towards *P. putida* the best inhibitory growth potential presented: Cubeb oil, *Piper cubeba* L., Fir balsam absolute Canada, *Abies balsamea* (Berje Inc.) the MIC = 12,5 mg/L, to *S. aureus* the most active were Violet leaves concrete, *Viola odorata* L., Onion Egypt essential oil, *Allium cepa* L, Garlic Egypt essential oil, *Allium sativum* L. (A. Fakhry & Co.), Porcini choco absolute, *Boletus edulis* (Hermitage oils) and Massoia essential oil *Cryptocarya massoia* (Oshadhi) the MIC of 50 mg/L. Against *C. albicans* the lowest MIC of 200 mg/L showed Turmeric oleoresin, *Curcuma longa* L. (Bordas S.A.); towards *A. brasiliensis* the lowest MICs of 25 mg/L presented Ginger, *Zingiber officinale;* Cypriol oil, *Cyperus scariosus*; Palmarosa oil, *Cymbopogon Martini* (DV Deo Industries), Rocket absolute, *Eruca vesicaria*; and Coriander leaf *Corriandrum sativum* L. (A. Fakhry & Co).

Conclusions

Natural fragrance raw materials have properties that inhibit the growth of microorganisms. The proven antimicrobial potential of natural fragrance raw materials is a basis for further research on the bactericidal and fungicidal potential, as well as their safety against the human. Natural fragrances can act as functional substances in end products from various industries by combining fragrance and antimicrobial properties.

ACKNOWLEDGMENTS

We would especially like to thank the producers of natural fragrance raw materials for providing research materials

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

Davy Moussango[1,2*], Sameza Modeste[2], Tchameni Severin[2], Ebong Priscile[2], Ampére Bédine[3], Jazet Dongmo[1], Chantal Menut[4].

[1]Department of Energy Engineering, University Institute of Technology, University of Douala, Douala, Cameroon.

[2]Department of Biochemistry, Faculty of Sciences, University of Douala, Douala, Cameroon.

[3]Department of Biochemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon.

[4]Glyco and nanovectors for therapeutic targeting" team, IBMM, Faculty of Pharmacy, Montpellier, France.

*E-mail: davy.moussango@yahoo.com

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

Objective

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

Methods

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

Results

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

Conclusions

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

REFERENCE

[1] Davy M., Sameza M., Tchameni S., Ntah A., Bédiné B., Youassi O., Kamsu P., Jazet M. (2020). Antifungal effects of clove (Syzygium aromaticum) essential oil against Colletotrichum gloeosporoïdes, the fungus associated with papaya (Carica papaya L.) fruit anthracnose. International Journal of Applied Microbiology and Biotechnology Research, 8: 2053-1818.

[2] Purkait A., Worede R., Baral D., Kumar H., Panja B., Kumar B., Kumar K. (2020). Development of nanoemulsion formulation of mustard oil, its chemical characterization and evaluation against post-harvest anthracnose pathogens. Indian Phytopathology 8: 236-237.

Photo‑protective effects of furocoumarins on citrus essential oils and the impact on natural perfumery

Clio Vidal[1] and Hannes Bitterling[1,*]

[1]Department of Analytical Development & Research, WALA Heilmittel GmbH, Dorfstrasse 1, 73087 Bad Boll/Eckwälden, Germany

*E-mail: hannes.bitterling@wala.de

Keywords: furocoumarin, essential oils, photo-oxidation, natural perfumery, aromatherapy

Abstract

Furocoumarins belong to the plant secondary metabolites mainly found in the *Apiaceae* and *Rutaceae* families, thus including all citrus fruits [1]. During periods of stress, such as fungal, bacterial and herbivore attack plants may defend themselves by furocoumarin biosynthesis [2]. During the production of cold-pressed essential oils, furocoumarins are also incorporated into the oil. By wavelengths between of 320 – 380 nm, furocoumarins are excited into a photo-activated state by electromagnetic radiation. Activated furocoumarins may decay into their ground state via fluorescence or phosphorescence by emitting light of higher wavelengths up to 500 nm. However, upon exposure to sunlight, sunburn-like skin injuries, so called photo-dermatitis, may occur [2]. Nevertheless, photo-toxicity is very compound-specific or even non-existent for some furocoumarins [3].

The aim of the present study was to investigate the different photo-oxidation behaviour of the tree essential agrumen oils lime ((*Citrus* x *aurantiifolia* Christm. et Panz.) Swingle), lemon (*Citrus limon* (L.) Osbeck), and bergamot (*Citrus bergamia* Risso et Poit) if they have been left in their natural, cold-pressed state or if they have been freed from furocoumarins.

For this purpose, the essential oils where freed from furocoumarins by precipitation in cold *n*-hexane. Such pre-treated oils as well as samples of these oils spiked with the separated furocoumarin fraction were irradiated upon UV-A light. All essential oils devoid of furocoumarins showed a massive degradation of the main terpenes *R*-(+)-limonene and *γ*-terpinene. For lime and lemon essential oils 10% and 7.5% of the initial *R*-(+)-limonene amount was degraded within ten days, respectively. In addition, a noticeable hydroperoxide formation was observed. For *γ*-terpinene, this effect was even more pronounced and in both, lime and lemon essential oil samples the terpene was entirely converted into *p*-cymene after six days under UV light. In comparison, addition of 5% plant specific natural furocoumarins to the essential oils decelerated the photo-degradation of *R*-(+)-limonene and *γ*-terpinene significantly. Odor quality was also improved by the protective effect of the furocoumarins. This is probably due to the bathochromic shifts of the emitted light to less harmful longer wavelengths.

In the applied fields of aromatherapy, natural perfumery, and traditional fine fragrances, lemon and bergamot are among the most commonly used essential citrus oils. Their unique top notes are fresh and uplifting. Furthermore, their great aromatherapeutic benefits, such as antiseptic, phlebotonic, choleretic and antidepressant properties have made them key ingredients to the “Cologne” fragrance family. Lime oil (with its higher furocoumarin content) is more difficult to use and therefore already plays a minor part, which is deplorable in view of its special aroma profile. An olfactory evaluation of different cold-pressed oils and their furanocoumarin-free alternatives with the audience will substantiate these scientific results, their qualitative question and their consequences for the application field of natural perfumery and aromatherapy.

REFERENCES

[1] Peroutka, R., Schulzová, V., Botek, P., & Hajšlová, J. (2007). Analysis of furanocoumarins in vegetables (Apiaceae) and citrus fruits (Rutaceae). Journal of the Science of Food and Agriculture, 87, 2152–2163. https://doi.org/10.1002/jsfa.2979

[2] Melough, M. M., Cho, E., & Chun, O. K. (2018). Furocoumarins: A review of biochemical activities, dietary sources and intake, and potential health risks. Food and Chemical Toxicology, 113, 99–107. https://doi.org/10.1016/j.fct.2018.01.030

[3] Scott, B. R., Pathak, M. A., & Mohn, G. R. (1976). Molecular and genetic basis of furocoumarin reactions. Mutation Research/Reviews in Genetic Toxicology, 39, 29–74. https://doi.org/10.1016/0165-1110(76)90012-9

Xanthoxylin from *Pulicaria incisa* essential oil: chemical composition of volatile fraction and their biological activities

Antonella Porrello[1,*], Natale Badalamenti[1,2], Maurizio Bruno[1,2], Viviana Maresca[3], Adriana Basile[3]

[1] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Palermo, 90128, Building 17, Italy.

[2] NBFC, National Biodiversity Future Center, Palermo, 90133, Italy.

[3] Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio 7, Naples, 80138, Italy.

* Email: antonella.porrello@unipa.it

Keywords: *Pulicaria incisa*; Asteraceae; essential oil; xanthoxylin; antioxidant activity

Objective

Xanthoxylin, a trisubstituited phenol, was isolated for the first time from *Xanthoxylum alatum*. This compound is commonly found in Rutaceae, Asteracee, and Euphorbiaceae family [1]. Xanthoxylin showed different biological activities: it exhibited fungicide actions, inhibited contractions induced by agonists and electrical stimulation of smooth and cardiac muscle [2]. Furthmore, extracts containing xanthoxylin have shown antioxidant, and insecticidal properties [3]. Xanthoxylin was, also, found in the essential oil extracted from *Pulicaria undulata* L. [4], a species belonging to the genus *Pulicaria* (Asteraceae) that consists of ca. 82 species distributed in many countries such as Europe, Western Asia, and North Africa. *Pulicaria* essential oils, characterized by the presence of oxygenated sesquiterpenes, other terpenoids and phenols, possess different biological properties such as insecticidal, antioxidant, antibacterial, and cytotoxic activities [5]. Objects of this work were the isolation and characterization of *Pulicaria incisa* Lam. essential oil by GC and GC-MS and the subsequently isolation of xanthoxylin from the essential oil. Both, essential oil and purified compound were tested for antioxidant and antitumoral activities.

Methods

Fresh aerial parts (leaves and flowers) of *P. incisa* were collected in Israel in February 2023. For the essential oil fresh samples were ground in a waring blender and then subjected to hydrodistillation for 3 h, according to the standard procedure of Pharmacopoeia. The sample yielded 0.15% of oil (*w*/*w*). GC-MS analysis was performed using an Agilent 7000C GC system equipped with an apolar DB-5MS column coupled to an Agilent Mass Selective triple quadrupole MSD 5973 detector. Xanthoxylin, isolated from the essential oil after crystallization with *n*-pentane under N_2, was characterized by ^{1}H-NMR, ^{13}C-NMR e mass spectrometry. Essential oil and the isolated xanthoxylin were tested for antioxidant activity *in vitro*, with DPPH, ABTS, and H_2O_2 radical methods, and for antiproliferative activity against acute myeloid leukemia (U937 cells).

Results

The composition of essential oil of *P. incisa* was determined by GC-MS analysis. After the isolation of xanthoxylin, thirty-seven components were identified representing 91.54% of the total oil content. In terms of compound classes, oxygenated monoterpenes (82.9%) dominate the essential oil composition, and the main compound was *trans*-chrysanthenol (65.7%) followed by carvotanacetone (10.8%). In contrast, monoterpene hydrocarbons, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes accounted respectively only for 2.0, 0.9, and 1.7%, while xanthoxylin, classified as other, constitutes 2.4% of *P. incisa* essential oil.

Conclusion

The present study has focused on determining the chemical composition and biological activities of *P. incisa* essential oil and of isolated phenolic compound xanthoxylin. In our work, the GC analysis of the *P. incisa* essential oil resulted rich in oxygenated monoterpenes, representing 82.9% of the total composition, with *trans*-chrysanthenol (65.7%) as the most abundant component. The essential oil, together with xanthoxylin, showed excellent antioxidant activity, and the isolated compound performed better results. Furthermore, both showed moderate antitumoral activity.

REFERENCES

[1] Brophy J.J. et al. (2011). Leaf oils of the genus *Acradenia* (Rutaceae), *Journal of essential Oil Research*, 13:2, 136-139.
[2] Calixto J.B. et al. (1990). Action of 2-hidroxy-4,6-dimethoxy-acetophenone isolated from *S. schottiana*, *Planta Medica*, 56:1, 31-35.
[3] Dolma S. K. et al. (2022). Characterization of *Triadica sebifera* (L.) small extracts, antifeedant activities of extracts, fractions, seed oil and isolated compounds against *Plutella xylostella* (L.) and their effect on detoxification enzymes, *Molecules*, 27, 6239.
[4] Mustafa A.M. et al. (2018). Chemical composition and biological activities of the essential oil from *Pulicaria undulata* (L.) C. A. Mey. Growing wild in Egypt. *Natural Product Research*, 34:16, 2358-2362.
[5] Ali N.A.A. et al. (2012). Chemical composition and biological activity of essential oil from *P. undulata* from Yemen. *Natural Product Communications*, 7:2, 257-270.

Circular Economy in the cosmetic industry: a systematic literature review

Alice Mondello[1,*], Roberta Salomone[1], Giovanni Mondello[1]

[1] Department of Economics, University of Messina, Messina, ITALY

* Email: alice.mondello@studenti.unime.it

Keywords: circular economy, cosmetic industry, systematic literature review, sustainability

Objective

Cosmetic industry is one of the most fast-growing sector worldwide and has a fundamental importance in the day-to-day life of consumers. In this context of continuous growth, cosmetic companies are looking for sustainable solutions aimed to reduce the potential negative impacts of their products in terms of economic, environmental and social aspects. The scientific community is also involved in this field and has started to investigate the concept of Circular Economy (CE), proposing it as a paradigm which could help cosmetic companies in the transition to a sustainable future. However, there is still a lack of information about the practical implementation of CE principles and its connection with sustainability-related concepts. For this reason, a systematic literature review is needed for providing an overview of CE practices and strategies which are or could be applied in the cosmetic industry.

Methods

A systematic literature review (Khan et al., 2003) is carried out with the aim of defining the state of the art of CE in the cosmetic industry and its connection with sustainability. The literature review is carried out in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) (Moher et al., 2009).

Results

A final sample of 86 articles and reviews has been extracted from Scopus and Web of Science databases. The analysis of the sample allowed classifying the proposed circular solutions in: input valorization, packaging optimization, output valorization, management and governance. Results show that the attention of scientific community is mainly focused on circular solutions for the input valorization to be used in the formulation of cosmetic products. In addition, among the proposed circular options a preeminent link with the agri-food, chemical and biochemical sectors is highlighted. The results confirm also the existence of a link between CE and sustainability, with a particular attention to the environmental dimension, while the social ones is the less investigated.

Conclusions

In conclusion, the interest of the scientific community in CE in the cosmetic industry is growing in the last years. The attention of academics is mainly focused on the input valorisation for the formulation of cosmetic products, but also other CE options are investigated, focusing on packaging, output valorisation and governance and management. The main findings of this review show also an interdisciplinary interest of authors. More in details, a connection with the agri-food, chemical and biochemical sectors is highlighted especially regarding the input valorisation. The agri-food sector has an important role due to the great amount of its waste and by-products from which raw materials could be extracted to be used as ingredients in the cosmetic formulations. The chemical and biochemical sectors have also an important role due to the need of analysing the raw materials properties and identifying innovative and sustainable extraction methods. Finally, this review highlights the connection between CE and sustainability in the cosmetic industry. Indeed, in almost all the proposed circular options the authors consider sustainability and its dimension, focusing especially on the environmental ones.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Italian Ministry of Education, PON scholarship Green n°1, CUP J35F21003690007.

REFERENCES

[1] Khan K.S., Kunz R., Antes G. (2003). Five steps to conducting a systematic review, Journal of the royal society of medicine, 96(3), 118-121
[2] Moher D., Liberati A., Tezlaff J., Altman D.G., The PRISMA Group (2009). Preferred reporting items for systematic review and meta-analyses: the PRISMA statement, PLoS Med., 6(7), 1000097

Commercial use of *Cannabis Sativa L.* essential oil for the production of the first alcohol-free craft beer.

Lorenzo Lamberti[1, 2*], Giorgio Grillo[2], Giancarlo Cravotto[2]

[1] Craft Brewery Baladin S.s.a., Piozzo (CN), ITALY
[2] Department of Drug Sciences and Technology, University of Turin, Turin, ITALY

* Email: lorenzo.lamberti@unito.it

Keywords: Industrial scale, Green extraction, Microwave-Assisted Hydro-Distillation, *Cannabis Sativa L.*, Alcohol-free beer.

Objective

The global trends in the consumption of beverages show a steadily increasing demand for healthier alcohol-free options. The brewing industry does not make any difference; all the major companies have already presented their alcohol-free line of products. The biggest challenge to overcome for this type of beverage is the long-term stability and the risk of contamination due to the absence of ethanol. Since craft breweries can not pausteurize nor microfiltrate, the possibility of contamination is even higher, making the addition of natural ingredients very risky since every biomass could carry spoiling agents. To overcome this challenge, we decided to extract the terpene fraction from Cannabis Sativa L. inflorescence and to use only the oils recovered for the aromatisation of the beer. To avoid the use of organic solvents, we decided to use a Microwave-Assisted Hydro-Distillation (MAHD) pilot scale system called ETHOS XL from Milestone S.r.l. company. This method led us to a high extraction yield of 5.9 ml/kg of essential oils compared to a modest 5.1 ml/kg using the standard hydro-distillation; moreover, this technology reduces the hot spots inside the reactor leading to the recovery of a higher essential oil quality. The other main problem faced was the delivery of the lipophilic extract inside an aqueous medium. We were able to stabilise the essential oils in the alcohol-free beer creating a stable emulsion, and we produced 16.000 cans in our first production batch (the stabilisation of the essential oils is currently under patent submission process). The new product, Baladin Botanic, was presented during the Beer&Food Attraction event in February and is now available in the Italian national market.

Methods

The Cannabis Sativa L. used for the extraction was cultivated by an Italian company that selected a THC-free variety. After the harvesting, the buds recovered were dried at a low temperature reaching 7% w/w humidity. The reactor of the ETHOS XL is capable of processing 5kg of buds per batch and took nearly 3 hours to complete the process (1 hour for the moistening and the preparation of the reactor chamber and 2 hours for the actual extraction). The characterization of the extract was done using a GC-MS system following a standardized protocol [1]

Results

The extraction rate reached with the optimized protocol led us to recover 120 ml of essential oil during a 10h working day. The GC-MS analysis showed a nearly perfect overlap between the chromatograms of our sample and the commercial data given by the supplier showing that the MAHD method is not only capable of reaching high extraction yields but also a high-quality product.

Conclusions

The extraction and delivery of the EO inside the aqueous medium were capable of bringing the desired aroma to the final product without the risk of contamination. This work also showed the possibility of implementing this process on the hops used in the production of classic beers since it would significantly reduce the quantity of raw material used.

ACKNOWLEDGMENTS

The Piedmont Region is acknowledged for financial support.

REFERENCES

[1] V. Gunjević, G. Grillo, D. Carnaroglio, A. Binello, A. Barge, G. Cravotto, (2021). Selective recovery of terpenes, polyphenols and cannabinoids from Cannabis sativa L. inflorescences under microwaves, *Industrial Crops and Products*, Volume 162.

Forgotten perfumery plants: hawthorn

Fragrance revelation for innovation

K. da Fonte[1*], A.S. Bouville[1,2], P. Portes[2], J.C. Lhommet[2], S. Azoulay[1], X. Fernandez[1]

[1] Institut de Chimie de Nice, UMR 7272 Université Côte d'Azur/CNRS, 06108 Nice, FRANCE
[2] Laboratoires M&L, L'Occitane Group, 04100 Manosque, FRANCE

* Email: kevin.da-fonte@univ-cotedazur.fr

Keywords: Hawthorn, *Crataegus monogyna* Jacq., forgotten perfumery plant, extractions, chemical composition, olfactory profile, biological activities

---

Objective

Plants have been used since Neolithic times to compose perfumes. Some of these plants are not used anymore. A collaboration between the Chemistry Institute of Nice (ICN, University Côte d'Azur-CNRS), the International Perfume Museum (MIP) and its Gardens (JMIP) as well as L'Occitane en Provence was set up to study and revalorize these forgotten perfumery plants, source of innovation. The floral, powdery, almondy, balsamic, green, 'rustic' and heady smell of common hawthorn has been reproduced by accords thanks to synthetic raw materials, that caused the disuse of the natural extracts.

Methods

Flowering aerial parts of *Crataegus monogyna* Jacq. were collected in June 2017 and in May 2018, in Lus-La-Croix-Haute (Drôme, France). Absolutes of fresh, frozen, and dried plant materials were obtained by 6 hours' hexane extraction and then analyzed using HS-SPME-GC-MS/FID. The volatile extract was also obtained by a 2 hours' hydrodistillation of the 2017 frozen plant material and then analyzed using DI-GC-MS/FID. The extracts were olfactory evaluated by a perfumer of L'Occitane en Provence. Tyrosinase, lipoxygenase, elastase hyaluronidase, collagenase and DPPH biological activities were carried out.

Results

Hydrodistillation of 2017 frozen material plant appeared to have a very poor essential oil yield (hexane trapping was necessary). 105 compounds were identified whose 48 for the first time. The main ones were: hexanal, (*Z*)-2-hexenal, linalool, nonanal, methyl salicylate, α-terpineol, geraniol, α-farnesene, nonadecane, heneicosane, and tricosane. The extract had a green, anise odor, recalling the fragrance of artichoke and cooked vegetables: truly different from the olfactive profile of the plant [1].

Absolutes of hawthorn were studied for the first time. They had acceptable yields (0,2-1,3%/32-50%). 184 compounds were identified whose 126 for the first time. The main compounds were: (*E*)-2-hexenoic acid, 2-phenylethanol, methyl salicylate, *p*-anisaldehyde, eugenol, *p*-methyl anisate, (*Z*)-jasmone, β-caryophyllene, α-farnesene, and nonadecane. The smell of the extracts was described as anisic, honeyed, flowery-white with almondy facets. The treatment and harvesting year had a significant impact on the plant but chromatographic profiles of absolutes were close [2]. The anti-ageing property of hawthorn extracts had never been used yet, but their anti-hyaluronidase, anti-elastase, and anti-collagenase activities could be valued in cosmetics [1,2].

Conclusions

The R&D study of forgotten perfumery plants brought to light and gave value to precious historical heritage. The hawthorn absolutes appeared to present a strong olfactory potential, especially the fresh plant one. New natural perfume ingredients were developed and products have been released by L'Occitane en Provence [3].

REFERENCES

[1] A-S. Bouville, P. Portes, J-C. Lhommet, S. Azoulay, X. Fernandez, (2023). Forgotten Perfumery Plants: Hawthorn volatile oil study, JEOR. [Submitted]
[2] A-S. Bouville, K. da Fonte, P. Portes, J-C. Lhommet, S. Azoulay, X. Fernandez, (2023). Forgotten Perfumery Plants - Part II: Hawthorn, Chemistry & Biodiversity. [Submitted]
[3] K. da Fonte, A-S. Bouville, P. Portes, J-C. Lhommet, X. Fernandez, (2022). Forgotten perfumery plants: fragrance revelation for innovation, Perfumer & Flavorist, 47, 42-50.

Effect of essential oils on biofilm formation in different bacterial species

Francesco Buccioni[1,*], Francesca Maggio[1], Annalisa Serio[1], Chiara Rossi[1], Chiara Purgatorio[1], Antonello Paparella[1]

[1] Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, ITALY

* Email: fbuccioni@unite.it

Keywords: Biopreservation, Essential oils, Biofilm, FICI, Food Industry

Objective

Biofilms are hierarchic structures that enhance the survival of bacteria in the environment, due to both social and physical interactions within involved cells. These structures increase the resistance of microorganisms to antimicrobial agents and therefore are a threat in food industries, where they reduce the effectiveness of physical, mechanical or chemical treatments. In recent times, Essential Oils (EOs) demonstrated a great anti-biofilm activity as a consequence of their complex composition that interferes with biofilm-forming mechanisms [1]. Therefore, this work aimed to evaluate the Minimal Inhibitory Concentration (MIC), the Fractional Inhibitory Concentration Index (FICI) and the effect on biofilm formation of different EOs on four important food related microorganisms.

Methods

The anti-biofilm activity of *Thymbra capitata* (L.) Cav. (TEO), *Origanum vulgare* subsp. *hirtum* (OEO) and *Rosmarinus officinalis* (REO), all obtained by Exentiae srl (Catania, Italy), was evaluated against *Listeria monocytogenes* ATCC 7644, *Pseudomonas fluorescens* ATCC 13525, *Staphylococcus aureus* ATCC 43300 and *Salmonella enterica* ser. Kasenyi, belonging to the microbial culture collection of the Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo (Italy). The MIC values for the different EOs were evaluated by broth microdilution method; FICIs for EOs combinations were evaluated by Checkerboard method [2]; biofilm-forming ability inhibition was evaluated on both stainless steel (SS) and polystyrene (PS) by adding the EOs during incubation. The results were evaluated by spectrophotometric readings, carried out at 590 nm wavelength.

Results

Concerning the evaluation of the MICs, TEO demonstrated activity against *S. aureus* and *L. monocytogenes* from 1.25 µl ml^{-1} concentration. OEO was found to be effective against *S. aureus*, *L. monocytogenes* and *S. enterica* ser. Kasenyi starting from 2.5 µl ml^{-1}. Differently, REO did not show any effectiveness even at the highest concentration used (20 µl ml^{-1}). *P. fluorescens* was the most resistant species, as it was inhibited by TEO and OEO at 10 µl ml^{-1} and 5 µl ml^{-1}, respectively. However, REO was active when combined with TEO and OEO, due to a synergic action starting from 0.31 µl ml^{-1}. As for biofilm-forming ability inhibition, doubled concentrations were used, supposing a higher resistance of biofilm to treatments compared to free-living cells. Results evidenced a significant inhibition of biofilm-forming ability by the EOs, when used both individually and in combination; the effect was observed starting from 0.13 µl ml^{-1} for TEO + CEO. Remarkably, REO showed anti-biofilm activity up to 90.1%, in contrast to the results obtained in the evaluation of the MIC.

Conclusions

The outcome of this study confirmed the possibility to use EOs to overcome biofilm formation by pathogenic and spoilage microorganisms in the food environment. Our data indicate the usefulness of Checkerboard in the preliminary evaluation of EOs, to select EOs combinations that maximize the inhibition of biofilm formation, obtaining significant effects starting from 0.13 µl ml^{-1}.

ACKNOWLEDGMENTS

The project was funded by the NOP Research and Innovation 2014-2020 under the action Innovative Industrial Doctorates, cycle XXXVII (CCI 2014IT16M2OP005).

REFERENCES

[1] Rossi C., Chaves-López C., Serio A., Casaccia M., Maggio F., Paparella A., (2020). Effectiveness and mechanisms of essential oils for biofilm control on food-contact surfaces: An updated review, *Critical Reviews in Food Science and Nutrition*, 62, 2172-2191.

[2] CLSI, Clinical and Laboratory Standards Institute, (2015). Performance standards for antimicrobial susceptibility testing, twenty-fifth informational supplement (M100-S25), Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Evaluation of *Juniperus communis* L. (Juniper) essential oil samples sold on the market in Türkiye in terms of European Pharmacopoeia 10.0 criteria

Timur Hakan Barak[1*], Duru Düşmen[2], Tuğba Buse Şentürk[1], Engin Celep[1]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Mehmet Ali Aydınlar University, 34750, Istanbul, Türkiye
[2] Faculty of Pharmacy, Mehmet Ali Aydınlar University, 34750, Istanbul, Türkiye

* Email: Timur.barak@acibadem.edu.tr

Keywords: *Juniperus communis* L, GC-MS, Essential Oil, European Pharmacopoeia, Juniper Oil

Objective: *Juniperus communis* L. (Juniper) essential oil is widely used in complementary medicine and aromatherapy. Scientific studies previously showed that juniper essential oil has a wide range of bioactivities such as antimicrobial, strong antioxidant and anti-inflammatory, anti-diabetic, anti-hyperlipidemic, anti-hypercholesterolemic, hepato-, renal-, gastro- and neuroprotective, anti-proliferative effects [1]. Pharmacopoeias are official publications that contain qualitative and quantitative analysis methods of active substances and excipients used in the manufacture of medicinal products. The international rules and methods enclosed in these publications must be followed both legally and scientifically. In this study, it was aimed to assess various commercial *Juniperus communis* L. (Juniper) essential oils on the Turkish market regarding the pharmacopoeial quality standards. All selected products are labelled to contain pure Juniper oils obtained by steam distillation from berry cones of *Juniperus communis* and licensed as a cosmetic product from Ministry of Health of Türkiye. Ten of the samples were purchased from pharmacies and five of them were selected from other sources such as Akhtar shops, websites and herbalists.

Methods: Characteristic tests (appearance and odor), fatty oils and resinified essential oils, relative density, refractive index, optical rotation and peroxide value tests were applied as stated in the EP monograph to fifteen different samples of Juniper oil sold on the market [2]. In addition, thin layer chromatography (TLC) was carried out for the qualitative determination of chemical profile while gas chromatography-mass spectrometry (GC-MS) analysis was carried out for elaborate quantitative investigation of phytochemical content.

Results: Results demonstrated that only one of the products fully met the standards stated in the European Pharmacopoeia 10.0. Some of the products showed clear signs of adulteration and few of them showed lesser deviations from criteria. Nonetheless, it was observed that the ratio of meeting the criteria for the products purchased from pharmacies was significantly higher when compared to other sources.

Conclusions: The quality insufficiency of Juniper oils on the market to meet criteria of Pharmacopoeia indicates the requirement of higher standards for regulation and auditing mechanisms in Türkiye. Until then, results showed that pharmacies are still the best option for public to obtain pure essential oils.

ACKNOWLEDGMENTS

This study is financially supported by a grant (2209-A) from Scientific and Technological Research Council of Turkey (TÜBİTAK)

REFERENCES

[1] Judžentienė, A. (2019). Juniperus communis L.: A review of volatile organic compounds of wild and cultivated common juniper in Lithuania. Chemija, 30(3), 184-193.
[2] Ph. Eur. 10.0, 1846 (01/2008). Juniper oil monograph, p.1493-1494, EDQM, Strasbourg.

Enantio-selective multidimensional gas chromatography coupled to isotopic ratio mass spectrometry for the authenticity assessment of Citrus essential oils

Lorenzo Cucinotta[1,*], Gemma De Grazia [2], Danilo Sciarrone[1], Luigi Mondello[1,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Capua 1880 Srl, Reggio Calabria, ITALY
[3] Chromaleont s.r.l., c/o, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: lcucinotta@unime.it

Keywords: Citrus, terpenes, IRMS, chiral, multidimensional gas chromatography

Objective
This research aimed to develop an enantio-selective gas chromatographic method coupled to isotopic ratio mass spectrometry (Es-GC-C-IRMS) to simultaneously evaluate isotopic and enantiomeric ratio of the main target terpenes in Citrus essential oils. With respect to previous analytical methods[1], this approach would be able to reduce total time analysis, as well as the consumption of electricity, samples and solvent. Due to the reduced reliability associated to co-elutions, a prototypal enantio-selective multidimensional gas chromatography (Es-MDGC) coupled to IRMS and a single quadrupole mass spectrometric detection (qMS) was also exploited.

Methods
Cold pressed citrus essential oils were analysed by both monodimensional Es-GC-C-IRMS analysis and multidimensional ones. Es-MDGC-C-IRMS/qMS analysis were performed by combining an apolar column in the first dimension and a chiral stationary phase in the second one.

Results
Due to the issues associated to low resolution, the employment of a single chiral gas chromatographic column was unable to guarantee a baseline resolution for each key component. Instead, the multidimensional gas chromatographic approach delivered pure peaks for IRMS and qMS detection, allowing a reliable measurement of both their isotopic and chiral ratio in a single gas chromatographic run. Dealing with chiral and isotopic results, all the data obtained were found to be in agreement with respect to previous literature data for cold pressed Citrus essential oils. Moreover, this coupling allows determining the specific isotopic ratio of each enantiomer separated. In this concern, typical trends were found in terms of $\delta^{13}C$ value between the enantiomers of the same chiral component, as in the case of (-) and (+) of α and β-pinene, suggesting a characteristic isotopic fractionation related to a specific biosynthetic pathway.

Conclusions
The current study reports for the first time the simultaneous achievement of both enantiomeric and isotopic data for the typical terpenes of Citrus essential oils, by employing a prototype system coupling enantio-selective multidimensional gas chromatography to isotopic ratio mass spectrometry. Besides the advantages associated to reduced total time analysis, this system demonstrated to be able to provide new interesting features for authenticity assessment, related to the specific $\delta^{13}C$ of each enantiomer.

REFERENCES

[1] Schipilliti L., Dugo P., Bonaccorsi I., Mondello L., 2011 Authenticity control on lemon essential oils employing Gas Chromatography–Combustion-Isotope Ratio Mass Spectrometry (GC–C-IRMS), *Food Chem.*, 131(4), 1523-1530

A comprehensive evaluation of hydrolates obtained from *Iris* rhizomes

Maria Filatova[1,*], Jana Hajslova[1], Petr Kastanek[2], Michal Stupak[1]

[1] University of Chemistry and Technology Prague, Faculty of Food and Biochemical Technology Department of Food Analysis and Nutrition, Technicka 3, 166 28 Prague, Czech Republic
[2] Ecofuel Laboratories s.r.o., Ocelarska 9, 190 00, Prague, Czech Republic

* Email: maria.filatova@vscht.cz

Keywords: Iris rhizomes, hydrolates, GC–MS, volatiles

Objective

Iris is the largest genus of the *Iridaceae* family comprising more than 300 species. Many of these plants are known for content of various bioactive compounds which occur mainly in rhizomes. To isolate volatile fraction from this matrix, hydrodistillation was chosen in this study. In particular case, that process enables to produce so cold "orris butter" and respective hydrolate. As limited information is available on the latter product, we focused on its comprehensive evaluation of phytochemicals present. Set of rhizomes obtained from 10 different cultivars (*I. pallida*, *I. germanica* Nepalensis, *I. germanica* Santana, *I. germanica* Florentina coerulea, *I. germanica* Florentina alba, *I. germanica* Carcassone, *I. pseudacorus*, *I. flavescens*, *I. versicolor*, *I. barbata elatior* Admiral) were provided by the research partner, Ecofuel Laboratories, Prague. To characterize profile of hydrolate volatiles, head-space solid-phase microextraction followed by gas chromatography coupled to high-resolution mass spectrometry (HS–SPME–GC–HRMS) was used. To evaluate the distribution of volatiles obtained by hydrodistillation between the aqueous and solid product ("orris butter"), its analysis was performed, too.

Methods

The rhizomes (60g) were subjected to hydrodistillation for approximately 5 h using a Clavenger-type apparatus. For the HS–SPME extraction, SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm, 1 cm) from Supelco (Bellefonte, USA) was used. The Agilent 7200B system consists of an Agilent 7890B gas chromatograph equipped with a multimode inlet, PAL RSI 85 for automated headspace–solid phase microextraction (HS–SPME) and direct injection, and quadrupole – time of flight mass spectrometer (Q-TOF) (Agilent Technologies, Palo Alto, California, USA) was employed. Chromatographic separation was performed at the HP–5MS (30 m x 250 µm x 0.25 mm) capillary column. "Orris butter" obtained by hydrodistillation, was after dissolving in hexane also analyzed by GC–HRMS system.

Results

In samples of hydrolates obtained from iris rhizomes were detected terpenic substances (cis-β-ocimene, linalool, trans-sabinene, α-terpineol, etc.) and fatty acid esters (methyl- and ethyl octanoate, ethyl decanoate, etc.). In hydrolates, among others, the characteristic compounds of iris plants such as cis-α-iron and cis-γ-iron were detected. The dominating substances occurring in "orris butter" were saturated fatty acids (decanoic, dodecanoic, tetradecanoic acids) and their esters (especially ethyl esters). Ethyl esters of unsaturated fatty acids (α-linoleic and α-linolenic acids) and higher alkanes (tricosane, tetracosane, pentacosane, etc.) were also detected. In addition, number of other minor substances, mainly terpenoids, such as α-cadinol, α-santalene, δ-guaien were present too. Individual *Iris* cultivars differed in patterns of volatile compounds obtained by hydrodistillation

Conclusions

Hydrodistillation was shown to be suitable strategy for isolation of volatiles from iris rhizomes. HS-SPME-GC-HRMS enabled detection and identification of many bioactive volatile compounds and distinguish the difference in volatile profiles of individual iris cultivars. depending on the cultivar.

ACKNOWLEDGMENTS

This work was financial supported from a specific university research A2_FPBT_2023_004.
The authors thank the Department of Food Analysis and Nutrition of UCT Prague for the contribution for the research.

Altitude-Dependent Variation in Chemical Composition of Essential Oil of *Origanum acutidens* (HAND-MAZZ.) IETSWAART

Furkan Coban[1,*], Hakan Ozer[1], Ramazan Cakmakci[2],

[1] Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, TÜRKİYE
[2] Department of Field Crops, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, TÜRKİYE

* Email: furkan.coban@atauni.edu.tr

Keywords: *Origanum acutidens*, essential oil, altitude, carvacrol, p-cymene

Objective

Altitude is considered a vital determinant for the quantity and quality of medicinal plants grown and produced. The relationship between essential oil content and altitude in medicinal plants is complex and can vary depending on several factors such as the plant species, geographic location, climate, soil type, and altitude range. *Origanum acutidens* (Hand-Mazz.) Ietswaart, which grows endemic in the Eastern Anatolia region of Turkey, an aromatic perennial from the Lamiaceae family, is one of the plant species that thrives in high-altitude regions [1]. Origanum species have a long-standing history of traditional usage as natural remedies for various health conditions. These include sedative, diuretic, degasifying, and sweating effects, as well as antiseptic properties. Moreover, *Origanum acutidens* (Hand-Mazz.) Ietswaart also has an excellent food aroma due to its pleasant scent [2]. The purpose of this study is to investigate the essential oil yield and compositions in the aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart at the time of flowering, and in three different altitudes (1150, 1650 and 2150 m) of Eastern Black Sea Region, Türkiye.

Methods

The aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart plants were collected during the flowering season at an altitudes of 1150, 1650 and 2150 m on August 2022 from the İspir, Erzurum, Türkiye, To extract the essential oil from the aerial parts, a water distillation method was employed. The obtained essential oils were separated from water, dried over anhydrous sodium sulfate and after filtration, stored at +4 ºC until tested and analyzed. The GC and GC/MS instruments were utilized to identify the compositions of the essential oil.

Results

According to the findings, the essential oil yield obtained from altitudes of 1150, 1650, and 2150 m were 0.75 ± 0.01 %, 0.86 ± 0.02 %, and 1.03 ± 0.02 %, respectively. In general, there was a noticeable increase in the essential oil level as the altitude increased. Calvacrol and p-cymene represents the predominant compounds in all plants from the different altitudes. The results indicate that there was a noticeable increase in the percentage of carvacrol and a decrease in the percentage of p-cymene with increasing altitude. Specifically, the percentage of carvacrol was 38.30%, 41.58%, and 58.76% at altitudes of 1150m, 1650m, and 2150m, respectively. On the other hand, the percentage of p-cymene decreased from 35.47% at 1150m to 22.75% at 1650m, and then to 17.12% at 2150m.

Conclusions

Generally, it has been observed that there is an increase in the essential oil content of *Origanum acutidens* (Hand-Mazz.) Ietswaart as the altitude increases. This is because higher altitudes are associated with colder temperatures, lower air pressure, and stronger UV radiation, which can result in increased production of secondary metabolites such as essential oils. The altitude had either a positive or negative effect on all the major components identified in the *Origanum acutidens* (Hand-Mazz.) Ietswaart essential oil. Further studies are highly recommended due to the geographical diversity in Turkey and the wide distribution of *Origanum acutidens* (Hand-Mazz.) Ietswaart, as well as the numerous medicinal applications of this plant.

REFERENCES

[1] Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish Origanum acutidens and its three components, carvacrol, thymol and p-cymene. Bioresource Technology, 99(18), 8788-8795.
[2] Cosge, B., Turker, A., Ipek, A., Gurbuz, B., Arslan, N. (2009). Chemical compositions and antibacterial activities of the essential oils from aerial parts and corollas of Origanum acutidens (Hand.-Mazz.) Ietswaart, an endemic species to Turkey. Molecules, 14(5), 1702-1712.

Fast chiral GC recognition of citrus essential oils evaluated with greenness metrics: is this analysis sustainable in industrial quality control?

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

[1] Department of Drug Science and Technology, University of Turin, 10125 Turin, Italy;

* Email: gaia.bechis@unito.it

Keywords: Citrus essential oils, Enantioselective fast GC-MS, Cyclodextrin derivatives, Environmental impact, Metrics

Objective

Citrus essential oils (EOs) are used in several fields around the world and require constant monitoring of their quality and authenticity to avoid risks of improper use or adulteration. Chiral analysis of these EOs is essential, not only to correlate enantiomeric distribution with organoleptic and biological properties, but also to authenticate genuine EOs and detect possible adulteration with cheaper synthetic substances or from different sources [1]. Enantioselective gas chromatography (Es-GC) with cyclodextrin derivatives as stationary phases is a well-established technique for chiral analysis of EOs, and has also been shown to be successfully used in fast GC mode after optimization of column dimensions and analytical conditions [2]. The use of fast GC is commonly regarded as one of the most important strategies to improve environmental performance of GC analyses, but few studies have "quantitatively" evaluated its impact in this respect. In addition, industrial quality control laboratories must not only face with increasing regulatory and public attention to environmental sustainability, but also with the multiplication of norms and quality standards requiring accurate and reliable measurements, as well as practical considerations such as productivity, cost, and simplicity of methods. This study therefore aims to: i) develop and speed up chiral recognition methods suitable for citrus essential oil authentication controls, and ii) determine, through a metric tool based on the additive RGB (Red-Blue-Green) color model [3], whether the methods are a suitable trade-off between limited energy and chemicals' consumption, high analytical performance, and reasonable laboratory productivity.

Methods

Cold-pressed essential oils of bergamot (*Citrus limon* (L.) Osbeck), sweet and bitter orange (*Citrus* × *aurantium* L., http://www.worldfloraonline.org) were analysed with MEGA-DEX DET-Beta capillary columns coated with 30% 2$^{I\text{-}VII}$-*O*-ethyl-3$^{I\text{-}VII}$-*O*-ethyl-6$^{I\text{-}VII}$-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin diluted in PS086 stationary phase (MEGA, Legnano, Italy). Standard methods carried out with a conventional column (25m x 0.25 mm d_c x 0.25 mm d_f.) were translated and speeded up with columns of reduced dimensions (15m x 0.18 mm d_c x 0.18mm d_f. and 10 m x 0.10mm d_c x 0.10mm d_f). Data elaboration was performed by supervised and unsupervised statistical treatment and the performance of the methods was evaluated through the RGB calculation model [3].

Results:

Es-GC-MS analysis of essential oils of citrus fruits allows to discriminate samples of different quality. In addition, the translation of the methods to shorter and narrower columns enables to reduce analysis time, while giving results comparable to those obtained with conventional columns. At the same time, fast GC enables a reduction in energy and gas consumption and a dramatic improvement in laboratory effectiveness.

Conclusions:

In a world increasingly moving towards "greenness", this study proposes an analytical approach to evaluate genuineness of citrus essential oils addressing the need to reduce the carbon footprint of analyses without altering analytical performance and increasing productivity.

REFERENCES

[1] G. Dugo and L. Mondello, Citrus Oils Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity. CRC Press: Boca Raton, 2011.
[2] C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, and C. Bicchi, "Separation of stereoisomers by gas chromatography - Handbooks in Separation Science: Gas Chromatography," in Gas Chromatography, 2nd Edn., Amsterdam (NL): Colin F. Poole Ed. Elsevier Science Publisher, 2021, pp. 581–614.
[3] P. M. Nowak and P. Kościelniak, "What color is your method? Adaptation of the RGB additive color model to analytical method evaluation," Anal. Chem., vol. 91, no. 16, pp. 10343–10352, 2019, doi: 10.1021/acs.analchem.9b01872.

Characterization of volatile and aroma active compounds in *Cupressus torulosa* needles essential oil by GC-MS and GC-O analyses

Piyush Bhalla [1*], Renuka Thergaonkar [2], V.K Varshney [1]

[1] Chemistry and Bioprospecting Division, Forest Research Institute, Dehradun, Uttarakhand, INDIA
[2] R & D Global Consultants Private Limited, 106-108, 1st Floor, B wing, Hamilton, Hiranandani Business Park, Hiranandani Estate, Patlipada, Godhbunder Road, Thane (W), Maharashtra, INDIA

* Email: piyush.imf@gmail.com

Keywords: *Cupressus torulosa*, essential oil, GC-MS, GC-O, AEDA

Objective

Cupressus torulosa D. Don (Family Cupressaceae) is an evergreen tree of the north western Himalayan region of India and its aromatic needles are known for various traditional uses. However, aromatic potential of the needles for use in aroma industry is not scientifically explored. The present study, was therefore, aimed to establish chemical composition and to identify the odour-active components of the needles' derived essential oil of *Cupressus torulosa*.

Methods

Fresh needles of *C. torulosa* were collected from the Ogla region of Pithoragarh district, Uttarakhand (India). The needles (800g, in triplicate) were hydro distilled using Clevenger-type apparatus. *C. torulosa* needles essential oil (CTEO) was separated from the hydro distillate using dichloromethane, dried over anhydrous Na_2SO_4 and yield was determined on a moisture-free basis. Composition of the CTEO was determined using GC-FID and GC-MS on a DB-5MS column (30 m x 0.25 mm x 0.25 µm). Quantification of the compounds was done according to the IOFI-recommended practice using methyl octanoate as an internal reference. Gas Chromatography-Olfactometry (GC-O) technique using aroma extract dilution analysis (AEDA) was employed to identify aroma-active components in CTEO. On the basis of GC-O and AEDA results, odour active value (OAV) and relative flavour activity (RFA) of the compounds were calculated. The oil was examined for its sensory properties by the trained perfumer.

Results

A greenish-yellow CTEO in 0.8% yield with top note: green, fresh leafy, cool, fruity, citrus; middle note: dry woody; and base note: spicy, dry was obtained. Altogether, 36 compounds representing 90.69% of total oil with δ-3-carene (18.34%), sabinene (11.25%), terpinen-4-ol (8.91%) and α-pinene (8.21%) as major components were identified. AEDA led to identification of 22 odour-active components in CTEO. Values of odour activity and RFA revealed linalool (1225000, 1.12), α-Pinene (432105, 0.62), limonene (392500, 0.53), α-Terpinene (296250, 0.58), camphor (130379, 2.04), sabinene (114795, 0.23), α-terpinolene (80243, 1.14), citronellol (75806, 0.43), terpinene-4-ol (74250, 0.59), umbellulone (67466, 0.79), γ-Terpinene (42100, 0.43), and β-Pinene (28600, 1) as the major odorants.

Conclusions

The results suggest that *Cupressus torulosa* needles essential oil could be a potential candidate for use in aroma industry.

ACKNOWLEDGMENTS

Financial assistance from the National Authority CAMPA, MOEF&CC for the project "Bioprospecting for Industrial Utilization of Lesser-Known Forest Plants" under the scheme "Strengthening Forestry Research for Ecological Sustainability and Productivity Enhancement" is gratefully acknowledged.

Fractionation of *Cryptomeria japonica* essential oil by sequential elution during hydrodistillation – a preliminary study

Filipe Arruda[1,2*], Ana Lima[1,3], Alexandre Janeiro[2], Tânia Rodrigues[2], José S. Rosa[2,4], José Baptista[1,3], Tanner Wortham[5], Elisabete Lima[1,3]

[1] Institute of Agricultural and Environmental Research and Technology, Azores University, Angra do Heroísmo, PORTUGAL
[2] Department of Biology, Faculty of Science and Technology, Azores University, Ponta Delgada, PORTUGAL
[3] Department of Physics, Chemistry and Engineering, Azores University, Ponta Delgada, PORTUGAL
[4] Biotechnology Centre of Azores, Azores University, Ponta Delgada, PORTUGAL
[5] The Perfumery, 621 Park East Blvd. New Albany, IN, USA

* Email: filipe.mp.arruda@uac.pt

Keywords: Azorean forestry wastes valorisation, *Cryptomeria japonica*, essential oil, hydrodistillation, sequential elution, fractions

Objective

Cryptomeria japonica (Thunb. ex L.f.) D. Don (Cupressaceae) is the most economically important tree species in the Azores archipelago, Portugal. As a result, a huge amount of biomass wastes is locally produced every year, such as leaves (CJL) that can serve as a rich source of essential oils (EOs). This study aimed, for the first time, to fractionate the EO from Azorean CJL through sequential elution during the hydrodistillation (HD) process, at specific distillation timeframes (DT), in order to produce EO fractions (EO–Frs) with different chemical profile and thus with different functional bioactivity value.

Methods

Azorean CJL, collected in São Miguel Island in July 2022, was subjected to HD process, using a Clevenger-type apparatus. Three EO–Frs were collected sequentially at the following DT: 0–2 min (EO–Fr_1), 2–60 min (EO–Fr_2) and 60–180 min (EO–Fr_3). All of the accumulated EO during each time interval was collected, separated from water phase and stored. In addition, a control sample (crude EO) was obtained from a straight 0–180 min non-interrupted distillation. The chemical composition of the crude EO and EO–Frs was analyzed by gas chromatography–mass spectrometry (GC–MS).

Results

EO–Frs collected during the HD process varied significantly in the concentrations (%) of their major terpene compounds. Specifically, the α-pinene, sabinene, myrcene and limonene percentage was higher at the beginning of distillation (EO–Fr_1) and gradually decreased over time. In contrast, EO–Fr_3 presented the highest percentage of elemol, β-eudesmol, α-eudesmol and phyllocladane. On the other hand, EO–Fr_2 was richer in δ-cadinene and bornyl acetate as compared to the other EO–Frs. In general, monoterpenes, which are the more volatile compounds (lower boiling point and higher vapor pressure) tend to vaporize more quickly and in greater quantities at the beginning of the distillation process (0–2 min). Sesqui- and diterpenes, due to their higher boiling points and lower vapor pressure, are less volatile, therefore are more likely to be present in the later EO–Frs.

Conclusions

Sequential EO elution during HD process is indeed an efficient method to fractionate the EO from Azorean CJL This finding could be helpful to the industry professionals seeking to identify EO–Frs with desirable compositions for use in pharmaceutical, medical, cosmetic and food industries. Further studies, which are ongoing, are warranted to explore the biological activity of each EO–Fr, particularly the final EO–Fr that is enriched in diterpenes. Such investigations may lead to the development of novel bioactive products with manifold applications and potential economic value.

ACKNOWLEDGMENTS

This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Filipe Arruda thanks FRCT for Doctoral Research Scholarship (refª M3.1.a/F/008/2021).

ISEO 2023

53rd INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

September 13-16, 2023 – Milazzo, Messina, Italy

POSTER PRESENTATIONS

Elettaria cardamomum (L.) Maton essential oil as potential acetylcholinesterase and butyrylcholinesterase inhibitor: A bio-guided fractionation approach

Marta Pavarino[1*], Cecilia Cagliero[1], Arianna Marengo[1], Carlo Bicchi[1], Patrizia Rubiolo[1], Barbara Sgorbini [1]

[1] Department of Drug Science and Technology, University of Turin, Turin, ITALY

* Email: m.pavarino@gmail.com

Keywords: cardamom essential oil, acetylcholinesterase inhibition, butyrylcholinesterase inhibition, essential oils, bio-guided fractionation

Objective

Elettaria cardamomum (L.) Maton is a plant of the Zingiberaceae family and has been used for centuries in traditional Ayurvedic and Chinese medicine for a variety of different purposes, as infusions, decoctions, distillates and essential oils, which we have focused on [1] The rapid spread of the use of the essential oil of this plant throughout the world and its use in food, medicine, and fragrance fields makes it a very interesting matrix in the research for specialized bioactive metabolites. Essential oils have traditionally been used to improve cognitive abilities and alleviate other symptoms associated with Alzheimer's disease (AD) [2]
Inhibitors of cholinesterase enzymes are currently used as drugs in AD treatment (e.g., galantamine, rivastigmine, donepezil) [3] Therefore, the aim of this study was to investigate the possible inhibitory activity of *E. cardamomum* essential oil towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and finally to find the compounds to which this activity could potentially be attributed using abio-guided fractionation approach.

Methods

Whole cardamom fruits (opened capsules and seeds) and, separately, seeds and fruits were hydrodistilled in a Clevenger apparatus for 4 hours. In addition, five batches of cardamom essential oil (supplied by Witt Italia) were investigated.
All essential oil samples were subjected to an AChE and BChE *in vitro* colorimetric assay by the Ellman method.
Fractionation was performed using a PuriFlash 450 column chromatography (Sepachrom, Italy), equipped with both a UV and Evaporative Light Scattering detector (ELSD). Petroleum ether and ethylacetate were used as mobile phase solvents; Sphera 50 µm silica cartridges (Sepachrom, Italy) were selected. The eluent flow was maintained at 25 ml/min and the volume of essential oil injected was 1 ml. Chemical characterization of the solutions of the essential oils and their respective fractions performed by GC-MS analysis.

Results

Cardamom essential oil was found to be active in inhibiting both cholinesterase enzymes. Bio-guided fractionation approach allowed us to isolate fractions/pure compounds that can be individually tested to evaluate their activity. The hydrocarbon fraction of cardamom essential oil was found to be inactive towards both AChE and BChE. The oxygenated fraction, on the other hand, was active towards both enzymes. Terpinyl acetate and 1,8-cineole were found to be the responsible of the inhibitory activity.

Conclusions

Cardamom essential oil was found to be active towards both cholinesterase enzymes, thus resulting very promising for further in-depth studies looking for complementary treatment to conventional therapy.

REFERENCES

[1] K. Ashokkumar, M. Murugan, M. K. Dhanya, and T. D. Warkentin, (Jan. 2020), 'Botany, traditional uses, phytochemistry and biological activities of cardamom [Elettaria cardamomum (L.) Maton] – A critical review', J Ethnopharmacol, vol. 246, p. 112244.
[2] C. Dobetsberger and G. Buchbauer, (Sep. 2011), 'Actions of essential oils on the central nervous system: An updated review.', Flavour Fragr J, vol. 26, no. 5, pp. 300–316.
[3] S. Zhou and G. Huang, (Feb. 2022), 'The biological activities of butyrylcholinesterase inhibitors', Biomedicine & Pharmacotherapy, vol. 146, p. 112556.

Differentiation and Characterization of Aromatic Moroccan Plants by Ambient Mass Spectrometry Combined with Chemometrics

Domenica Mangraviti[1,*], Francesca Rigano[1], Paola Dugo[1,2], Luigi Mondello[1,2,3]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[3] Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, Rome, ITALY

* Email: dmangraviti@unime.it

Keywords: Lamiaceae family, Moroccan plants, functional compounds, REIMS-QToF, DART-QDa

Objective

Nowadays, a growing interest in industry, agriculture, and health sciences is aimed to Medicinal & Aromatic Plants (MAPs), due to the beneficial effects on human health significantly related to the presence of functional compounds in these natural sources (phenols, flavonols/flavonoids, alkaloids, polypeptides, carotenoids, etc). On the other hand, the essential oils extracted from aromatic plants are characterized by valuable flavors widely employed in food and fragrance industry for production of beverages, cosmetics, and perfumes. Such important properties explain the great economic interest around MAPs, and at the same time, making them target of fraudulent activities. In order to deal such illegal practices more competitive tools, as fast and innovative fingerprinting analytical methods, were employed in the last decade for the reliable identification of "precious" foodstuffs. In this regard, Ambient Mass Spectrometry (AMS) represents a valuable strategy for which a minimal of absent sample preparation is required, and matrices of interest are analyzed in its native form under ambient conditions.

Methods

Moroccon species of Lamiaceae family, originated from different pedo-climatic areas (*Mentha pulegium*, *Mentha suaveolens*, *Melissa officinalis*, *Calamintha nepeta*, *Thymus zigis*, *Sideritis incana*), were analyzed by distinct AMS approaches, Rapid Evaporative Ionization Mass Spectrometry (REIMS), coupled to a quadrupole-time of flight (Q-ToF), and Direct Analysis in Real Time (DART) connected to a single quadrupole (Qda) respectively, for metabolome screening, and their differentiation by means of data statitical analysis. No sample preparation procedure was performed before REIMS-QTOF analysis and ionization processes occured directly on raw material, whereas a minimal extraction method was carried out prior DART-QDa approach.

Results

In the present work, two different AMS approaches were employed for differentiation of six Moroccan plants, as fast and reliable methods to be used for preserve the biodiversity and local economies. Specifically, REIMS-QTOF and DART-QDa techniques were explored to obtain, in a single analysis, the whole metabolome of the plants under investigation. These systems, differently to the most commonly target separation methods used for specific chemical classes analysis, do not required laborious sample preparation, long analysis and relevant researcher involvement for method optimization and data processing. Moreover, classification models based on Principal Component Analysis and Linear Discriminant Analysis were subsequently employed for Lamiaceae species discrimination according to different geographical origin.

Conclusions

The exploited techniques led to a really rapid and valuable detection of functional compounds in Moroccan APs in a very short time. The comparison between the mass spectra profiles and statistical models of both AMS strategies revealed their ability to be applied for the reliable differentiation of aromatic plants and consequently as valid tools against fraud activities.

ACKNOWLEDGMENTS

The authors are grateful to Waters Corporation and Merck KGaA for their support.

Analysis of oxygen heterocyclic compound in cold-pressed *Citrus* essential oils and fragrances using supercritical fluid chromatography coupled to tandem mass spectrometry method

Maria Rita Testa Camillo[1,*], Marina Russo[1], Paola Dugo[1,2], Luigi Mondello[1,2.]

[1] Department of Chemical Biological Pharmaceutical and Enviromental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont S.r.l., c/o Department of Chemical Biological Pharmaceutical and Enviromental Sciences, University of Messina, Messina, ITALY

* Email: testacamillom@unime.it

Keywords: furocoumarins, fragrances, supercritical fluid chromatography, *Citrus* essential oils, quality control.

Objective
Oxygen heterocyclic compounds (OHCs) are secondary metabolites mainly present in the non-volatile fraction of cold-pressed *Citrus* essential oils [1]. Under this denomination coumarins, furocoumarins and polymethoxyphlavones are included. These compounds possess numerous beneficial properties for human health; however, the interaction of furocoumarins with UVA rays could be toxic to human health [2]. Due to their photoactivity, furocoumarins levels are constantly monitored by opinions and regulations issued by the International Fragrance Association (IFRA) and the European Food Safety Authority [3].
The present research is focused on the development of an environmental-friendly analytical method using supercritical fluid chromatography-triple quadrupole mass spectrometry (SFC-QqQ/MS) technique.

Methods
The SFC-QqQ-MS analysis was performed on a Shimadzu Nexera-UC system (Shimadzu, Kyoto, Japan). An Ascentis Express F5 (150 × 2.1 mm, 2.7 μm) column (Merck KGaA, Darmstadt, Germany) was used. The mobile phase chosen was composed by CO_2 and methanol. Thirteen cold-pressed *Citrus* essential oils and thirty *Citrus* flavored commercial fragrances were analysed without any pre-treatment.

Results
The method was validated by constructing the calibration curves of twenty-eight OHCs reference materials using spiked lemon distilled essential oil and, the method was validated according to the EURACHEM guidelines. Linearity, limit of detection (LoD), limit of quantification (LoQ), accuracy, intraday and inter-day precision were then calculated. All the validation parameters resulted satisfactory, with low LoQs (0.0014 and 0.1536 mg L^{-1}) and LoDs (0.0004 and 0.0470 mg L^{-1}), that could allowed the quantification of OHCs even when they are contained at trace level in finished products (such as foods and cosmetics).
The quali-quantitative profiles of the cold-pressed essential oils analyzed resulted quite coherent with the data reported in litterature [1]
Analysis of *Citrus* flavored commercial perfumes showed the presence of OCHs. In detail, coumarin was found in most of the sample analysed, because is normally employed in cosmetic industry to fix aroma. Polymethoxyphlavones were quantificated in most perfumes suggesting the use of cold-pressed grapefruit, mandarin and, sweet orange essential oils as flavours. None of the Citrus flavoured perfumes showed furocoumarins concentration level higher than limit set by IFRA regulation.

Conclusions
The developed method permits the reduction of solvent consumption and overall analysis time, while keeping a high separation power as well as good identification and quantification capabilities through the use of tandem MS data. The SFC-QqQ-MS method is then clearly suitable to be applied for the quality control of OHCs in *Citrus* essential oils and for trace analysis of these compounds present in *Citrus* flavored commercial perfumes.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the continuous support from Merck Life Science and Shimadzu Corporations.

REFERENCES

[1] Russo M, Rigano F, Arigò A, Dugo P, Mondello L., (2021). Coumarins, psoralens and polymethoxyflavones in cold-pressed Citrus essential oils: a review, Journal of Essential Oil Research, 33, 1-18.
[2] Hung WL, Suh JH, Wang Y., (2016) Chemistry and health effects of furanocoumarins in grapefruit, Journal of Food and Drug analysis, 25, 1-13.
[3] IFRA Standard. 48th Amendement. Citrus oils and other furocoumarins containing essential oil, 2015.

Effect of drying methods on the volatiles profile of Chamomile

Natalia Pachura[1,*], Adam Figiel[2], Antoni Szumny[1] and Jacek Łyczko[1]

[1]Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences, 50-375 Wrocław, Poland
[2]Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, 51-630 Wrocław, Poland

* Email: natalia.pachura@upwr.edu.pl

Keywords: *Matricaria chamomilla*, SPME-arrow, drying, essential oils, sensory analysis

Objective

Matricaria recutita, as a representative of the Asteraceae family, is a small but well-known and highly valued medicinal herb which contains coumarins (umbelliferone and its methyl ester heniarin), and flavonoids (apigenin, quercetin, rutin). One of the main constituents is also essential oils, whose content ranges from 0.2% to 2%. Chamomile has a very broad spectrum of health properties. These include antibacterial, anti-inflammatory, antispasmodic, anti-ulcer and antiviral. Our team's research aimed to design a comprehensive study on multiple drying techniques (CD - convection drying, VMD - microwave-vacuum drying , CPD-VMFD - convective pre-drying and vacuum-microwave finish-drying) influence on *Matricaria recutita* flowers and explore the possibilities of applying drying to improve the dried products' expected quality.

Methods

Chamomile flowers of the Złoty Łan variety were obtained from the local company Zioła z Doliny Bobru. Before undertaking the drying process, the chamomile flowers were checked to prevent chemotype diversity. As drying methods, convection drying (CD) at three temperatures (50, 60 and 70 °C), microwave-vacuum drying at three powers (240, 360 and 480W) and convective pre-drying and vacuum-microwave finish-drying (CPD-VMFD) were selected. The drying kinetics model was created according to the Page model to predict the decrease in water content during drying. Fresh and dried by various methods chamomile flowers, were examined for changes in essential oil content and released aromas. For this purpose, a distillation process was carried out using a Deryng apparatus, and the aromas emitted by the plant matrix were analyzed using Arrow headspace-SPME. All samples were analyzed using GC-MS (Shimadzu GCMS QP 2020, Kyoto, Japan) equipped with a Zebron ZB-5 MSi capillary column (30 m×0.25 mm×0.25 147 µm; Phenomenex, Torrance, CA, USA). In addition, sensory analysis of chamomile flowers was carried out to compare the results obtained with the profile of emitted aromas from SPME-Arrow.

Results

As a result, two profiles were obtained For the essential oil profile, 54 compounds were identified where the main components were α-bisabolol oxide (A and B), bisabolone A, chamazulene, E-β-farnesene and Z-spiroether. On the other hand, for the aroma profile, 65 compounds were identified, where the main compounds turned out to be E-β-farnesene, α-bisabolol and its oxide B, bicyclogermacrene. And their content ranged from 40 to 4%, respectively. The profile of the essential oils and the profile of the emitted VOCs from the plant material are similar, but the two methods should be considered complementary. The drying methods used had a significant effect on changing the content of almost all bioactive compounds with the exception of α-Bisabolol oxide B.

Conclusions

Quantitative and qualitative analysis showed that drying (CD-60/360W) was the most conservative for volatile fractions. The CD-70 variant generated the highest losses (approximately 48%) in volatile fractions. The results show that increasing the temperature or power of the magnetrons does not proportionally affect the recovery of essential oils. It is also important to note the technical problem of microwave-vacuum drying in damaging the delicate chamomile flowers as a result of their constant repositioning in the rotating drum allowing the material to be heated evenly in the non-uniform microwave field. However, the main argument in favour of using microwave-vacuum drying for the preservation of herbal raw materials is the relatively short drying time and the possibility to change the process parameters at any time. In addition, the reduced time allows for reduced operating costs and adaptation to external conditions to harvest the raw material at the best time and dry it efficiently before adverse microbiological changes occur.

Chemical composition and biological activity of essential oil from *Platycladus orientalis*

Marta Klemens[1,*], Jacek Łyczko[1], Antoni Szumny[1], Przemysław Bąbelewski[2], Tomasz Gębarowski[3]

[1] Department of Food Chemistry and Biocatalysis, Wrocław University of Life Science and Environment, Wrocław, POLAND
[2] Department of Horticulture, Wrocław University of Environmental and Life Sciences, Wrocław, POLAND
[3] Department of Biostructure and Animal Physiology, Wrocław University of Environmental and Life Sciences, Wroclaw, POLAND

* Email: marta.klemens@upwr.edu.pl

Keywords: Platycladus orientalis, essential oils, bioactive compounds, cell cultures, GC-MS analysis

Platycladus orientalis is a coniferous plant belonging to the cypress family. It is mainly found in Asia, but is also cultivated in Europe in the Black Sea and Mediterranean. Plants of this species are poisonous by the thujones in them. They are irritating to the skin, and when consumed cause convulsions and degenerative changes in the kidneys, liver and stomach. *Platycladus orientalis* is used as an ornamental plant.

Objective

The purpose of this study was to evaluate the qualitative and quantitative chemical composition of the essential oils of thuja (*Platycladus orientalis*) and to assess potential biological properties which include cytotoxicity studies and potential anticancer activity. Four varieties of thuja *Platycladus orientalis*, *Platycladus orientalis* 'Aurea Nana', *Platycladus orientalis* 'Sieboldii' and *Platycladus orientalis* 'Aurea' were used. The plants compared were 1-year, 2-year and 3-year plants.

Methods

Samples were weighed from each year - about 5 g of fresh *Platycladus orientalis* material and the other varieties were weighed at 10 g each. The oils were distilled using a Deryng apparatus. 2-Undecanone standard of 0.5mg/ml in cyclohexane was added. The profile of the tested essential oils was evaluated using the GC-MS technique (Shimadzu Company, Kyoto, Japan).

Biological activity on cell cultures studies are underway. The studies will use human cell lines - human dermal fibroblasts (NHDF) purchased from LONZA (Verviers, Belgium) and human cancer cell lines - MCF7 (breast cancer), A549 (lung cancer) and LOVO and HT29 (colorectal adenocarcinomas). Cells will be cultured under standard conditions of 37 °C and 5% CO2. Activity assessment will be performed in accordance with the National Cancer Institute's guidelines for screening human cancer lines and based on our studies [1, 2].

Results

The identification of all of the components was based on a comparison of the mass spectra with the mass spectra of the compound obtained experimentally, available in the NIST20 and FFNSC databases. In the plants, there were eighteen dominant compounds identified including α- pinene, sabinene, terpinolene, myrcene, cedrol and 3-carene. Quantitative and qualitative differences in essential oils were shown between varieties and also between 1-year-old, 2-year-old and 3-year-old plants.

The results from biological activity on cell cultures will be prepared after the study is completed and presented at the conference.

Conclusions

The data collected allow us to conclude that the chemical composition of *Platycladus orientalis* for individual varieties varies from year to year, but also between varieties of this plant. Cytotoxicity and anticancer activity on cell lines, or the absence thereof, will only be able to be evaluated after the study is completed. Depending on the results from biological activity on cell cultures, further activities will be planned.

REFERENCES

[1] Arendt-Pindel, A.; Marszałek-Harych, A.; Gębarowska, E.; Gębarowski, T.; Jędrzkiewicz, D.; Pląskowska, E.; Zalewski, D.; Gulia, N.; Szafert, S.; Ejfler, J. Design and functionalization of bioactive benzoxazines. An unexpected ortho-substitution effect. New J. Chem. 2019, 43, 12042–12053.
[2] Iwiński Hubert, Różański Henryk, Pachura Natalia Aleksandra Wojciechowska, Tomasz Gębarowski, Antoni Szumny. In Vitro Evaluation of Antiprotozoal Properties, Cytotoxicity Effect and Anticancer Activity of New Essential-Oil Based Phytoncide Mixtures Molecules, 2023, vol. 28.

Chemical composition of essential oils from different parts of Azorean Cryptomeria japonica (Thunb ex. L.F.) D. Don and their in vitro antimicrobial activity

Ana Lima[1,2], Filipe Arruda[1,3*], Alexandre Janeiro[1], Tânia Rodrigues[1,3], José Baptista[1,2], A. Cristina Figueiredo[4], Elisabete Lima[1,2]

[1] Institute of Agricultural and Environmental Research and Technology, University of Azores, PORTUGAL
[2] Department of Physics, Chemistry and Engineering, University of Azores, PORTUGAL
[3] Department of Biology, Faculty of Science and Technology, Azores University, Ponta Delgada, PORTUGAL
[4] Centro de Estudos do Ambiente e do Mar (CESAM Ciências), Faculdade de Ciências da Universidade de Lisboa, BV, DBV, C2, Piso 1, Campo Grande, 1749-016 Lisboa, PORTUGAL

* Email: filipe.mp.arruda@uac.pt

Keywords: Azores, *Cryptomeria japonica*, essential oil, seed and pollen cones, α-pinene, phyllocladene

Objective

Biomass residues of *Cryptomeria japonica* (Thunb ex. L.F.) D. Don, a commercially important forest tree throughout Asia and in Azores Archipelago (Portugal), are still raw materials that can be converted into eco-friendly and high value-added products, such as essential oils (EOs), with social, economic, and environmental impacts [1]. Thus, the present study aimed to evaluate the chemical composition of the EOs from different parts of Azorean *C. japonica* and its antimicrobial activity.

Methods

The EOs of needles, foliage, seed and pollen cones from Azorean *C. japonica*, were obtained by hydrodistillation and subsequently analysed by gas chromatography (GC–FID) and gas chromatography-mass spectrometry (GC–MS), as in [2]. The biological activities (antibacterial and antifungal) were examined using the agar disk diffusion according to Kirby-Bauer methodology.

Results

The EOs were obtained in yield of 0.67–2.63% (w/w on dry basis), and their analysis allowed the identification of 50 components, representing 92.0–96.7% of the total EOs. The EOs chemical profile revealed the dominance of monoterpene hydrocarbons in all different plant parts, although some quantitative variance was noticed between them. α-Pinene was the main component in all samples, with its levels decreasing in the following order: seed cones > pollen cones > needles > foliage, whereas phyllocladane displayed the opposite tendency. The EOs exhibited weak to moderate activity against gram positive bacteria, with no more than 13 mm inhibition growth zones and no activity against gram negative bacteria. Only the seed and pollen cones OEs showed activity against the *Penicillium* spp., probably due to the higher monoterpene hydrocarbons concentration in these plant parts.

Conclusions

The EOs chemical profile of all studied samples was similar between them, with α-pinene being the main component. Comparing the present data with those previously reported in literature [1–3], the studied EOs displayed analogous chemical profiles, except regarding the diterpene hydrocarbons class, probably due to genetic or environmental/climatic differences. Since the studied crude EOs exhibited only a weak inhibitory effect against gram positive bacteria, further studies are ongoing in order to obtain EO fractions with differential chemical profile and, consequently, to increase its biological activity.

ACKNOWLEDGMENTS. Thanks to Marques, S.A. from São Miguel, Azores, who provided the *Cryptomeria japonica* samples. This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Filipe Arruda thanks FRCT for Doctoral Research Scholarship (refª M3.1.a/F/008/2021). Thanks to FCT/MCTES through national funds, under CESAM UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020.

REFERENCES

[1] Lima A. et al. (2021). Appl. Sci. 11: 11097.
[2] Figueiredo et al. (2021). Nat. Prod. Commun. 16: 1-10.
[3] Ruas et al. (2022). Molecules 27: 3572.

Application of *Conobea scoparioides* Cham. & Schltdl essential oil in association with parabens as natural cosmetic preservative under a challenge test

Cristina M. T. S. Miguel[1], Cristina H. Serra[1], Paulo R. H. Moreno[1,2,*]

[1] Programa de Pós-graduação de Fármaco e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, BRAZIL

[2] Departamento de Química Fundamental. Instituto de Química, Universidade de São Paulo, São Paulo, BRAZIL

* Email: prmoreno@iq.usp.br

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

Objective

The aim of the present study was to evaluate the preservative action of *Conobea scoparioides* Cham. & Schltdl essential oil in topical preparations using a challenge test. The potential of essential oils indigenous to Brazil has not been sufficiently exploited as functional ingredients in cosmetic formulations.

Methods

Conobea scoparioides Cham. & Schltdl, essential oil was a commercial sample obtained from International Flavors & Fragrances (IFF) (Santana do Parnaíba, São Paulo, Brazil). Efficacy Test of the Preservative System was carried out using a non-ionic emulsion (Polowax®), using 4 different concentrations of the preservative mixture essential oil/parabens. The ideal concentration for the preservative mixture was previously determined by a simplex network analysis [1]. In the challenge test were assayed the ideal concentration plus two higher concentrations (2 and 4 times the ideal concentration) and one below the ideal.

The test was performed accordingly to USP 2015 [2] and was based on the deliberate contamination of the product with *Aspergillus brasiliensis* (ATCC 6404), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027). *Burkholderia cepacia* (ATCC 25416) and *Staphylococcus aureus* (ATCC 6538). The test duration was 28 days, with samples collected on the 7th, 14th and 21st days, ending on the 28th day after inoculation.

Results

In all test formulations, a decay of 5 logarithmic growth cycles was observed for all microorganisms after seven days with no further growth until the end of the test. Therefore, this result indicates that all the associations essential oil/parabens are in accordance with the pharmacopeial specifications for preservative systems, that considers as acceptable a reduction of 2 logarithm growth cycles of the initial CFU on the 14th day for bacteria, and no increase in the initial CFU for fungi. Additionally, all the challenged microorganisms must remain without an CFU increase.

Conclusions

The association of *C. scoparioides* essential oil with parabens constitutes an option for their use as a preservative system for cosmetic formulations in order to minimize the amount of synthetic preservatives added in cosmetics.

ACKNOWLEDGMENTS

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

REFERENCES

[1] Miguel M. T. S., Scotti M. T., Serra C. H., Moreno, P. R. H., (2022) Association of Conobea scoparioides Cham. & Schltdl essential oil with parabens. In: Lochynski S., Strub D. J., Surowiak A. K., Balcerzak, L. (eds) Program and Book of Abstracts from the 52nd International Symposium on Esssential Oils, College of Physiotherapy, Wrocław

[2] USP. Microbiological Tests (51) Antimicrobial Effectiveness Testing. Usp38Nf33, n. c, p. 111–114, 2015.

Photoselective screens, chlorophyll fluorescence and essential oil composition in basil

Mirian Nomura[1], José Magno Q. Luz[1,*], Arie F. Blank[2], Carlos R. Rodrigues[3], Sérgio M. Silva[4], Roberta C. Oliveira[1], Mércia F. Alves[2], Daniela A. C. Nizio[2]

[1]Institute of Agricultural Sciences, Universidade Federal de Uberlândia-UFU, Uberlândia-MG, BRAZIL
[2]Department of Agronomic Engineering, Universidade Federal do Sergipe, São Cristóvão-SE, BRAZİL.
[3]Goiano Federal Institute, Rio Verde-GO, BRAZİL.
[4]Department of Agronomy, Universidade Federal dos Vales do Jequitinhonha e Muruci, Unaí-MG, BRAZİL.

* Email: jmagno@ufu.br

Keywords: *Ocimum basilicum* L., chlorophyll fluorescence, environmental, seasonality

The objective of this work was to evaluate the effect of photoselective screens (pearl, blue, red and black) on four basil (*Ocimum basilicum* L.) cultivars (Anise, Cinnamon, Italian Large Leaf, and Maria Bonita), grown in two planting times, in the index chlorophyll, chlorophyll fluorescence, and essential oil content and composition. The two experiments were implemented in a randomized block design, testing in the plots, five cultivation environments (three photoselective screens in the colors pearl, blue and red, all with 20% shading, a black screen with 30% shading, acquired from the company Chromatinet®, and the control - cultivation in full sun), and in the subplots, four cultivars of basil (Maria Bonita, Cinnamon, Anise and Italian Large Leaf). In the evaluation of the experiment, the content of chlorophyll a and b, the fluorescence of chlorophyll and the content and composition of essential oil were measured. Contrast was performed, using the Scheffé test, to compare the culture under screens and in full sun. The data of the two growing seasons were submitted to joint analysis of variance and compared by the tukey test at 5% probability. The cultivation of basil under photoselective screens increased the height of plants. The spring/summer growing season provided better conditions for the functioning of the photosynthetic apparatus of the plants and provided higher levels of essential oil and its main constituents. The cultivar Maria Bonita showed the better performance of photosynthetic stooe and, consequently, higher content of essential oil. The screens did not promote variation in the chlorophyll fluorescence indices and essential oil content, but they did change the concentration of the main constituents of essential oils in all cultivars. The blue screen in the spring/ summer crop increases the linalool content in the Cinnamon and Maria Bonita cultivars. The red screen increased the linalool content in the Italian Large Leaf cultivar and methyl chavicol in the Anise and Cinnamon cultivars in spring/ summer cultivation. The pearl mesh in spring/summer cultivation increased the content of methyl (E) -cinnamon in the cultivar Cinnamon. There are different responses among cultivars depending on the photoselective screen and growing season.

ACKNOWLEDGMENTS

The authors would like to thank the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES) and their institutions for supporting this research.

In vivo elicitation improves essential oil content and modifies terpene composition in *Salvia rosmarinus* leaves.

Ana Claudia Pacheco[1,*], Mateus Modesto Bosisio[1], Tiago Benedito dos Santos[1], Hélida Mara Magalhães[2], Silvia Gracielle Hulse de Sousa[2], Alessandra Ferreira Ribas[3], Cícero Deschamps[3], Roger Raupp Cipriano[3]

[1] Center of Study in Plant Ecophysiology, Wester Paulista University (UNOESTE), Presidente Prudente, BRAZIL
[2] Department of Agronomy, University of Paraná (UNIPAR), Umuarama, BRAZIL
[3] Department of Plant Science, Federal University of Paraná (UFPR), Curitiba, BRAZIL

* E-mail: anaclau@unoeste.br

Keywords: growth regulator, biostimulant, sustainable production.

Essential oils in plants are produced in very low concentrations and elicitation stands out among the techniques used to increase their productivity. The type of elicitor, timing of treatment and concentration must be considered for good results in individual species. This work evaluated the potential elicitor of salicylic acid (SA) or seaweed extract (SE) on the biomass yield and essential oil production in *Salvia rosmarinus* L. plants from Brazil. The experiment was conducted in potted plants under greenhouse condictions and followed a completely randomized design with 3 treaments and 30 repetitions, totaling 90 experimental units (plants). The elicitors were applied through foliar spray in four serial applications (30, 60, 90 and 120 days after transplanting the seedlings to the pots - DAT), in concentrations of 1 mM (SA – Sigma Aldrich) and 6 mL L^{-1} (SE – comercial product Acadian). The control plants were sprayed with distilated water. Plants were harvested at 150 DAT for biometric and phytochemical evaluations. The essential oil (EO) content was determined in the fresh leaves, through hydrodistillation in a Clevenger-type apparatus during an extraction period of 3 h. The analysis of the chemical composition of the EO was carried out in a gas chromatography-mass spectrometry (GC- MS) system. There were no changes on plant height, number of branches and shoot dry mass (SDM) in response to elicitation. On the other hand, the essential oil content and its components were significantly affected by elicitors treatments. The maximum content of essential oil was obtained with SE (21.53 mg g^{-1} dry mass or 2.15%) followed by SA (17.02 mg g^{-1} dry mass or 1.7%), which represented average increases of 83.88 and 45.35% when compared to control plants. The major components of rosemary EO were also altered by the elicitors, with higher amounts of beta-myrcene (increase of 9.32 % for SE), α-pinene (increases of 22.68% for SA and 59.85% for SE, respectively) and 1,8- cineole (increase of 6.87% for SE) and lower amounts of camphor (reduction of 15.6% for SE) when compared to control plants EO. According to these findings, elicitation with salicilic acid or seaweed extract can be an simple, low cust and effective agricultural practice to enhance the quantity and quality of rosemary essential oil. Comparing the elicitors studied, the seaweed extract allows greater results.

ACKNOWLEDGMENTS

We are grateful to Fundação de Amparo à Pesquisa no Estado de São Paulo (FAPESP) for funding assistance (Process 2021/07166-8).

Composition of the Essential Oil from the Needles and Twigs of Organic Tyrolean Spruce - *Picea abies* (L.) H. Karst.

Alin Bosilcov*, Clemens Vergeiner, Katrin Joas

Brüder Unterweger GmbH, 9911 Assling, AUSTRIA

* E-mail: alin.bosilcov@unterweger-oils.com

Keywords: Spruce, Picea abies, Pinaceae, Tyrol, Essential oil composition

Objective

The objective of this work was to evaluate the chemical composition of the essential oil from the needles and twigs of organic spruce (*Picea abies*) growing in Tyrol, a region comprising South-West Austria and Northern Italy. *Picea abies*, also known as Common European or Norway spruce, is the most wide-spread montane conifer in western Europe and an important timber species. The essential oil has a light, balsamic pine-needle type odour. It is used mainly in fine fragrances, air-fresheners, room deodorants, bath oils and soap perfumes. For this study, 22 samples of *Picea abies* essential oil have been analysed, covering a period from 2017 to 2023.

Methods

Fresh twigs and needles of *Picea abies* have been subjected to steam distillation yielding 0.12 to 0.24% of a colourless to pale yellow essential oil. The composition was determined by GC-MS and dual channel GC-FID. The enantiomeric distribution of some relevant constituents has been assessed by enantio-GC using a chiral cyclodextrin-based stationary phase.

Results

The essential oil comprised mainly monoterpene hydrocarbons, of which α-pinene (22 – 33%), β-pinene (25 – 36.5%), limonene (7 – 10%) and β-phellandrene (3.7 – 6%) were the major constituents. Chiral analysis has shown that for these compounds the levorotatory enantiomer prevailed.

Conclusions

Our results for the Tyrolean *Picea abies* essential oil differ to some extent from those reported in the literature, especially with regard to the distribution of monoterpenes. It appears that the high concentration of β-pinene is characteristic for this geographic area.

REFERENCES

[1] Eckenwalder, J.E., (2009). Conifers of the World, Timber Press, London
[2] Adler, W., Oswald K., Fischer R., (1994). Exkursionsflora von Österreich, Ulmer Verlag, Stuttgart und Wien
[3] Arctander, S., (1960). Perfume and Flavor Materials of Natural Origin, Elizabeth, New York

GC-O and Sniff&Trap (S&T): an alternative 2D approach in sensorial aroma investigation

Thomas Albinus[1], Andrea Caretta[2*]

[1] GERSTEL GmbH & Co KG. Eberhard-Gerstel-Platz 1 - 45473 Mülheim an der Ruhr, Germany
[2] Via Alla Castellana, 3 - 20063 Cernusco s/N (MI) - Italy, ITALY

* Email: carretta@srainstruments.com

Keywords: GC-O, Thermal Desorbtion, sensory analysis, aroma investigation

Abstract

GC-O is a powerful technique able to combine analytical results arising from GC-MS and sensorial perception. Recently introduced Olfactory Detection Port 4 (ODP4), By Gerstel GmbH, consists of a dual-detection system, pushing such combination of analytical and sensorial results to a new level of reliabilty.

A sample injected in a standard GC sistem is diverted at the end of the column by means of splitting device: part of the effluent reaches the MS detector, while a second aliquote is directed to an olfactometric port where a trained operator sniffs in a proper way. The two traces (i.e. **chromatogram** and **olfactogram**) are then overlayed to combine sensorial and analytical results.

İt often happens that coeluting compounds are not clearly detectable either by detector or operator; such situation can be elegantely fixed using the S&T option provided by Gerstel GmbH ODP4. Briefly, instead of a funnel suitable for sniffing, at the ODP exit is installed a desorbtion tube able to trap the analytes. Such tube is then desorbed in a GC-MS system where a different column is used: this allows to separate the previously eluting compounds in order to identify them.

A further improvement is related to the new Gerstel OID2, wich provides a streamlined GC-O data processing workflow, featuring deconvolution followed by RI-MS library search and compatibility with commercial user-generated databases.

Characterization of avocado leaf volatiles for food flavouring applications

Fabrizio Cincotta[1], Concetta Condurso[1], Maria Merlino[1,*], Anthea Miller[1], Martina Buda[1], Ivana Lidia Bonaccorsi[2], Antonella Verzera[1]

[1] Department of Veterinary Sciences, University of Messina, Messina, ITALY
[2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: maria.merlino@unime.it

Keywords: avocado leaves, aroma compounds, food flavoring, agro-waste valorization

Objective

Avocado (*Persea americana* L.) leaves are known to be an excellent source of flavor and antioxidant compounds with anti-inflammatory properties [1]. The leaves have different flavors that can range from an anise-like flavor and subtle notes of nutmeg, mint, and basil in relation to the avocado variety. They are used in Mexican and Central American cuisine in stews, soups, and sauces, and also to make herbal teas and are fast gaining recognition elsewhere in the world. Overall, the use of avocado leaves as a culinary herb could be a great way to add unique flavor and health benefits to various dishes. In this context, the aim of our research was to characterize the volatile aroma compounds of avocado leaves of different varieties cultivated in Sicily in order to exploit their possible application as new commercial food flavoring.

Methods

Avocado leaves were collected in an avocado field sited in Giarre (Catania, Italy) from four different *Persea americana* L. avocado varieties namely *Reed*, *Hass*, *Fuerte*, and *Bacon*. The fresh leaves were characterized for the volatile aroma compounds by HS-SPME-GC-MS.

Results

Globally more than 100 volatile aroma compounds were identified in the volatile fraction of the avocado leaves, with significant differences according to the cultivar. C_6 volatile aldehydes and alcohols, such as hexanal, E-2-hexenal, and Z-3-hexenol, responsible for green and leafy aroma, characterized the volatile fraction of *Hass* and *Reed* cultivars. Differently, the volatile fraction of *Fuerte* and *Bacon* leaves were characterized by terpenes, mainly estragole, eucalyptol, and methyl eugenol responsible for sweet, anice-like, and fennel odor and for antimicrobial, antifungal, anthelmintic, and antioxidant activities [2].

Conclusions

The results of the research underlined as the aroma composition of avocado leaves greatly depend on the cultivar. *Fuerte* and *Bacon* leaves are the most promising to be used as a food flavoring being richer in terpenoids and more active aroma compounds responsible for the typical anice-like odor and biological activity.

REFERENCES

[1] Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., Soto-Covasich, J. (2021). Pulp, leaf, peel and seed of avocado fruit: A review of bioactive compounds and healthy benefits, Food Reviews International, 37(6), 619-655.
[2] Coêlho, M. L., Islam, M. T., Laylson da Silva Oliveira, G., Oliveira Barros de Alencar, M. V., Victor de Oliveira Santos, J., Campinho dos Reis, A., Amélia de Carvalho Melo-Cavalcante, A. (2022). Cytotoxic and Antioxidant Properties of Natural Bioactive Monoterpenes Nerol, Estragole, and 3, 7-Dimethyl-1-Octanol, Advances in Pharmacological and Pharmaceutical Sciences.

Essential oil components as GC-compatible hydrophobic deep eutectic solvents to extract regulated compounds from water-based fragrances

Cecilia Cagliero[1*], Gaia Bechis[1], Arianna Marengo[1], Barbara Sgorbini[1], Carlo Bicchi[1], Patrizia Rubiolo[1]

[1] Department of Drug Science and Technology, University of Turin, 10125 Turin, Italy;

* Email: cecilia.cagliero@unito.it

Keywords: Essential oil components, Natural hydrophobic eutectic solvents, Dispersive liquid-liquid microextraction, Gas chromatography, Fragrances

Objective

Essential oils (EOs) are an excellent source of compounds that can be used in several fields. They belong to different chemical classes (mono- and sesquiterpenes and terpenoids, phenols, ...) and are generally hydrophobic. Therefore, they are optimal candidates for the preparation of hydrophobic natural deep eutectic solvents (NADES), i.e. solvents formed by a combination of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) with a lower melting point than one of the two components and characterized by low toxicity and environmental impact, ease of preparation, wide range of applications and low raw material costs. They have been widely applied as extraction phases [1] but few studies report the combination of NADES-based extraction and gas chromatographic analysis [2]. Indeed, GC analysis of regulated substances and possible cross-contamination in perfumes is the approach of choice in fragrance quality control laboratories. Conventional analytical methods require either large amounts of organic solvents or a direct injection of samples, which imply possible interferences and/or frequent maintenance of the chromatographic system, especially for fragrances with high water content. This research work aims to develop a more environmentally friendly NADES based approach using essential oil components to extract and enrich volatile compounds from water-based perfumes.

Methods

Thymol, eugenol, 1,8-cineole, and nootkatone isolated from different EOs and mixed in the appropriate molar ratio were used to prepare NADES. The NADES were dispersed in water-based fragrances by dispersive liquid-liquid microextraction (DLLME) to isolate a set of suspected allergens and cross-contaminants. Thanks to the volatile nature of the adopted terpenoids and phenolic compounds, the enriched NADES were directly analyzed by GC-FID and GC-MS.

Results

After optimization of the NADES composition and extraction conditions, the investigated EO component-based solvents were succesfully applied to recover the target volatile components from water-based perfumes with good figures of merits (accuracy, precision, enrichment factor, recovery, linearity). Direct analysis of the enriched NADES is possible not only via GC-FID but also via GC-MS. The solvent components must be chosen to avoid interference with the elution of the target analytes since MS acquisition has to be interrupted during HBA and HBD elution.

Conclusions

Natural resources have recently become very important in terms of sustainable growth and development. This study shows that EOs are a valuable source of phytochemicals that can also be used to generate green solvents for several applications, including analytical scale extraction.

REFERENCES

[1] Mastellone G., Marengo A., Sgorbini B., Rubiolo P., Cagliero C. (2021) New phases for analytical scale extraction from plants: Current and future trends. TrAC Trends in Analytical Chemistry 141, 116288

[2] Bechis G., Mastellone G., Marengo A., Sgorbini B., Cagliero C.;,Rubiolo P. (2022) Hydrophobic Natural Eutectic Solvents for the Gas Chromatographic Determination of Suspected Allergens in Fragrances by Dispersive Liquid-Liquid Microextraction. Separations, 9, 318.

IKnife Based on Rapid Evaporative Ionization Mass Spectrometry Technology for the Safeguard of Saffron Quality

Francesca Rigano[1*], Cinzia Cafarella[1], Domenica Mangraviti[1], Carmela Sottile[1], Paola Dugo[1,2], Luigi Mondello[1,2]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont SRL, c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: frigano@unime.it

Keywords: saffron, food authenticity, ambient mass spectrometry, REIMS, chemometrics

Objective

Saffron is the spice represented by the dried stigmas of the *Crocus sativus* flower, a plant of the Iridaceae family. In recent years, the authenticity and traceability of saffron are topics of great interest, being the most expensive spice in the world due to the high production costs. Nowdays, interest in the spice is growing due to the therapeutic properties ascribed to its main components: crocin, picrocrocin and safranal. Due to its antibacterial and antinflammatory properties, the essential oil of saffron could be used as a natural antioxidant resource in food industry. The high scientific interest, the growing use and the cost explain why the product is largely subject to fraud and adulteration. The present study employed an innovative Ambient Mass Spectrometry approach, such as the iKnife, for the rapid screening of the total metabolome of different saffron samples, coming from various areas of Northern and Southern Marocco, seat of the Slow Food "Saffron of Taliouine" presidium. The goal was, through the total metabolomic analysis of the different samples, to be able to distinguish the geographical origin and highlight any discriminating feautures to protect the Taliouine Slow Food presidium from fraud such as misleading labeling. For this reason, a multivariate analysis was performed, using a dedicated software which allowed the construction of a chemometric model to be used for the real-time differentiation of saffron samples.

Methods

A high resolution tandem mass spectrometry system (Xevo G2-XS QTof from Waters Corporation, Wilmslow, UK), equipped with Rapid evaporative-ionization mass spectrometry (REIMS) source and coupled with an electroknife as a sampling device was used to analyze 15 stigmas of Crocus Sativus, coming from different regions of Northern and Souther Marocco (including the Slow Food Presidium of Taliouline). The multivariate statistical software LiveID^{TM} (Waters Corporation, Wilmslow, UK) was used as a model builder and recognition tool. Lastly, in order to validate the iKnife technique, a conventional analysis by HPLC-PDA/MS was performed.

Results

The analysis of the stigmas highlighted the presence of numerous compounds belonging to different chemical classes, such as phenolic acid and carotenoids. The most intense ions correspond to the picocrocin, picocrocin derivatives and crocetin. No qualitative differences were registered among the samples, for this reason chemometric analysis was required to enable rapid and automatic differentiation. HPLC-PDA/MS analysis confirmed the results obtained with the iKnife technique, resulting more appropriate for a quantitative analysis. However, through the iKnife technique it was possible to identify a higher number of compounds, probably due to the absence of a sample preparation procedure that can alter the native profile of the matrix itself.

Conclusions

The results achieved were very promising in the discrimination of different samples of saffron. The combination of an "ambient" MS technique with chemometrics represented a powerful tool for checking the authenticity and traceability of saffron in its native form.

Volatile Compositions of Three Different Commercial Herbal Teas and Statistical Analyses

Damla Kirci[1*], Betül Demirci[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Selçuk University, Konya, TURKEY
[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, TURKEY

* Email: damla.kirci@selcuk.edu.tr

Keywords: Herbal tea, SPME, volatile compound, infusion

Objective

Herbal teas vary widely in their composition; they are made from natural products, mainly a variety of herbs, and have various benefits for human health. Plant materials used in herbal teas include fresh or dried roots, stems, leaves, fruits, flowers, seeds, bark, or whole plants from one or more herbal tea plant species. Most herbal tea infusions use edible medicinal plants as raw materials and are consumed daily.

In recent years, the discovery of herbal tea's rich biological activities and health benefits has become a driving force for researchers interested in its development as a functional food [1,2].

Methods

Within the scope of this research, three different commercial herbal tea (Ht1, Ht2 and Ht3) from the Swedish market were purchased, and infusions were prepared at 10 min. Volatile compounds of the infusions were analysed by headspace-solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). Principal Component Analyses (PCA) and Hierarchical Cluster Analysis (HCA) were performed, utilizing major components of three herbal tea. Also, a Venn diagram was used to demonstrate chemical variations of the volatile compounds.

Results

Linalyl acetate (18.7%) and 3,4-dimethyl-5-pentyliden-2(5H)-furanone (10.4%) were found as the main constituent of the Ht1. It was included in black tea, citrus flowers, and cornflower petals. The Ht2 was included in black tea, black currant, and rose petal and was characterized as *α*-terpineol (23.8%), methyl salicylate (10.5%), and linalyl acetate (9.7%). The main components of the Ht3 were 3,4-dimethyl-5-pentyliden-2(5H)-furanone (12.4%), *trans*-*β*-ionon-5,6-epoxide (9.8%), neryl acetate (7.3%) and linalyl acetate (7.0%). The Ht3 is contained black tea, lemongrass, rose and cornflower petals.

Conclusions

a Venn diagram was used to determine any difference in the presence of the identified components in the infusions. According to the results, thirty-three compounds were found in common in the three infusions. Also, the only plant common to all three infusions was black tea. HCA analysis of main components was revealed two primary clades. The similarity level of Ht1 and Ht2 was found as 33.85%.

REFERENCES

[1] Liu, Y., Guo, C., Zang, E., Shi, R., Liu, Q., Zhang, M., ... & Li, M. (2023). Review on herbal tea as a functional food: classification, active compounds, biological activity, and industrial status. Journal of Future Foods, 3(3), 206-219.
[2] Liu, Y., Ahmed, S., & Long, C. (2013). Ethnobotanical survey of cooling herbal drinks from southern China. Journal of ethnobiology and ethnomedicine, 9, 1-8.

Characterization of the volatile profile of essential oil samples distilled from drug type *Cannabis Sativa L.* flowering tops

Giuseppe Micalizzi[1, *], Valentina Chiaia[1], Filippo Alibrando[1], Pietro Maida[2], Luigi Mondello[1,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Arma dei Carabinieri, Department of Scientific Investigations (RIS), Messina, ITALY
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: giumicalizzi@unime.it

Keywords: *Cannabis Sativa L.*, gas chromatography, cannabis essential oils.

Objective

The main aim of this research is the characterization of the volatile fraction in distilled narcotic *Cannabis Sativa L.* essential oils by using gas chromatography techniques. The research project was performed in collaboration with the Scientific Investigations Department, RIS Carabinieri of Messina, that provided the oils extracted from the seized psychoactive Cannabis flowering tops. All samples were distilled by using a microwave-assisted hydro distillation (MAHD) system. The essential oils were characterized by using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) instrumentations. Quali- and quantitative data were processed in order to characterize the volatile substances fingerprint in narcotic *Cannabis Sativa L.* essential oils. Several compounds showed similar trend in all analyzed samples indicating the phenotypic tract of this plants.

Methods

Cannabis Sativa L. narcotic inflorescence samples were obtained by the Carabinieri – Department of Scientific Investigations, RIS Carabinieri of Messina. The essential oils were obtained through the microwave assisted hydro-distillation (MAHD) method. The characterization of the volatile fraction was carried out by using GC-MS and GC-FID analyses. Two different internal standards were used for quantitative purpose (concentration levels expressed in mg g^{-1})

Results

A total of 107 compounds including monoterpenes, sesquiterpenes, and oxygenated derivatives were identified in the samples analysed, representing about 90% of the whole volatile fraction. In order to visualize the difference between the analysed Cannabis essential oils, the absolute concentrations (mg g^{-1}) were inserted within a diagram defined "heatmap". The graph highlighted that α-pinene, β-pinene, myrcene, limonene, linalool, fenchyl alcohol, and α-terpineol were the most abundant compounds of the monoterpene fraction, while the (E)-caryophyllene, α-humulene, α-,(E)-bergamotene, and selinene derivatives including β-selinene, α-selinene, selina-4(15),7(11)-diene, and selina-3,7(11)-diene were prevalent in the sesquiterpene fraction.

Conclusions

This research showed that MAHD process was able to distillate the *Cannabis Sativa L.* narcotic inflorescences, while the GC-MS and GC-FID analyses showed the terpene fingerprint of the distilled essential oils. Several compounds showed similar trends in all analyzed samples indicating the phenotypic tract of this plants.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corporation and Merck Life Science for their continuous support.

Evaluation of Arabica and Robusta essential oils on the USA market

Rima Juskelis[1*], Mark Krzeszowiec[1], Jonathan DeCenzi[1]

[1] NOW Foods, Bloomingdale, IL 60108, USA

* Email: rima.juskelis@nowfoods.com

Keywords: coffee essential oil, chemical composition, fatty acids, GC-FID/MS, Q Exactive

Objective

Coffee essential oil is a relatively new product to consumers, but it is quickly gaining popularity due to its pleasant aroma. Coffee essential oil is derived from coffee seeds coming from two different species: *Coffea arabica* (Arabica) and *C. canephora* (Robusta). The aim of this study was to analyze the composition and evaluate the quality of the coffee essential oils available on the US market.

Methods

Fourteen samples of commercial Robusta and Arabica coffee essential oils were obtained from local and online stores. These were labeled to be either cold press, supercritical CO2, or steam distillation extracted. Four whole roasted coffee samples were purchased to establish identification markers for the two species. The beans were ground, and hexane extracted for the determination of the fatty acid profile. Methanol extracts of the extracted oil were used for the determination of the marker compounds of Arabica and Robusta coffee using high resolution accurate-mass (HRAM) LC-MS (Thermo QExactive, Waters T3 2.7µm 2.1 x 100mm) and Compound Discoverer software was used for data analysis.

The fatty acid profile was determined by saponifying oil triglycerides with sodium hydroxide and derivatizing fatty acids with boron trifluoride in methanol. The hexane phase (1µL) was then injected into the gas chromatograph with flame ionization detection (Agilent 7890 GC-FID, Restek Rt-2560, 100m-0.25mm-0.2µm).

Some of the oils that did not contain fatty acids were analyzed neat (0.25 µL) by gas chromatography-mass spectrometry system (Agilent 7890 GC / 5977 MS). The volatile components were separated on a non-polar column (Agilent DB-5MS, 30m- 0.25mm-0.25µm), and identified by comparing mass spectra with those available in databases (NIST and Wiley).

Results

The chemical composition of coffee beans depends on various factors, such as species, geographic origin, soil conditions, storage, time, and roasting temperature. However, the fatty acid compositions of essential oil samples were very similar for all samples with no significant difference between oils obtained with different extraction methods. This research reports a comprehensive characterization of the fatty acid composition profile of coffee oil: linoleic (40-50%), palmitic (30-40%), oleic (5-12%) and stearic (4-8%).

In this study, a LC-Orbitrap MS qualitative method was established to distinguish Arabica and Robusta coffee oil samples. Multivariate statistical methods were employed to find potential classification markers (m/z 297.18473 and m/z 271.20612) with the highest contributions to the observed chemical differences between the coffee species.

Of the coffee essential oil samples analyzed, 21% were formulated fragrances. The main component of these oils was solvent - diethyl phthalate (66-74%), followed by antioxidants such as butylated hydroxytoluene (3%), and fragrances such as benzyl benzoate (13-14%), ethyl vanillin (14%), vanillin (4-5%), 4-methoxy-benzaldehyde (1.5%), ethyl maltol (0.4%), benzaldehyde (0.3%), and phenethyl alcohol (0.2%). There was no confirmation of steam distilled coffee essential oil among the purchased samples.

Conclusion

The fatty acid profile composition test is not specific enough for species level identification. The identification of the marker compounds is necessary for the complete identification and confirmation of authenticity of the Arabica and Robusta essential oils. However, 21% of the samples analyzed were adulterated, highlighting the need for quality control and regulation in the production of coffee essential oil. Further research is needed to identify the marker components for Arabica and Robusta coffee essential oils.

Geographic variation and diversity of aroma characteristics of *Alpinia zerumbet*

Eisuke Kuraya[1,*], Akiko Touyama[2]

[1] Science and Technology Division, National Institute of Technology, Okinawa College, Nago, JAPAN
[2] Research and Development, Okinawa Flora Co. Ltd., Nago, JAPAN

* Email: kuraya@okinawa-ct.ac.jp

Keywords: *Alpinia zerumbet*, aroma characteristic, geographic variation

Objective
The aromatic *Alpinia zerumbet* (Pers.) Burtt & Smith (Zingiberaceae) is widely distributed in tropical and subtropical regions of the world, including India, Malaysia, Taiwan, Brazil, and Japan. In Japan, it is found in several regions, ranging from southern Kyushu to the Amami Islands, Okinawa Islands, Daito Islands, and Sakishima Islands. Its flowers, leaves, and rhizomes have a characteristic fragrance. The essential oil extracted from the leaves is used for fragrances and cosmetics due to its pleasant scent and strong antioxidant effect. In recent years, five species of *Alpinia* have been reported to be reticulate-hybridized within the genus in Taiwan [1]. Previous studies have revealed that the fragrant properties of the leaves and essential oil of *A. zerumbet* growing in the Ryukyu Islands are diverse regardless of the island or region. The essential oil obtained from its leaves has a wide variety of fragrant properties [2]. Since *A. zerumbet* is a subtropical perennial, it is challenging to cultivate it in regions north of Kagoshima Prefecture. However, there have been reports of cases of cultivation in greenhouses and overwintering measures leading to flowering even in the Main Island of Japan. In this study, in addition to the aromatic characteristics of *A. zerumbet* grown in the Ryukyu Islands analyzed so far, we newly investigated the geographical variation and diversity in chemical composition among the *A. zerumbet* collected from the three Ryukyu Islands and Tokushima. *A. zerumbet* leaves grown in Tokushima were collected in June 2022 from an individual plant grown in the Herb Garden of Tokushima Bunri University. For the Ryukyu Islands' *A. zerumbet* leaves used for comparison, aroma data from 199 plants from 21 islands in the central and southern Ryukyu Islands were used between May 2017 and March 2023.

Methods
After collection, the leaves and stems were separated, cut, and dried at 45 °C until the moisture content was 10% or less; 0.2 g of each leaf and stem was taken in a 27 mL vial, heated at 60 °C for 10 min. Air was poured through activated carbon at 100 mL/min for 10 min to collect aroma in TENAX TA-filled glass tubes. The dynamic headspace method and thermal desorption-gas chromatography–mass spectrometry were used to analyze the collected aroma components. The identified aroma components were subjected to principal component analysis (PCA) and hierarchical cluster analysis (HCA) using SIMCA (ver. 13.01; UMETRICS) to determine the chemical composition of the fragrance of the leaves of the *A. zerumbet*.

Results
The major aroma components of *A. zerumbet* leaves are α-pinene, camphene, limonene, β-phellandrene, 1,8-cineole, *p*-cymene, camphor, linalool, methyl cinnamate, among others. The composition of aroma constituents in the Tokushima *A. zerumbet* leaf was low in α-pinene, *p*-cymene, 1,8-cineole, cryptone, and methyl cinnamate, and high in camphor, myrcene, linalool, borneol, α-terpineol, humulene, and humulene epoxide II. PCA and HCA were conducted with specimen 199 from the Ryukyu Islands. It was found that this specimen had the same aroma characteristics as the specimen growing in the Ryukyu Islands, even though the soil and climatic conditions in Okinawa are very different from those in the Ryukyu Islands.

Conclusions
Although the details of how the plant variety was introduced to Tokushima are unknown, the characteristic aroma composition of the newly obtained *A. zerumbet* leaves was attributed to the same group, distributed only on three islands near Iheya Island. These results suggest that the aromatic characteristics of *A. zerumbet* depend more on the plant specimen than on climatic or geographical variation.

REFERENCES

[1] Liu S., Lu C., Wang J., (2009). Reticulate hybridization of Alpinia (Zingiberaceae) in Taiwan, J. Plant Res. 122, 305–316.
[2] Kuraya E., Yamashiro R., Touyama A., Nakada S., Watanabe K., Iguchi A., Itoh S., (2017). Aroma Profile and Antioxidant Activity of Essential Oil from Alpinia zerumbet, Nat. Prod. Commun. 12, 1321–1325.

Aroma characteristics of Okinawa finger lime (*Citrus australasica*)

Akiko Touyama[1,*], Eisuke Kuraya[2]

[1] Research and Development, Okinawa Flora Co. Ltd., Okinawa Nago, JAPAN
[2] Science and Technology Division, National Institute of Technology, Okinawa College, Okinawa Nago, JAPAN

* Email: touyama@flora.okinawa

Keywords: *Citrus australasica*, finger lime, four variety, aroma characteristic

Objective
Finger lime (*Citrus australasica)* is a citrus fruit native to Australia, with more than 250 varieties. It produces fruits of various shapes and colors. The cut fruit is noted for its unique texture, its juice-vesicle structure that emerges from the inside when pinched, and its distinctive high aroma. The fresh aroma spreads in the mouth only after the juice vesicles of lime are crushed, which is a topic of discourse at high-end restaurants, where it is sold at a high price. Twenty varieties were introduced to Okinawa six years ago and are now under full-scale cultivation and production in the northern region of Okinawa Island. In recent years, finger limes have been actively cultivated in many countries worldwide, including the United States, Italy, and Thailand, and their aromatic characteristics have been reported [1,2]; however, to the best of our knowledge, there have been no studies on the aromatic characteristics of finger limes cultivated and harvested in Japan. This study reports the aroma characteristics of finger lime fruit peels grown and harvested from four Okinawa finger-lime cultivars and different rootstocks. Depending on their variety, finger limes have different peel colors (yellow, red, and green) and juice vesicle colors (clear, yellow, pink, red, and green). This study analyzed the aroma characteristics of three varieties with red peels (Wauchope, Pink Ice, and Red Ruby) and one variety with green peel. Green varieties were grafted onto *Poncirus trifollata* and *Citrus depressa* rootstocks, and fruits harvested from seedlings grown for three years were analyzed.

Methods
To analyze the aroma components, juice vesicle of finger lime was removed, and the outer peel was cut into 5 mm pieces and placed in a vial. Next, 2 mL of *n*-hexane was added and sonicated for 10 min to extract the aroma components from the peel, which were then analyzed by gas chromatography–mass spectrometry（GC–MS).

Results
Principal component analysis (PCA) was performed using SIMCA (ver. 13.01; UMETRICS) to analyze the aroma characteristics of fruits from different rootstocks. The GC–MS analysis revealed that the major aroma components of finger lime varied widely among the cultivars. In both varieties, limonene (70.57%–91.00%) was found to be the major component, as is the case in common citrus fruits, but its content varied among the varieties. The Wauchope variety contained γ-terpinene (11.29%), α-citronellol (2.46%), β-pinene (1.73%), and α-pinene (1.24%). In contrast, the Pink Ice variety contained β-phellandrene (4.55%), menthone (3.27%), menthol (1.77%), germacrene B (0.73%), and other components. Depending on the variety, there were apparent differences in their sensory effects, owing to the presence or absence of specific components and their quantities. In particular, Pink Ice contains menthone and menthol and has a minor aroma in addition to a lime-like flavor. The variety Red Ruby also contained *cis*-β-ocimene (1.64%), α-citronellol (3.80%), and bicyclogermacrene (2.82%), and had a lime-like and floral aroma. The fruits of the green varieties contained bicyclogermacrene (5.15%–6.03%), γ-terpinene (1.82%-3.17%), and myrcene (1.47%–1.95%) and wielded a green aroma in addition to a lime-like flavor.

Conclusions
Fruits from the *C. depressa* rootstock showed an increase in the number of components contributing to the green aroma, such as hexane-3-one, 2-hexanone, *cis*-3-hexanal, *cis*-3-hexenyl acetate, and *cis*-3-hexen-1-ol. In contrast, the contents of terpenoids, such as α-thujene, α-terpinene, β-phellandrene, γ-terpinene, and humulene, decreased or were absent, suggesting that the aroma characteristics were significantly affected by the rootstock. These results were clearly different from the PCA results, which revealed differences in the aroma characteristics of finger lime depending on the rootstock.

REFERENCES
[1] D'Auria M., Racioppi R., (2022), Volatile organic compounds from Citrus australasica growing in Basilicata (Southern Italy), Nat. Prod. Res. 25, 1-4. doi: 10.1080/14786419.2022.2063857
[2] Delort E., Jaquier A., Decorzant E., Chapuis C., Casilli A., Frérot E., (2015), Comparative analysis of three Australian finger lime (Citrus australasica) cultivars: Identification of unique citrus chemotypes and new volatile molecules, Phytochem. 109, 111–124. doi: 10.1016/j.phytochem.2014.10.023.

Phytotoxic potential of five essential oils to control *Chenopodium album* L. germination.

Nieves Melero-Carnero[1,*], Natalia Torres-Pagán[1], David López-González[2], Amparo Blázquez[3], Herminio Boira[1], Adela Sánchez-Moreiras[2], Mercedes Verdeguer[1]

[1] Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, SPAIN
[2] Departamento de Bioloxía Vegetal e Ciencias do Solo, Universidade de Vigo, Vigo, SPAIN
[3] Departament de Farmacologia, Facultat de Farmàcia, Universitat de València, Valencia, SPAIN

* Email: niemecar@upv.es

Keywords: essential oil, natural product, weed control, Chenopodium album, bioherbicide

Objective

Intensifying crop systems to supply food to global population has led to an over-reliance on herbicides to control weeds, which has resulted in the development of weed-resistant biotypes [1]. In recent years, it has been an increasing interest in the use of essential oils (EO) as herbicides for its allelopathic properties, which can be used to develop multi-target site activity herbicides [2]. *Chenopodium album* L. (Amaranthaceae) is a problematic weed in a wide range of crops, completely diminishing productivity when left uncontrolled [3]. This study aimed to determine the phytotoxic potential of the EOs of *Lantana camara* L., *Eucalyptus camaldulensis* Dehnh., *Eriocephalus africanus* L., *Cistus ladanifer* L. and *Seriphidium caerulescens* subsp. *gallicum* (Willd.) Soják against *C. album* germination. Aerial parts of *L. camara*, *E. camaldulensis* and *E. africanus* were collected from plants growing in landscaped areas in Valencia city, *S. caerulescens* and *C. ladanifer* aerial parts were collected from wild plants growing in their natural ecosystems in Torreblanca (Castellón province) and San Lorenzo del Escorial (Madrid province), respectively, all located in Spain. EOs were extracted from fresh leaves through hydrodistillation using a Clevenger apparatus.

Methods

Twenty seeds of *Chenopodium album* L. were sowed in 9 cm diameter *Petri* dishes between four disks of filter paper 50g/m^2. Five doses were tested for each EO: 0 (control), 0.125, 0.25, 0.5 and 1 µL/mL in distilled water and 4 mL of the corresponding treatment were applied in each Petri. Five repetitions were performed per each treatment. The dishes were sealed with Parafilm and incubated in a germination chamber, set at 30.0±0.1ºC 16h light and 20.0±0.1ºC 8h darkness. Dishes were monitored, checking germination after 3, 5, 7, 10 and 14 days.

Results

The germination results of *C. album* are shown in Table 1. Only *E. camaldulensis* EO was capable to reduce up to 57% *C. album* germination at the highest dose applied. On the contrary, at the two lowest doses tested it showed a stimulatory effect on *C. album* germination. Also *E. africanus* EO displayed a stimulatory effect on *C. album* germination at all doses applied. The other treatments did not show significant differences with control, although a slightly increasing trend in *C. album* germination was observed for *L. camara* EO.

Table 1. Germination of *C. album* (%) treated with EOs after 14 days post sowing.

Dose (µL/mL)	*L. camara*	*E. camaldulensis*	*E. africanus*	*C. ladanifer*	*S. caerulescens* subsp. *gallicum*
Control	35,0 ± 0,0a	35,0 ± 5,2b	35,0 ± 5,2b	42,0 ± 3,2a	42,0 ± 6,0a
0,125	52,0 ± 4,6a	56,0 ± 2,9a	68,0 ± 3,7a	23,0 ± 4,1a	27,0 ± 4,9a
0,25	50,0 ± 7,7a	57,0 ± 4,6a	60,0 ± 5,7a	27,0 ± 2,5a	36,0 ± 3,3a
0,5	56,0 ± 5,6a	39,0 ± 8,7b	63,0 ± 3,7a	25,3 ± 8,9a	28,8 ± 5,5a
1	47,0 ± 5,8a	15,0 ± 4,5c	55,0 ± 5,7a	33,0 ± 7,5a	26,0 ± 4,0a

Conclusions

The EOs tested did not showed a great potential to control *C. album* germination at the doses tested, only *E. camaldulensis* EO was able to reduce *C. album* germination: However, *C. ladanifer* and *S. caerulescens* subsp. *gallicum* EO's decreased it but with no significant effects. To study furthermore the herbicidal activity of these EOs on *C. album*, doses tested should be higher than 1 µL/mL. *E. africanus* EO and *L. camara* would be discarded as suitable natural herbicides to control *C. album.*

REFERENCES

[1] Chauhan B. S. (2020). Grand challenges in weed management. *Frontiers in Agronomy*, 1, 3.
[2] Verdeguer M., Sánchez-Moreiras A. M., Araniti F. (2020). Phytotoxic effects and mechanism of action of essential oils and terpenoids. *Plants*, 9(11), 1571.
[3] Bajwa A.A., Zulfiqar U., Sadia S., Bhowmik P., Chauhan B. S. (2019). A global perspective on the biology, impact and management of *Chenopodium album* and *Chenopodium murale*: two troublesome agricultural and environmental weeds. *Environmental Science and Pollution Research*, 26, 5357–5371

The Chemical Composition of the Essential Oil of *Asterothamnus molliusculus* Novopokr from the Mongolian Eastern-South Gobi

IN MEMORY OF OUR DEAREST FRIEND PROFESSOR JAN KARLSEN

Shatar Sanduin[1], Altantsetseg Shataryn[1], Nicolas Baldovini[2, *]

[1] Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences (MAS) 211051, Ulaan-Baatar, MONGOLIA.
[2] Institut de Chimie de Nice, UMR 7272, Université Côte d'Azur, Parc Valrose 06108 Nice, FRANCE.

* Email: baldovin@unice.fr

Keywords: *Asterothamnus molliusculus* Novopokr, Mongolia, Gobi.

Asterothamnus molliusculus Novopokr (Asteraceae) is restricted to Mongolian Eastern-South Gobi [1]. This endemic and relict plant is a very rare essential oil bearing plant, which grows only in red mud and sand of dried ocean from Kainozoic-Era (third period of geochronological scale).

The objective of this work was to investigate the chemical composition of the essential oil of this species. The plant material was collected in full grown flower stage between July-August from the experimental field Eastern-South Gobi of Mongolia. The aerial parts of the plant were submitted to hydrodistillation in Clevenger type apparatus, following classical procedure [2, 3] to yield essential oils which were further analyzed by GC-MS and GC-FID, using R. P. Adams MS database [3]. Significant differences in chemical compositions were observed between the different samples of *Asterothamnus molliusculus*. The total amount of identified compounds accounted for 99.4% of the total composition. The most abundant components of the herb essential of *Asterothamnus molliusculus* were camphor (32.9%), β-pinene (13.1%), sabinene (9.8%), 1,8-cineole (9.2%), limonene (5.8%), α-pinene (4.9%), geranyl acetate (4.6%), spathulenol (4.1%), bicyclogermacrene (3.6%) and myrcene (3.5%).

REFERENCES

[1] Grubov V. I., (1982). Key to the Vascular plants of Mongolia, Nauka, Leningrad.
[2] Adams R. P., (1991). Cedar Wood Oil - Analysis and Properties, in Modern Methods of Plants Analysis: Oils and waxes. Springer Verlag, Berlin.
[3] Adams R. P. (1995). Identification of Essential oil components by Gas chromatography/mass spectroscopy. Allured publishing, Carol Stream, Illinois.

The Chemical Composition of the Essential Oil of *Carduus crispus* L. from Mongolia

IN MEMORY OF OUR DEAREST FRIEND PROFESSOR JAN KARLSEN

Shatar Sanduin[1], Altantsetseg Shataryn[1], Nicolas Baldovini[2,*]

[1]Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences (MAS) 211051, Ulaan-Baatar, MONGOLIA.
[2] Institut de Chimie de Nice, UMR 7272, Université Côte d'Azur, Parc Valrose 06108 Nice, FRANCE.

* Email: baldovin@unice.fr

Keywords: *Carduus crispus* L., Mongolia, Middle Gobi.

Carduus crispus L. (Asteraceae) is native to Southeast Siberia and Mongolia where it grows in meadows and wetlands [1] and is wildly utilized in Mongolian traditional medicine [2, 3].
The aim of this paper was investigate the chemical composition of the essential oil of *Carduus crispus* L. from Mongolia. The plant material was collected from the Middle-Gobi region of Mongolian Steppe-Gobi, in full flower stage between August-September 2016 in the experimental field "Middle Gobi" of Mongolia.
Leaves and flowers of *Carduus crispum* L. were subjected separately to hydrodistillation in a Clevenger type apparatus to yield essential oils which were further analyzed by GC-MS and GC-FID. Significant differences were observed in the chemical compositions of these essential oil. Herbs oil were characterized by the presence of the following constituents (typical percentage): α-pinene-(1.41%), camphene (0.11%), α-terpinene (0.10%), sabinene (0.11%), cymene (0.81%), limonene (0.92%), β-pinene (0.45%), γ-terpinene (0.41%), terpinolene (0.40%), terpinen-4-ol (0.32%), methylchavicol (2.58%), methyleugenol (21.45%), β-farnesene (0.31%), γ-decalactone (0.87%), methylvanillin (0.41%), elemicin (12.42%), (*E*)-nerolidol (53.0%), spathulenol (6.12%), caryophyllene oxide (1.22%), α-bisabolol (2.09%) as well as traces of linalool and α-terpineol.

REFERENCES

[1] Grubov V. I., (1982). Key to the Vascular plants of Mongolia, Nauka, Leningrad.
[2] Markova L. P., Belinovskaya L. M., (1985). Wild growing useful plants of Mongolia, Nauka, Leningrad.
[3] Blinova K. F., Kuvaev V. B., (1961) Medicinal Plants for Tibetan Medicine of Baykal, in "To question Pharmacognosy" pp. 135-136.

The Chemical Composition of the Essential Oil of leaves, stems, fruits and roots of the *Peucedanum hystrix* Bge. from the Mongolian Steppe-Gobi

IN MEMORY OF OUR DEAREST FRIEND PROFESSOR JAN KARLSEN

Shatar Sanduin[1], Altantsetseg Shataryn[1], Nicolas Baldovini[2,*]

[1] Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences (MAS) 211051, Ulaan-Baatar, MONGOLIA.
[2] Institut de Chimie de Nice, UMR 7272, Université Côte d'Azur, Parc Valrose 06108 Nice, FRANCE.

* Email: baldovin@unice.fr

Keywords: *Peucedanum hystrix* Bge (Asteraceae); Mongolia, Steppe-Gobi.

Peucedanum hystrix Bge (Asteraceae) is native to Southeast Siberia and Mongolia [1, 2] and is widely used in Mongolian traditional medicine. This species contains in its roots coumarins which possess antiswelling properties [3] and sesquiterpenic lactons with high tuberculostatic and fungastatic activity [4].
This study investigates the chemical diversity of *Peucedanum hystrix* Bge essential oils and the quantitative changes of its main constituents according to the plant organs considered. The plant materials (leaves, stems, fruits and roots) were collected in Mongolian Steppe-Gobi and subjected separately to hydrodistillation in Clevenger type apparatus following classical procedure [5] to yield essential oils which were further analyzed by GC-MS and GC-FID. These analyses revealed significant differences in chemical compositions of the essential oils of different organs.
Eudesma 3.7(11)-dien-8-one was detected in all essential oils at content ranging from 8.54% to 27.86%. The leaf oil was characterized by the presence of α-pinene (6.28%), sabinene (8.76%), myrcene (5.59%), β-phellandrene (6.84%) and eudesma-3.7(11)-dien-8-one (8.65%).
The essential oil of steams contained α-pinene (5.15%), sabinene (7.47%), β-selinene (1.22%), germacren-B (2.20%), caryophyllene oxide (1.30%), selina-4(15)7(11)-diene (3.23%), selina-3.7(11)-diene (5.35%), spathulenol (3.24%), α-bisabolol (1.67%), eudesma-3.7(11)-dien-8-one (14.32%), costol (4.72%).
The essential oil of fruits was characterized by the presence of α-pinene (2.63%), sabinene (17.81%), β-pinene (1.24%), myrcene (7.46%), β-phellandrene (8.44%), β-selinene (1.42%), germacrene-B (1.73%), caryophyllene oxide (1.22%), selina-4(15)7(11)-diene (2.33%), selina-3.7(11)-diene (4.38%), spathulenol (1.21%), α-bisabolol (1.43%), eudesma-3.7(11)-dien-8-one (10.24%) and costol (1.16%).
The essential oil of roots contained α-pinene (8.11%), β-selinene (3.72%), α-selinene (1.09%), selina-4(15)7(11)-diene (2.23%), selina-3.7(11)-diene (1.96%), spathulenol (3.16%), eudesma-3.7(11)-dien-8-one (27.86%) and costol (1.55%).

REFERENCES

[1] Grubov V. I., (1982). Key to the Vascular plants of Mongolia, Nauka, Leningrad.
[2] Markova L. P., (1985). Wild growing useful plants of Mongolia, Nauka, Leningrad.
[3] Tsetlin A. L et al. (1965). To question on antiswelling (tumour) activity the coumarins. Journal of Vegetation Resource 1, 507-511.
[4] Vithkanova C. A., Adgina B. B., Rubenthyk M. A., (1977). Antibacterial and Antilingual property Natural lactones. Journal of Vegetation Resource 18, 428-435.
[5] Adams R. P., (1991). Cedar Wood Oil - Analysis and Properties, in Modern Methods of Plants Analysis: Oils and waxes. Springer Verlag, Berlin.
[6] Adams R. P. (1995). Identification of Essential oil components by Gas chromatography/mass spectroscopy. Allured publishing, Carol Stream, Illinois.

Changes in accumulation and spectrum of volatiles in peppermint as result of elicitation

Zámboriné Németh, É.*, Kandoudi, W., Tavaszi-Sárosi, Sz.

[1] Department of Medicinal and Aromatic Plants, Hungarian University of Agricultural and Life Sciences, Budapest, H

* Email: zamborine.nemeth.eva@uni-mate.hu

Keywords: *Mentha piperita*, methyl jasmonate, essential oil, menthol, menthon

Objective

Jasmonates are small molecule plant hormones, involved in many physiological processes of plants and defense responses. Their application for stimulating the formation of secondary metabolites *in vitro* bireactors has been introduced, however, *in vivo* application is still a challange [1]. Therefore, the goal of our study is to reveal the potential effects of *in vivo* treatment with MeJa on peppermint. In this presentation we are focusing and summarizing the results of elicitation on its volatile compounds.

Methods

Two open field (randpomized block design in 3 replicates) experiments and two other ones in climatic chamber (10 plant individuals/treatment) were conducted in 2020-21 with peppermint to explore the effect of methyl jasmonate (MeJa) on the essential oil (EO) content and composition (except the first phytotron trial, where only EO content was determined). 2mM MeJa (dissolved in water, mixed with 0.3% ethanol) was applied by spraying the aerial parts of the plants twice (one and two weeks before harvest).

20 g of dried leaves were dydro-distilled in three replicates in a Clevenger-type apparatus filled with 500 mL of water for 1.5h. and expressed as mL/100 g of dry matter. The compositional analysis was carried out by GC–MS: an Agilent Technologies 6890N instrument equipped with HP–5MS capillary column and an Agilent Technologies MS 5975 inert mass selective detector. 10 µL of EO was diluted with n-hexane to 1 mL and from this, the injected quantity was 0.2 µL. Further parameters and temperature program was as described earlier [2]. Identification of compounds: linear retention indices, mass spectral libraries (NIST MS Search 2.0 library, Wiley 275) and a mass spectra library [3].

Results

The EO accumulation was enhanced by the MeJa treatment in all experiments, except the first open field trial. In the first and second phytotron experiments, the increase was 19% and 9% respectively, while in the second open field trial it reached 35%.

In the phytotron experiment the MeJa increased the proportions of both of the two major components menthone and menthol by appr. 16%. On the other hand, menthofuran, its precursor pulegone and isomenthyl-acetate were reduced significantly. In the first open field experiment, the elicitation had no significant effects on the ratio of the evaluated major components, while in the second one remarkable changes were determined. Similarly, to the phytotron, menthone was elevated while pulegone and isomenthyl-acetate were reduced by 50% and 48%, respectively. However, the menthol content decreased, too (by 13%). Significantly increased ratios were found also for limonene and 1.8-cineole in these samples.

Conclusions

We concluded, that MeJa treatments showed promising effects in influencing the accumulation of biologically active compounds and the quality of the peppermint drugs. Our results indicate however, that the effects may also be influenced by the phenological and developmental stages of the plant, therefore further systematic studies are needed to optimize the technology *in vivo*.

REFERENCES

[1] Kandoudi, W., Zámboriné Németh, É. (2022): Stimulating secondary compound accumulation by elicitation – is it a realistic tool in medicinal plants *in vivo*? Phytochemistry Reviews vol. 21, p.2007–2025

[2] Kandoudi, W.; Radácsi, P.; Gosztola, B.; Németh, É.Z. (2021): Elicitation of Medicinal Plants In Vivo— Is It a Realistic Tool? The Effect of Methyl Jasmonate and Salicylic Acid on Lamiaceae Species. *Horticulturae* 2021, *8*, *5*.

[3] Adams RP. (2007). Identification of essential oil components by gas chromatography / mass spectrometry. 4th ed. (vol 456), Allured publishing corporation, Illinois

Role of volatile compounds on the specificity of Portuguese honeys

Alexandra M. Machado[1,*], Maria Graça Miguel[2], Miguel Vilas-Boas[3,4], Ana Cristina Figueiredo[1]

[1] Centro de Estudos do Ambiente e do Mar (CESAM Ciências), Faculdade de Ciências da Universidade de Lisboa (FCUL), Biotecnologia Vegetal, DBV, C2, Campo Grande, 1749-016 Lisboa, PORTUGAL
[2] Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento (MED), Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, PORTUGAL
[3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, PORTUGAL
[4] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, PORTUGAL

* Email: ampmachado@fc.ul.pt

Keywords: Monofloral honeys, volatile compounds, distinctive aromas, Portuguese honeys

Objective

Honeys have distinctive organoleptic characteristics variable between honey types. Monofloral honeys typical aromas and flavours constitute unique patterns, related with the presence of volatiles responsible for honey´s fragrance features [1]. The mainland Portugal and Azores and Madeira Islands are characterised by a rich and varied honey flora, contributing to the production of a great diversity of local monofloral honeys [2]. This study aimed to find the volatile compound´s specificity of selected Portuguese monofloral honeys by comparison with the volatiles of monofloral honeys with different geographical origins.

Methods

Honey volatiles were isolated from 51 honey samples of 12 monofloral-labelled Portuguese honey types, by solid-phase microextraction (SPME) and hydrodistillation (HD). The volatiles were analysed by Gas Chromatography (GC) and GC coupled to Mass Spectrometry (GC-MS) as in [2]. Honeys volatiles were used in the evaluation of chemical correlation among the samples and compared with the volatiles of the same honey types but produced in different countries [3].

Results

Agglomerative cluster analysis of honey HD volatiles evidenced two main clusters, in which alkanes and fatty acids were dominant, followed by oxygen-containing monoterpenes, and aromatic amino acid derivatives in lower amounts. The comparison between honey volatiles reported in the literature from different geographical origins and Portuguese chestnut, eucalyptus, heather, lavender, orange, rape, raspberry, sunflower and strawberry tree honeys, revealed marker compounds. Aromadendrene, for instance, was characteristic of eucalyptus honey, β-copaene in sunflower honey and veratrole, 2,3,5-trimethylphenol, as well as 3,4,5-trimethylphenol in strawberry tree honey.

Conclusions

The results highlighted the occurrence of specific volatile compounds identified in some Portuguese monofloral honeys, contributing to the knowledge and valorisation of this distinctive product.

ACKNOWLEDGMENTS

Thanks are due to FCT/MCTES for the PhD grant SFRH/BD/117013/2016 to AMM, and to financial support to CESAM UIDB/50017/2020 + UIDP/50017/2020, CIMO UIDB/00690/2020, SusTEC LA/P/0007/2021, CEAUL UIDB/00006/2020, MED UIDB/05183/2020, FEDER, PT2020 PACompete 2020.

REFERENCES

[1] Escriche et al. (2017). Volatile profile in the accurate labelling of monofloral honey. The case of lavender and thyme honey, *Food Chemistry*, 226, 61-68.
[2] Machado et al. (2021). Volatile profile of Portuguese monofloral honeys: significance in botanical origin determination, *Molecules*, 26, 4970.
[3] Machado et al. (2020). Honey volatiles as a fingerprint for botanical origin—A review on their occurrence on monofloral honeys, *Molecules*, 25, 374.

Herbicidal potential of *Eucalyptus camaldulensis* Dehnh. essential oil against Mediterranean weed seeds

Natalia Torres-Pagán[1,3*], Nieves Melero[1], David López-González[2], Adela Sánchez-Moreiras[2], Alessandra Carrubba[3], Mercedes Verdeguer[1]

[1] Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, SPAIN
[2] Departamento de Bioloxía vexetal e ciencias do solo, Universida de Vigo, Vigo, SPAIN
[3] Dipartamento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Palermo, ITALY

* Email: Natorpa@upv.es

Keywords: essential oils, bioherbicides, integrated weed management, *Eucalyptus camaldulensis*, natural products

Objective

The objective of this work was to determine the herbicidal potential of *Eucalyptus camaldulensis* leaves essential oils (EOs) from two Mediterranean locations, Valencia (Spain) and Sparacia (Italy), against the germination of different weeds important in Mediterranean crops.

Methods

Aerial parts from *Eucalyptus camaldulensis* Dehnh. were collected to obtain the EO in October 2007 in Valencia (Spain) and in October 2009 in Sparacia (Italy). Both EOs were obtained by hydrodistillation, with a Clevenguer type apparatus (EO from Valencia) and with an extractor for EOs from Albrigi Luigi (Verona, Italy) (EO from Sparacia). The analysys of the EOs was carried out by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS). For testing the herbicidal potential of the EOs, 20 seeds of each weed species (*Amaranthus hybridus* L., *Portulaca oleracea* L., *Chenopodium album* L., *Erigeron canadensis* L. and *Parietaria judaica* L.) were placed in 9 cm diameter Petri dishes (5 dishes per treatment) and treated with 4 mL of distilled water (control) or with the corresponding doses of EO: 0.125, 0.25, 0.5 and 1µL/mL, respectively. To evaluate seed germination, counts were made 3, 5, 7, 10 and 14 days after treatment. An analysis of variance (ANOVA) was applied to the results obtained, using Fisher's multiple comparison test (LSD, Least Significant Difference) to separate the means, with a confidence level of 95% ($P \leq 0.05$).

Results

The effects of E. camaldulensis EOs from Valencia and Palermo against weed seed germination are showed in Table 1. The EO from Valencia demonstrated higher herbicidal potential than the EO from Sparacia. Most compounds were the same in both EOs, but the content of spathulenol was much higher in the EO from Valencia.

Table 1 Germination of Amaranthus hybridus, Portulaca oleracea, Chenopodium album, Erigeron canadensis and Parietaria judaica seeds treated with Eucalyptus camaldulensis essential oils from Valencia and Sparacia.

Concentration (µL/mL)	Germination (% ± standard error)						
	Eucalyptus camaldulensis EO from Valencia (Spain)					*Eucalyptus camaldulensis* EO from Sparacia (Italy)	
	Amaranthus hybridus*	***Portulaca oleracea****	***Chenopodium album***	***Erigeron canadensis***	***Parietaria judaica***	***Portulaca oleracea***	***Erigeron Canadensis***
0 (control)	87.0 ± 6.4 a	100.0 ± 0.0 a	35.0 ± 5.2 b	78.0 ± 9.4 a	23.0 ± 4.9 a	88.0 ± 2.0 a	64.0 ± 2.4 a
0.125	0.0 ± 0.0 b	0.0 ± 0.0 b	56.0 ± 2.9 a	0.0 ± 0.0 b	0.0 ± 0.0 b	84.0 ± 3.3 ab	60.0 ± 6.3 b
0.250	0.0 ± 0.0 b	0.0 ± 0.0 b	57.0 ± 4.6 a	0.0 ± 0.0 b	0.0 ± 0.0 b	64.0 ± 11.6 b	19.0 ± 5.8 c
0.5	0.0 ± 0.0 b	0.0 ± 0.0 b	39.0 ± 8.7 b	1.0 ± 1.0 b	0.0 ± 0.0 b	35.0 ± 10.2 c	0.0 ± 0.0 b
1	0.0 ± 0.0 b	0.0 ± 0.0 b	15.0 ± 4.5 c	0.0 ± 0.0 b	0.0 ± 0.0 b	35.0 ± 10.2 c	0.0 ± 0.0 b

The results for *Amaranthus hybridus* and *Portulaca oleracea* have been already published [1].

Different letters in the same column indicate significant differences.

Conclusions

Both *E. camaldulensis* EOs inhibited weed seed germination. *E. camaldulensis* EO from Valencia showed an interesting herbicidal potential in all weeds, being *C. album* the most resistant. Spathulenol could be responsible of the higher herbicidal potential of *E. camaldulensis* from Valencia.

REFERENCES

[1] Verdeguer. M., Blázquez. M. A., Boira. H., (2009). Phytotoxic effects of Lantana camara, Eucalyptus camaldulensis and Eriocephalus africanus essential oils in weeds of Mediterranean summer crops. Biochemical Systematics and Ecology, 37(4), 362-369.

Minerals elements, essential oïl composition, antimicrobial activity of Algerian *Melissa officinalis* plant.

Fahima Abdellatif[1,*], Dahmane dahmane[1], Mohammed Messaoudi [2]

[1]Laboratoire de Recherche sur les Produits Bioactifs et la Valorisation de la Biomasse, Ecole Normale Supérieure Kouba, Alger, Algérie. B.P. 92, Kouba Alger, Algérie Tel 21321297511 ; Fax : 021282067 Fahima.abdellatif@g.ens-kouba.dz
[2]Nuclear Research Centre of Birine, Djelfa, P.O. Box 180,17200 Ain Oussera, Algeria

* Email: zina_fahima@yahoo.fr

Keywords: *Melissa officinalis* L.; medicinal plants; mineral content; essential oils composition; antibacterial activity.

This study describes the minerals elements, chemical composition, antimicrobial activity of Algerian *Melissa officinalis* plant. The essential oil (EO) was extracted by hydrodistillation (HD) using a Clevenger-type apparatus of dry leaves of *M. officinalis* and was analyzed by two techniques, gas chromatography coupled with flame ionization (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). Eighteen minerals comprising both macro- and microelements (As, Br, K, La, Na, Sb, Sm, Ba, Ca, Ce, Co, Cr, Cs, Fe, Rb, Sc, Th, and Zn) were determined using neutron activation analysis technique for the first time from Algerian *Melissa officinalis* plant. Seventy-eight compounds were identified in the essential oil, representing 94.090% of the total oil and the yields were 0.470%. The major component was geranial (45.060%). Other predominant components were neral (31.720%) and citronellal (6.420%). The essential oil presented high antimicrobial activity against microorganisms, mainly five human pathogenic bacteria, one yeast, Candida albicans, and two phytopathogenic fungi. The results can be used as a source of information for the pharmaceutical industry and medical research.

Evaluation of half-sib progenies of *Varronia curassavica* harvested in two seasons

Arie F. Blank[1,*], Vanderson S. Pinto[1], W. S. Jesus[1], José C. F. Sá Filho[1], Daniela A. C. Nizio[1], Paulo C. L. Nogueira[2], Maria T. S. Leite Neta[3]

[1] Department of Agronomic Engineering, Federal University of Sergipe, São Cristóvão, Sergipe, BRAZIL
[2] Department of Chemistry, Federal University of Sergipe, São Cristóvão, Sergipe, BRAZIL
[3] Department of Food Technology, Federal University of Sergipe, São Cristóvão, Sergipe, BRAZIL

* Email: arie.blank@gmail.com

Keywords: Cordiaceae, genotypes, essential oil, viridiflorol, seasonality

Objective

Varronia curassavica Jacq. is an aromatic, shrubby, and cross-pollinated species with significant pharmacological and medicinal importance. This species exhibits biological activity against pests of agricultural and zootechnical importance, such as the protozoan *Ichthyophthirius multifiliis*. A study conducted with the essential oil of *V. curassavica* at the Federal University of Sergipe observed that this biological activity is due to the presence of E-caryophyllene and viridiflorol, which resulted in 100% mortality of the trophonts and tomonts of *I. multifiliis*. The objective of the research was to evaluate the performance of half-sibling progenies of *V. curassavica* in two harvest seasons for leaf dry mass.

Methods

Seeds were collected from plants of the VCUR-503 accession, characterized by the E-caryophyllene/viridiflorol chemotype, and sown. The obtained seedlings constituted the half-sibling progenies. These, along with the maternal parent, were transferred to the field with a spacing of 1.0 x 1.0 m. Harvests took place in January 2019 (dry season) and June 2019 (rainy season). Agronomic data were subjected to analysis of variance, while the chemical composition was analyzed using multivariate clustering and principal component analysis. The harvested materials were defoliated, and the leaves and inflorescences were placed in a forced-air circulation oven at 40°C±1 for five days. The dried plant material was weighed to obtain the dry leaf mass per plant. For the determination of EOC and EOY, 35g of the mixture of dried leaves+inflorescences were weighed in triplicate per plant. Hydrodistillation for 140 minutes with a Clevenger apparatus was used to extract the essential oil. Chemical analyses were performed using a GC/MS-FID equipped with an AOC-20i automatic sampler (Shimadzu).

Results

The progenies VCUR-503-42 and VCUR-503-58 produced biomass exceeding 500 g/plant in both harvests. The EOC (1.21-2.38%) and EOY (2.80-6.35 mL/plant) were higher in the dry season. The clustering analysis revealed the presence of two chemical groups: Group I [α-pinene and E-caryophyllene], which included 20 half-sibling progenies plus the parental in the dry season and 18 progenies plus the parental in the rainy season; Group II [α-pinene, E-caryophyllene, and viridiflorol], which included 19 progenies in the dry season and 21 progenies in the rainy season. The E-caryophyllene content ranged from 3.40 to 24.66% in the dry season and from 4.07 to 24.91% in the rainy season, considering the progenies that produced this compound. A higher average was observed in the rainy season (13.33% vs. 10.84%). Meanwhile, the viridiflorol content ranged from 0.45 to 64.74% in the dry season and from 0.46 to 59.31% in the rainy season, considering the progenies that produced this compound. Highest means for viridiflorol was observed in the dry season (23.75% vs. 22.08%).

Conclusions

The half-sibling progenies VCUR-503-01, VCUR-503-02, VCUR-503-45, VCUR-503-66, VCUR-503-88, and VCUR-503-119 exhibit promising agronomic and chemical characteristics to be released as cultivars in the breeding program of *V. curassavica*. The chemical composition of the essential oils showed stability in the evaluated seasons.

ACKNOWLEDGMENTS

This study was financed in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq), the Fundação de Apoio à Pesquisa e a Inovação Tecnológica do Estado de Sergipe (Fapitec/SE) - Brasil, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001)

Antimicrobial activity of essential oils from *Croton grewioides* Baill. on *Xanthomonas campestris* pv. *campestris*

Maria F. Arrigoni-Blank[1,*], Taíse C. Rodrigues[1], Itamara B. Gois[1], Roberta P. M. Fernandes[2], Maria T. S. Leite Neta[3], Arie F. Blank[1]

[1] Graduate Program in Agriculture and Biodiversity, Federal University of Sergipe, São Cristóvão, BRAZIL
[2] Departament of Physiology, Federal University of Sergipe, São Cristóvão, BRAZIL
[3] Department of Food Technology, Federal University of Sergipe, São Cristóvão, Sergipe, BRAZIL

* Email: fatima.blank@gmail.com

Keywords: Euphorbiaceae, volatile oil, eugenol, black rot disease, minimum bactericide concentration

Objective

The productivity of species of Brassicacea family can be affected by *Xanthomonas campestris* p.v. *campestris*, who is the causal agent of the black rot disease. The work aimed to evaluate the antimicrobial activity of *Croton grewioides* Baill. leaf essential oil from Brazil against *Xanthomonas campestris* p.v. *campestris*.

Methods

The antimicrobial activity was performed for essential oils of five access from *Croton grewioides*: CGR-107, CGR-108, CGR-125, CGR-209, and CGR-318. The essential oils were obtained by hydrodistillation and the separation and identification of the compounds were performed by a gas chromatograph coupled to a mass spectrometer (Agilent Model). The *Xanthomonas campestris* pv. *campetris* 629IBSBF (Xcc-629IBSBF) isolates were obtained from the Instituto Biológico de São Paulo (São Paulo, Brazil). The inhibitory (MIC) and bactericide (MBC) concentrations were determined by the microdilution method based on the protocol established by CLSI (Clinical and Laboratory Standards Institute). The positive control was the streptomycin sulfate, and the negative control was the YM medium with DMSO (2%). The MIC was defined as the lowest concentration of the essential oil that inhibited 100% of the Xcc-629IBSBF growth. The MBC was defined as the concentration of the essential oil that does not present bacteria growth after 24h in the YM culture medium. The permeability assay of the layer was performed with Propidium Iodide (PI). The identification of the compounds related to bacteriostatic activity was carried out using the Partial Least Square – Discriminant Analysis (PLS-DA).

Results

The essential oil CGR-108 showed the lowest MIC (1000 µg.mL^{-1}), and MBC of 2000 µg.mL^{-1}. The values of MIC and MBC for the essential oil CGR-107 were equal to 2000 µg.mL^{-1}. The essential oil CGR-125 presents MIC of 2000 µg.mL^{-1} and MBC of 4000 µg.mL^{-1}. For the essential oil CGR-318, the MIC was 4000 µg.mL^{-1}, but it was not observed bactericide activity to the tested concentrations. The tested concentrations for the essential oil CGR-209 were not enough to inhibit bacterial growth. The compound related to the bacteriostatic activity that presented major importance in the projection (VIP – variable importance in the projection) in the PLS-DA analysis was Eugenol, which is the majority compound of the essential oil CGR-108 (MIC = 1000 µg.mL^{-1}). The increase in the permeability of the membrane was observed after 30 minutes of the exposition to essential oil CGR-108 at the concentrations 1x and 2x MIC, compared to treatment without the essential oil (negative control).

Conclusions

The cell viability of *Xanthomonas campestris* pv. *campestris* is rapidly reduced after contact with CGR-108 essential oil, whose majority compound is eugenol, causing structural alterations in the cytoplasmatic layer.

ACKNOWLEDGMENTS

This study was financed in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil (CNPq), the Fundação de Apoio à Pesquisa e a Inovação Tecnológica do Estado de Sergipe (Fapitec/SE) - Brasil, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001).

Scaling down the sample amount in hop essential oil analysis

Humberto R. Bizzo*, Rosemar Antoniassi, Paola E. Gama, Marcos J. O. Fonseca

Embrapa Agroindústria de Alimentos, Rio de Janeiro, BRAZIL

* Email: humberto.bizzo@embrapa.br

Keywords: hop essential oil, *Humulus lupulus*, beer, quality parameters, hydrodistillation

Objective

Hop (*Humulus lupus* L., family Cannabaceae) has been introduced in Brazil in late 19th century, but its production in commercial scale has succeeded only in the last 20 years, prompted by the exponential increase in artisanal breweries. Use of the American Society of Brewing Chemists' (ASBC) official method [1] for volatile oils implicates in a 100 g dried hop sample per replicate (300 g for a triplicate result). Either in breeding studies or small scale production, intended to artisanal breweries, this is a prohytitive hop quantity to be set apart for testing. The objective was to reduce the amount of hop necessary for the distillation of the essential oil from hop cones in control quality analysis, without changing oil yield and composition.

Methods

Three different distilling devices were tested, namely a Clevenger-type apparatus according to the Brazilian Pharmacopoeia [2] (the same as in the European Pharmacopoeia), a modified Clevenger-type used in routine at our lab [3] and a microdistillation device, as proposed by Bicchi and collaborators [4]. A single homogeneous batch of dried hop was used for all tests. Triplicate analyses were run in each of the apparatus listed, using 10, 15, 20 and 100 g of dried hop. Oil yield was expressed in volume by weight. Oils were analysed by GC-FID in a DB-5 column (25 m x 0.25 mm x 0.25 µm) with detection by FID, with internal standard and response factors correction. Oil yield and composition were compared. Data was treated using a series of Excel pre-programmed electronic sheets [5]. Statistic comparison of results by software Statgraphics®.

Results

No differences were observed for the oil yield with reduced sample quantities (10, 15 and 20 g) when compared to the original 100 g sample for the Pharmacopoeia model apparatus. For the other distillers tested, no oil separation was observed. Regarding oil composition, no significant differences were observed in oil profile as well as in quantitative data for 10 control compounds: β-pinene, myrcene, 2-methyl isobutyrate, linalool, undecanone, methyl geranate, β-caryophyllene, α-humulene, β-farnesene and β-selinene. The smallest standard deviations were observed for the 15 g samples.

Conclusions

Reduction of sample size from 100 g to 15 g did not impart significant quantitative nor qualitative differences in hop volatile oil analysis. This reduction will make hop quality testing feasible for small-scale production and breeding studies.

ACKNOWLEDGMENTS

Authors thank Mrs. Marcelly C. S. Santos for technical assistance. H.R.B. thanks research grants from CNPq (311021/2021-2) and FAPERJ (210.049/2020).

REFERENCES

[1] American Society of Brewing Chemists, (2011). ASBC Methods of Analysis, ASBC, Saint Paul, MN.
[2] Agência Nacional de Vigiância Sanitária, (2019). Farmacopeia Brasileira, 6th ed., ANVISA, Brasília.
[3] Gottlieb O. R., Magalhães M. T., (1960). Modified Distillation Trap. Chemist-Analyst, 49, 114.
[4] Bicchi C., D'Amato A., Nano G. M., Frattini C., (1983). Improved method for the analysis of small amounts of essential oils by microdistillation followed by capillary gas chromatography. Journal of Chromatography, 279, 409-416.
[5] Bizzo H. R., Barboza E. G., Santos M. C. S., Gama, P. E., (2020). A set of electronic sheets for the identification and quantification of constituents of essential oils. Quimica Nova, 43, 98-105.

Synergism in two-component insecticides with dillapiole

Murilo Fazolin[1], André F. M. Monteiro[1], Maria E. C. Lima[2], Natalia S. Maisforte[3], Paola E. Gama[4], Humberto R. Bizzo[4,*].

[1] Embrapa Acre, Rio Branco AC, BRAZIL
[2] Universidade Federal do Acre, Rio Branco AC, BRAZIL
[3] Instituto Federal do Acre, Rio Branco AC, BRAZIL
[4] Embrapa Agroindústria de Alimentos, Rio de Janeiro, BRAZIL

* Email: humberto.bizzo@embrapa.br

Keywords: *Piper aduncum*, *Spodoptera frugiperda*, Essential oils, Bioinsecticides.

Objective

Essential oils (EOs) have been used as low risk, biodegradable insecticides, reducing environmental contamination [1]. The EO of *Piper aduncum* L. (Piperaceae), a native species from the Amazon, is rich arylpropanoids, mainly dillapiole. The insecticidal activity of this class of compounds has been associated to inhibition of detoxification enzymes in insects, such as monooxygenases, esterases and glutathione *S*-transferase [2]. Bioactivity in EOs is frequently associated to their major compounds, nevertheless synergic interactions with minor constituents may lead to higher biological activity than isolated compounds [3]. The objective of this work is to evaluate the occurrence of synergic interactions regarding insecticide activity in binary combinations of dillapiole with other arylpropanoids and terpenoids originally present as constituents of the EO from *P. aduncum*.

Methods

The EO from the leaves of *P. aduncum* was obtained by hydrodistillation. Dillapiole, asaricin and safrole were isolated by fractional distillation. Other compounds tested were supplied by Sigma Aldrich. The insecticidal activity of isolated compounds and standards were tested by topical contact *in vitro*, using *Spodoptera frugiperda* (J. E. Smith, 1797) larvae as target insect, to determine the lethal dose (LD_{50}) and the lethal concentration (LC_{50}) [4].

Results

For all compounds individually tested against *S. frugiperda* larvae, dillapiole proved to be the most toxic one, with an average toxicity 6.9 times higher than other arylpropanoids, 18.5 times in comparison with sesquiterpenes and 40 times more toxic when compared to monoterpenes present in the oil. When tested in binary combinations, most of the other common constituents of the EO showed synergism factor (SF) > 1. Best synergic effects were observed for the combinations of dillapiole with β-caryophyllene (SF = 12.0), with methyl eugenol (SF = 7.5), and α-humulene (SF = 7.0).

Conclusions

Synergistic interactions were observed in binary combinations of dillapiole and selected compounds originally present in the EO of *P. aduncum*. These data can be used on more efficient insecticide formulation.

ACKNOWLEDGMENTS

Authors thank Mrs. Marcelly C. S. Santos for technical assistance. H.R.B. thanks research grants from CNPq (311021/2021-2) and FAPERJ (210.049/2020).

REFERENCES

[1] Nollet L. M. L., Rathore, H. S., (2017). Green Pesticides Handbook, CRC Press, Boca Raton.
[2] Liu N., (2015). Insecticide resistance in mosquitoes: impact, mechanisms, and research directions, Annual Reviews of Entomology, 60, 537-559.
[3] Hummelbrunner L. A., Isman M. B., (2001). Acute, sublethal, antifeedant, and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, Spodoptera litura (Lep., Noctuidae), Journal of Agricultural and Food Chemistry, 49, 715-720.
[4] Estrela J. L. V., Fazolin M., Catani V., Alécio M. R., Lima M. S., (2006). Toxicidade de óleos essenciais de Piper aduncum e Piper hispidinervum em Sitophilus zeamais, Pesquisa Agropecuária Brasileira, 41, 217-222.

Lipid peroxidation inhibitory potential of the selected terpenoids

Jelena S Lazarević[1,*], Jelena Zvezdanović[2]

[1] Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000, Niš, SERBIA
[2] Faculty of Technology, University of Niš, Bulevar oslobođenja 124, 16000, Leskovac, SERBIA

* Email: jelena_lazarevic@iseoils.com

Keywords: terpenoids, antioxidant activity, lipid peroxidation, TBA-MDA method

Objective

In conditions of oxidative stress, in interaction with generated free radicals, polyunsaturated fatty acids can undergo oxidative damage, which results in the initiation of the lipid peroxidation (LP) process. Oxidative stress and the resulting LP are involved in the pathogenesis of numerous chronic and degenerative diseases which seriously impair the quality of life. That is why numerous researches are focused on finding substances with antioxidant properties, preferably from natural sources. Terpenoids are one of the most abundant and diverse groups of naturally occurring compounds, widely distributed in human diet, known for extensive range of biologic activities. It has also been reported that terpenoids reduce reactive oxygen species and malondialdehyde (MDA) production through their antiinflammatory and antioxidant properties and increase the activity of superoxide dismutase in radical scavenging [1]. The objective of our work was to evaluate *in vitro* antioxidant activity of analitical standards of eleven terpenoids, frequently encounted in essential oils, on the LP process.

Methods

Antioxidant properties of selected terpenoids on the peroxidation of the phospholipid mixture (Phospholipon®90) were investigated in an *in vitro* model. LP and the percentage of its inhibition in the presence of terpenoids as tested compounds were calculated by determining the concentration of MDA. The concentration of MDA was evaluated spectrophotometrically, by measuring the absorbance of the thiobarbituric acid-malondialdehyde (TBA-MDA) conjugate at 530 nm, according to the slightly modified procedure described by Lazarević et al. [2] and Brizzolari et al [3]. The same experiments were done using BHA, BHT, trolox and quercetin as standards.

Results

The *in vitro* results of selected terpenoids on the peroxidation of the model phospholipid mixture have showed that all compounds at tested concentrations (5 mM - 0.1 mM) had antioxidant properties and are proved to be relatively good scavengers of radicals that are induced in the LP process. LP inhibition values displayed by carvacrol, thymol, camphor, linalool and menthone ranged between 1.2 ± 0.2 mM and 5.2 ± 2.1 mM (IC_{50} value extrapolated from our data). At concentrations below 1 mM, a somewhat stronger LP inhibition effect can be observed for α-bisabolol (IC_{50} = 0.2 ± 0.1 mM), thymyl-acetate (0.4 ± 0.1 mM), carvacryl acetate (0.4 ± 0.1 mM), menthol (0.7 ± 0.1), *p*-cymene (0.9 ± 0.5 mM) and caryophyllene oxide (0.9 ± 0.3 mM). None of the compounds had a LP inhibition results comparable to antioxidant standards.

Conclusions

Six out of eleven compounds have LP inhibition effect, which indicates direct antioxidant properties. This result also suggests that the role of terpenoids in plant lipoperoxidation observed by some authors is due more to some regulatory effect on oxidative stress in cells than to intrinsic ability of the molecules to react with the lipoperoxyl radicals.

ACKNOWLEDGMENTS

The work was funded by the Ministry of Science and Technological Development of Serbia Project 451-03-47/2023-01/200113 and Faculty of Medicine, University of Niš Internal project No. 40

REFERENCES

[1] Baccouri B and Rajhi I (2021) Potential Antioxidant Activity of Terpenes. Terpenes and Terpenoids - Recent Advances. IntechOpen.
[2] Lazarević J., Šmelcerović A., Zvezdanović J., Yancheva D., Casati S., Ottria R., Ciuffreda P., (2020). Lipid peroxidation inhibition study: A promising case of 1,3-di([1,1'-biphenyl]-3-yl)urea, Chemico Biological Interactions 326:109137.
[3] Brizzolari A., Foti M.C., Saso L., Ciuffreda P., Lazarević J., Santaniello E., (2022). Evaluation of the radical scavenging activity of some representative isoprenoid and aromatic cytokinin ribosides (N6-substituted adenosines) by in vitro chemical assays. Natural Product Research 36(24):6443-6447.

The effect of *Arthemisia arborescens* essential oil and its constituents on the inhibition of lipid peroxidation

Jelena S Lazarević[1*], Jelena Zvezdanović[2], Gordana Stojanović[3]

[1] Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000, Niš, SERBIA
[2] Faculty of Technology, University of Niš, Bulevar oslobođenja 124, 16000, Leskovac, SERBIA
[3] Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, SERBIA

* Email: jelena_lazarevic@iseoils.com

Keywords: *Artemisia arborescens*, 1,8-cineol, camphor, chamazulene, lipid peroxidation

Objective

Bringing to close connection with oxidative stress in vivo, lipid peroxidation (LP) has received a great deal of attention. In recent years, considerable interest has been focused on finding substances with antioxidant properties, preferably from natural sources. The aim of this work is to examine *in vitro* antioxidant activity of essential oil (EO) obtained by the hydrodistillation of cultivated *Artemisia arborescens* and 1,8-cineol, camphor and chamazulene as its components, all previously reported to exert anti-inflammatory activity [1-3], on LP process.

Methods

Artemisia arborescens EO was obtained by the hydrodistillation, and chamazulene was isolated by isocratic column chromatography on earlyer occasion from *Matricaria* sp essential oil. To measure LP, one of the most commonly used assays a thiobarbituric acid-malondialdehyde (TBA-MDA) test, was used to calculate the percentage of LP inhibition in the presence of tested compounds by determining the concentration of MDA. The concentration of MDA was evaluated spectrophotometrically, by measuring the absorbance of the TBA-MDA conjugate at 530 nm, according to the slightly modified procedure described by Lazarević et al. [4] and Brizzolari [5]. The same experiments were done using BHA, BHT, trolox and quercetin as standards.

Results

Antioxidant properties, assessed on the basis of LP inhibition effect of *A. arborescens* EO and its constituents: chamazulene, as isolated and 1,8-cineole and camphor, as purchased standards, were investigated *in vitro* using phospholipid mixture Phospholipon®90 as a model. *Artemisia arborescens* EO showed LP inhibitory effect expressed as IC_{50} = 0.06 ± 0.06 mg/cm^3, as did chamazulene, having 0.05 ± 0.02 mg/cm^3, 1,8-cineol with IC_{50} = 0.04 ± 0.02 mg/cm^3 and camphor, having IC_{50} = 0.03 ± 0.02 mg/cm^3.

Conclusions

Preliminary results have shown that *A. arborescens* EO and its currently three assayed components, by inhibiting the LP process, are all having antioxidant properties. Antioxidant activity of *A. arborescens* oil can be partially attributed to the presence of chamazulene, 1,8-cineol and camphor and to the ability of the oil sample to neutralize ROS free radicals generated in LP process: lipid peroxyl radicals and hydroperoxides, that our results, showing significant antioxidant activity in terms of direct scavenging of free radicals, have confirmed.

ACKNOWLEDGMENTS

The work was funded by the Ministry of Science and Technological Development of Serbia (Projects 451-03-47/2023-01/200113 and 451-03-68/2023-14/200124) and Faculty of Medicine, University of Niš Internal project No. 40.

REFERENCES

[1] Juergens U.R., (2014) Anti-inflammatory properties of the monoterpene 1.8-cineole: current evidence for co-medication in inflammatory airway diseases. Drug Research 64(12):638-46.
[2] Xiao S., Yu H., Xie Y., Guo Y., Fan J., Yao W., (2021) The anti-inflammatory potential of Cinnamomum camphora (L.) J. Presl essential oil in vitro and in vivo. Journal of Ethnopharmacol. 267:113516.
[3] Safayhi H., Sabieraj J., Sailer E. R., Ammon H. P., (1994) Chamazulene: an antioxidant-type inhibitor of leukotriene B4 formation. Planta Medica 60(5):410-3.
[4] Lazarević J., Šmelcerović A., Zvezdanović J., Yancheva D., Casati S., Ottria R., Ciuffreda P., (2020). Lipid peroxidation inhibition study: A promising case of 1,3-di([1,1'-biphenyl]-3-yl)urea, Chemico Biological Interactions 326:109137.
[5] Brizzolari A., Foti M.C., Saso L., Ciuffreda P., Lazarević J., Santaniello E., (2022). Evaluation of the radical scavenging activity of some representative isoprenoid and aromatic cytokinin ribosides (N6-substituted adenosines) by in vitro chemical assays. Natural Product Research 36(24):6443-6447.

Plasticizer contamination of commercial Tunisian essential oils: a preliminary study

Angela Giorgia Potortì[1], Vincenzo Lo Turco[1], Ambrogina Albergamo[1], Federica Litrenta[1], Rossana Rando[1], Hedi Ben Mansour[2], Giuseppa Di Bella[1,*]

[1]Department of Biomedical, Dental, Morphological and Functional Images Sciences, University of Messina, Messina, ITALY
[2]Research Unit of Analysis and Process Applied to Environmental-APAE UR17ES32, Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, TUNISIA

* Email: gdibella@unime.it

Keywords: Tunisian essential oils, plasticizers, PAEs, NPPs

Objective

Commercial essential oils (EOs, SW), namely cypress leaf (*Cupressus sempervirens* L.), Tasmanian blue gum leaf (*Eucalyptus globulus* Labill.), lavender flower (*Lavandula officinalis* Chax.), pennyroyal mint leaf (*Mentha pulegium* L.), thyme herb (*Thymus vulgaris* L.), rosemary herb (*Rosmarinus officinalis* L.) and clove flower buds (*Syzygium aromaticum* L.), were evaluated for phthalate ester (PAE) and non-phthalate plasticizer (NPP) contamination and discussed in relation to their potential application in food and cosmetics. In fact, although there is still a noticeable gap in regulating these contaminants in food, the Reg. (EC) No. 1223/2009 expressely bans the presence of dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), butylbenzyl phthalate (BBP), di(2-methoxyethyl) phthalate (DMEP), di-n-pentyl phthalate (DnPP), diisopentyl phthalate (DiPP), n-pentyl isopentyl phthalate (DPP), and diisobutyl phthalate (DiBP) in cosmetics.

Methods

In 2022, several EOs were provided in glass bottles closed with plastic screw caps by the "Aroma CAP" company (Mahdia, Tunisia). Each EO was suitably diluted with a *n*-hexane solution of the internal standards DBP-d4, DEHP-d4, DEHA-d4 and DEHT-d4. Then, each sample was analyzed for PAEs and NPPs by a validated GC-qMS method reported in our previous study [2]. The statistical comparison of each EO was performed by one-way ANOVA followed by a Tukey's HSD test, and the statistical significance was accepted at $p \leq 0.05$.

Results

PAEs, such as DEP, DPrP, DiBP, DBP, BBP, DEHP, and DPhP, and NPPs, such as DEHT and DiBA, were detected in all EOs. Specifically, DEP, DEHP, DEHT were the most abundant compounds (≥1mg/Kg); while DPrP and DiBA the least concentrated ones (≥0.05 mg/Kg). Clove EO was the most contaminated sample, followed by pennyroyal mint and thyme EOs, which had a similar contamination degree. These results may be explained by the fact that EOs come into contact with leaching plastic materials during the production and the packaging (e.g., plastic cap) which, consequently, need to be revised. The incorporation of such EOs into food may result in a greater exposure to plasticizers, whose risks to human health are already known. Therefore, it is necessary to complement the migration limits of plasticizers from food contact materials already established by the Reg. (UE) 1245/2020, with maximum levels of such contaminants allowed in food, given the multiple plasticizer sources potentially encountered during food processing. Likewise, the employment of such EOs in cosmetics should be carefully evaluated, since according to the Reg. (EC) 1223/2009 all samples contained banned DBP, BBP, DEP, DEHP, and DiBP, which may be detected in final products based on the amount of oil used (usually 2-3%).

Conclusions

This study suggests that the chemical safety of EOs should be carefully assessed before they are employed in various applications, including food and cosmetics. In the next future, a greater number of Tunisian EOs and contaminant classes will be evaluated to get a more in-depth contamination pattern.

REFERENCES

[1] Saitta M., Di Bella, G., Dugo, G. (2012). Pesticides and plasticizers in Citrus essential oils: An ordinary history of research. Journal of Essential Oil Research, 24, 171-180.
[2] Jebara A., Albergamo A., Rando R., Potortì A.G., Lo Turco V., Mansour H.B., Di Bella, G. (2021). Phthalates and non-phthalate plasticizers in Tunisian marine samples: Occurrence, spatial distribution and seasonal variation. Marine Pollution Bulletin, 163, 111967.

Commercial Tunisian essential oils as potential food antimicrobials and antioxidants and screening of their element profile

Ambrogina Albergamo[1], Vincenzo Nava[2], Vincenzo Lo Turco[1], Benedetta Sgrò[1], Giovanni Bartolomeo[1], Asma Beltifa[3,4], Giuseppa Di Bella[1,*]

[1]Department of Biomedical, Dental, Morphological and Functional Images Sciences, University of Messina, Messina, ITALY
[2]Department of Veterinary Science, University of Messina, Messina, ITALY
[3]Research Unit of Analysis and Process Applied to Environmental‑APAE UR17ES32, Higher Institute of Applied Sciences and Technology Mahdia, University of Monastir, Monastir, TUNISIA
[4]Direction d'hygiène du milieu et de la protection de l'environnement à la direction régionale de santé de Mahdia, Mahdia, TUNISIA

* Email: gdibella@unime.it

Keywords: Essential oils, *E. coli* O157:H7, *Klebsiella pneumoniae subsp. pneumoniae*, DPPH activity, inorganic elements

Objective

An array of commercial Tunisian essential oils (EOs, SW) obtained from different Mediterranean species were elucidated for their potential as natural food preservatives. To this purpose, EOs were investigated for their *in vitro* antimicrobial activity against two food-borne bacteria, namely *Escherichia coli* O157:H7 (Migula) Castellani and Chalmers (ATCC 43895) and *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan (ATCC 700721), and *in vitro* antioxidant activity as well. Additionally, volatiles and inorganic elements of EOs were screened and discussed in relation to the biological activities. In fact, besides the unquestionably relevant study of the volatile active fraction, the screening of inorganic elements may also contribute to assess the effectiveness and safety of EOs as preservatives in food [1].

Methods

The antibacterial activity was evaluated by the agar-well diffusion method and by calculating minimum inhibitory concentration (MIC), according to the microbroth dilution method. The antioxidant activity was determined by a DPPH UV–Vis spectrophotometric assay. Main volatiles were qualitatively and quantitatively elucidated by GC–MS and GC-FID, respectively. Minerals, essential trace elements, and potentially toxic trace elements were elucidated by ICP-MS. A statistical comparison of each EO was performed by one-way ANOVA, followed by a Tukey's HSD post-hoc test and the statistical significance was accepted at $p \leq 0.05$. A Pearson correlation analysis investigated the potential relationship between inorganic elements and biological activities of a given EO.

Results

Almost all selected EOs inhibited the growth of at least one reference strain. A variety of volatiles, such as carvone (in spearmint EO), thymol (in bigroot geranium EO), 1,8-cineol (in spearmint, rosemary and lavender EOs), linalool (in rosemary and lavender EOs), α-terpineol (in lavender and Scots pine EOs), and limonene (in spearmint and Scots pine EOs), as well as certain combinations of volatiles (i.e., 1,8-cineol and camphor) may justify their bioactivity [2]. Spearmint, sage and rosemary EOs had the lowest MICs against *E. coli* O157:H7 (0.09, 0.09, 0.07 mg/ml, respectively); whereas sage EO was effective against *K. pneumoniae* at 0.41 mg/ml. All EOs also displayed a promising antioxidant activity. Specifically, spearmint, bigroot geranium, and sage EOs showed the lowest IC50 values (0.024, 0.046 and 0.052 mg/ml), which were similar to that of the synthetic antioxidant BHT. EOs exhibited peculiar element profiles. Among minerals, Mg and K were in the range 1.72–14.12 mg/Kg and 0.57–20.90 mg/Kg; while Cu and Fe were the most abundant trace essential metals (0.07–1.02 mg/Kg 0.20–2.98 mg Kg, respectively). Low and safe levels of heavy metals were also revealed. The statistical correlation analysis pointed out a significant positive correlation between some elements (K, Cu, and Fe) and the biological activities displayed.

Conclusions

Based on the results from this study, Tunisian EOs may have effective applicability as antibacterial and antioxidant additives in food industry.

REFERENCES

[1] Fu L., Xie H., Shi S. (2018) Multielement analysis of Zanthoxylum bungeanum Maxim. essential oil using ICP-MS/MS. Analytical and Bioanalytical Chemistry, 410, 3769–3778
[2] Friedman M., Henika P.R., Mandrell R.E.(2002). Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes,and Salmonella enterica. Journal of Food Protection, 65, 1545–1560.

Evaluation of Volatile Fraction and Oxygen Heterocyclic Compounds of Mandarin essential oils.

Federica Vento[1*], Filippo Alibrando[2], Giuseppe Micalizzi[1], Marina Russo[1], Ivana Bonaccorsi[1], Cristian Reale[1], Paola Dugo[1], Luigi Mondello[1,2]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

* Email: federica.vento1@studenti.unime.it

Keywords: Mandarin oil, chiral analysis, oxygen heterocyclic compounds, GC-MS, GC-FID

Objective

Mandarin essential oil (*Citrus deliciosa*) is used for a wide range of application, as perfume, cosmetic, food and beverages industry. The essential oil of mandarin is currently extracted by using the Pelatrice and the Food Machinery Corporation Inline Extractor (FMC). According to ISO 3528 of 1977 this oil is presented as a mobile liquid with a color that can vary from greenish yellow to reddish orange, depending on the degree of ripeness of the fruit from which it is extracted, with a slight blue fluorescence. As regards its chemical composition, the volatile fraction represents about 96-98% while the remaining 2-4% is the non-volatile fraction. Citrus essential oils are very valuable and expensive, for this reason, adulteration activities by addition of less valuable products are very common. These adulterations affect not only the organoleptic qualities, but also the health properties. In this contest, the evaluation of authenticity and quality through the determination of the volatile and non-volatile fraction is of fundamental interest.

Methods

For the determination of the volatile fraction, especially for the qualitative analysis of the sample, mass spectrometry coupled to a gas chromatographic system (GC-MS) was employed. Quantitative and enantiomeric analyses have been effected on a flame ionization detector coupled to a gas chromatographic system (GC-FID). For both qualitative and quantitative analysis a capillary column of fused silica SLB-5 ms was used, while for enantiomeric analysis a capillary column MEGA-DEX DET-Beta was selected. The analysis of the non-volatile fraction was performed by using a LC-2040C 3D Nexera-i PDA integrated UHPLC System. For the separation, an Ascentis Express HPLC column was selected, water/methanol/THF (85:10:5 v/v) and methanol/THF (95:5 v/v) were used as solvent A and B respectively.

Results

Qualitative and quantitative results have been compared with the samples analysed previously by our research group. The results showed volatile fraction of mandarin oil varied in dependence of the production season, allowing us to differentiate the oils obtained at the beginning of the season from green mandarin, from those obtained from yellow and red mandarins. The non-volatile fraction analyses were carried out according to the analytical method, previously developed and validated by Arigò et al. [1], which allowed the identification of 35 oxygenated heterocyclic compounds.

Conclusions

The analyses were carried out on essential oils from samples collected in the years 2020-2023, obtained with peeling and sfumatrice extraction techniques. The results led to investigate deeply the qualitative and quantitative differences due to different pedo-climatic conditions and genetic variations, respect to the latest available data.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corp. and Merck KGaA for their support and Simone Gatto S.r.l and F.lli Branca S.p.a. for providing the samples.

REFERENCES

[1] Arigò A., Rigano F., Micalizzi G., Dugo P., Mondello L., (2019). Oxygen heterocyclic compound screening in Citrus essential oils by linear retention index approach applied to liquid chromatography coupled to photodiode array detector, Flavour Fragr. J., 34, 349-364.

Evaluation of Volatile Fraction and Oxygen Heterocyclic Compounds of sweet orange essential oils.

Federica Vento[1*], Filippo Alibrando[2], Giuseppe Micalizzi[1], Marina Russo[1], Ivana Bonaccorsi[1], Elisa Irrera[1], Paola Dugo[1], Luigi Mondello[1,2]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy

* Email: federica.vento1@studenti.unime.it

Keywords: Sweet orange oil, FMC, oxygen heterocyclic compounds, GC-MS, GC-FID

Objective

The composition of citrus essential oils "cold-pressed" is regulated by international regulations that describe the intervals of variability of chemical-physical characteristics and volatile components. However, citrus essential oils are often subject to attempts of adulteration, either through the addition of by-products from the citrus industry, or through the use of synthetic substances. Sweet orange essential oil (Citrus sinensis) is traditionally produced by cold pressing of citrus peels. Its chemical composition consists of a volatile fraction mainly characterized by mono-terpene hydrocarbons and oxygenated terpenes, and a non-volatile fraction constituted by oxygen etherocyclic compounds as coumarins, psoralens and polymethoxyflavones. Volatile and non-volatile metabolites possess beneficial activities: antioxidant, anti-inflammatory, anti-cancerogenic, cardiovascular and neuroprotective properties [1], and are used to evaluate the quality and authenticity of the oils.

Methods

For the determination of the volatile fraction, especially for the qualitative analysis of the sample, mass spectrometry coupled to a gas chromatographic system (GC-MS) was employed. Quantitative and enantiomeric analyses have been effected on a flame ionization detector coupled to a gas chromatographic system (GC-FID). For both qualitative and quantitative analysis a capillary column of fused silica SLB-5 ms was used, while for enantiomeric analysis a capillary column MEGA-DEX DET-Beta was selected. The analysis of the non-volatile fraction was performed by using a LC-2040C 3D Nexera-i PDA integrated UHPLC System. For the separation, an Ascentis Express HPLC column was selected, water/methanol/THF (85:10:5 v/v) and methanol/THF (95:5 v/v) were used as solvent A and B respectively.

Results

The analyzed samples were produced in 2020-2021 with a Food Machinery Corporation Inline Extractor (FMC) extraction procedure. For the determination of the volatile fraction, samples were directly analysed, identified by using linear retention index (LRI), calculated respect to a C7-C40 n-alkane reference solution, quantified as mean of three replicates of relative percentage area and compared with samples produced in 2010. An enantiomeric study was also done. For the determination of the non-volatile fraction, the samples were analysed after dilution, following the method previously validated by our research team.

Conclusions

Samples of sweet orange essential oils obtained by an FMC extraction process were characterized and quantified, and the range content of metabolites found was compared with the samples previously analyzed.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corp. and Merck KGaA for their support and Simone Gatto S.r.l and F.lli Branca S.p.a. for providing the samples.

REFERENCES

[1] Oboh G., Olasehinde T.A., Ademosun A.O., (2017), Inhibition of enzymes linked to type-2 diabetes and hypertension by essential oils from peels of orange and lemon, Int. J. Food Prop, 20, S586–S594.

Characterization of the essential oil, absolute and extract of Egyptian Calendula (*Calendula officinalis* L.)

Federica Vento[1*], Emanuela Trovato[1], Domenica Mangraviti[1], Francesca Rigano[1], Hussein A. Fakhry[2], Fatma Salaheldin[2], Paola Dugo[1,3], Luigi Mondello[1,3,4]

[1] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[2] A. Fakhry & Co.. 1081 Comiche El-Nil, Cairo 1145l, Egypt
[3] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy
[4] Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, Rome, Italy

* Email: federica.vento1@studenti.unime.it

Keywords: *Calendula officinalis*, bioactive compounds, REIMS-QToF, GC-MS, GC-FID

Objective

Calendula officinalis L. is an annual herbaceous species presents in central and southern Europe, northern Africa, southwestern Asia and Macaronesia region. The major constituents of Calendula include steroids, terpenoids, triterpenoids, flavonoids, phenolic acids and carotenoids. The aim of this research is to provide a rapid characterisation of the main compounds present in Egyptian Calendula Essential oil produced by steam distillation and products derived from solvent extraction. For this purpose direct Ambient Mass Spectrometry (AMS), gas chromatographic-mass spectrometry (GC-MS) and gas chromatographic-flame ionization detector (GC-FID) analysis were applied.

Methods

The essential oil obtained by steam distillation, the absolute and the solvent extract, were produced starting from fresh flower. All samples were analyzed by a shotgun MS approach, exploiting an innovative ambient ionization source named REIMS (Rapid Evaporative Ionization Mass Spectrometry) coupled to a quadrupole-time of flight (Q-ToF) detector, capable to generate mass accuracy data. The volatile fraction was analysed by GC-MS and GC-FID analysis for qualitative and quantitative purposes, respectively. For the separation, a poly(5% diphenyl/95% dimethyl siloxane) fused-silica capillary column was chosen. The identification was performed by using a linear retention index (LRI), calculated respect to a C7-C40 *n*-alkane reference solution.

Results

The employment of REIMS-QToF method led to a rapid untargeted screening of all samples in a very short time (2-3 sec), without the need of clean-up procedures or chromatographic separations. The peculiar fingerprints obtained by means of MS high-resolution analysis took to detection of the main phytochemicals compounds belonging to different chemical classes (carotenoids, lipids, phenolic and volatile compounds), by matching the mass accuracy data in commercial and online database. The volatile fraction was confirmed by GC-MS and GC-FID analysis. In particular, alcohols and terpene hydrocarbons were the most abundant classes revealed in all the samples. Moreover, esters compounds were also identified principally in solvent extract, whereas acids in absolute sample and solvent extract.

Conclusions

The method here applied led to detect and identify a great number of compounds in a very short time, respect to conventional analytical techniques, that require a long time analysis and a great amount of solvents. In this context, REIMS-QTof technology proved to be a rapid, green and reproducible analytical approach. Concerning the volatile fraction, 177 compounds belonging to different chemical classes, including aldehydes, alcohols, ketones, esters, furans and terpenes were identified by the analysis of the three different Calendula samples.

ACKNOWLEDGMENTS

The authors thank Waters Corporation, Shimadzu Corp., Merck KGaA for their support, and A. Fakhry & Co. for providing the samples.

Comparison between Satsuma mandarins' (*Citrus unshiu)* essential oil components sampled from Fort Beaufort farms, South Africa

Vuyokazi Mazwi[1], A.O. Oyedeji[1,*], and O. O. Oyedeji[2]

[1] Department of Natural Science, Walter Sisulu University, Private Bag X1, Mthatha, South Africa
[2] Department of Chemistry, University of Fort Hare, Private Bag X1314, Alice, South Africa

* Email: aoyedeji@wsu.ac.za

Keywords: *Citrus unshiu*, bioactive compounds, Hydro-distillation, GC-GC/MS

Citrus fruit, popular for its tasty juice, is grown in different parts of the world including South Africa mainly cultivated for export purposes. The essential oil contained in the oil glands of the peels of these fruits find wide applicability due to phytochemicals contained in it. On harvesting, the fruits are either eaten raw or processed for various purposes with the peels discarded as waste in most cases. This study therefore seeks to compare the peels and leaf essential oils of *Citrus unshiu* grown in South Africa for the purpose of understanding their chemical constituents to mitigate the negative effects that emanate from discarding the peels and the leaves to the environment. Extraction of oils was done using hydro-distillation, which were then analysed with a GC-GC/MS.
The d-Limonene and γ-terpinene were identified as a major compound in most of the peel and leaf samples, respectively. The results further reveal variation between same species cultivated in different farms of the same town. These peel and leaf samples that are currently regarded as waste would find wide applicability in different sectors for economic gain.

Chemical compositions and anti-microbial activity of *Heliotropuim Bacciferum* & *Anethum graveolens* essential oils

Maha Hasni[1,*], Abdelmadjid Saidani[1,*], Nasser Belboukhari[1], Khaled Sekkoum[1], Mebarka Belboukhari[1], Abdelaziz Berghioua[1]

[1]Bioactive Molecules and chiral separation Laboratory (BMCSL), PO Box 417, University of bechar, 08000, Algeria

* Emails: hasni.maha@univ-bechar.dz, saidani.abdelmadjid @univ-bechar.dz

Keywords: Heliotropium Bacciferum, Anethum graveolens, GC-MS, anti-microbial, activity.

Abstract

Essential oils are natural plant products that contain a complex blend of ingredients and therefore have many antimicrobial properties. Most of EO's antimicrobial activity appears to derive from oxidized terpenoids, specifically phenolic terpenes, phenylpropanoids, and alcohols. For this purpose, they are often used in medicine and in the food industry. The aim of this study was to evaluate the chemical composition and antimicrobial activity of two species from two different families essential oils, the Heliotropuim Bacciferum is a paramount medicinal herb[1]. This plant was collected in the lahmar region 35.2 Km away from Bechar (Algeria), is a species that is commonly used to treat various ailments. Anethum graveolens is an aromatic plant of the Apiacea family known as SOWA or SOYA [2], This plant was collected in the Abadla region 865 Km away from Bechar, Algeria.

The essential oils was obtained by hydrodistillation, The volatile chemical compositions of the essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS). The essential oils was a complex blend composed mostly of monoterpenes and sesquiterpenes. The volatile chemical compositions of both essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). This analysis indicated that the main compounds in the *H.Bacciferium* volatile oil are Agarospirol (15.15%), 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetram (9.41%), Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methyletheny (8.96%), respectively. In the other hand it indicated that the main compositions in the *Anethum* volatile oil are (+)-m-Mentha-1(6),8-diene (15.19%), alpha.-phellandrene (9.93%), Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- (8.19%), respectively.

These two essential oils were screened *in vitro* for antimicrobial activity against a common pathogen *Enterococcus faecalis* using disc agar diffusion technique, as a reslut *both of them* were very effective against *Enterococcus faecali, when the inhibtions zones were* (13mm , 15.5mm) respectively.

REFERENCES

[1] R. A. Sharma, B. Singh, D. Singh, and P. Chandrawat, "Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India," Journal of Medicinal Plants Research, vol. 3, no. 13, pp. 1153–1175, 2009

[2] Rizwana, K., Mohd, A., Shahid Shah, C., & Professor, A. (2019b). Ethnobotany and Unani Perspective of Tukhme Soya (Anethum sowa Roxb.). https://doi.org/10.22270/jddt.v9i3.2823

Identification of *Hedera helix L* essential oils composition collected from northern region of Algeria and prediction of the biological effect of major compounds by ADMET

Belazougui Katia[1], Mecherara-Idjeri Samira[1,*]

[1] Laboratory of Functional Organic Analysis, Faculty of Chemistry, University of Science and Technology Houari Boumediene (USTHB)

* Emails: samira.idjeri@usthb.edu.dz, mecherarasamira@yahoo.fr

Keywords: *Hedera helix* L., Essential oils, variability, Admet method, cytotoxicity

Abstract

Hedera helix L. (Common ivy) is an evergreen dioecious woody liana, one of the 15 species of the genus *Hedera*, Araliaceae family. The ivy was considered as an anti-inflammatory, analgesic and antimicrobial agent in alternative medicine. The aim of this work was to enhance the value of essential oil from six different regions harvested in the north of Algeria of Hedera Helix L leaves by identification of its chemical compounds. In vivo acute toxicity was also tested on an extract. Then, SEM technique was carried out to determine the morphology of glandular trichomes containing the essential oil. Finally, six chemotypes established during the identification was submitted to ADMET pharmacokinetic study.

The results showed a high content non-oxygenated sesquiterpene, which according to a surface scanning electron microscope view performed before and after hydroditillation was located in a peltate glandular trichomes. The physicochemical, drug likeness and pharmacokinetic properties of the target molecules revealed no significant violation of drug likeness rules, a significant bioavailability score and a good permeability, approving these molecules as an ideal drug candidate. The experimental acute toxicity and toxicity predicted by the ADMET were in accordance.

Morphological changes induced by *Thymus Vulgaris* essential oil on *Phyllosticta Citricarpa*: alternative antifungal strategies

Bheki Thapelo Magunga[1,*]

[1] Department of Environmental Health, University of Johannesburg, South University of Johannesburg P.O. Box 524, Auckland Park 2006 Johannesburg 2006 South Africa

* Email: Bhekim@uj.ac.za

Objective

The antifungal resistance currently being reported in the treatment of citrus black spot (CBS), couple with toxic and carcinogenic effects of non-biodegradable synthetic fungicides to human and food systems is a major concern to citrus growers. As such Natural-based products, such as essential oils (EO's) may be used to solve this problem. EO's are gaining global attention to researchers as they are biodegradable, eco-friendly, economical, and safe. The EOs reported in various studies have shown to exhibit antifungal properties by targeting structures responsible for the life cycle of fungal organisms such as ascospores and conidia in different fresh produce. *P. citricarpa* depends on these structures for reproduction and dispersal. Purpose: To investigate the effect of *Thymus vulgaris* essential oil against conidia structure of *P.citricarpa*.

Methods

The effect of thyme oil on *P. citricarpa* conidia structures was evaluated by bio-assay preparation and MICs, the morphological changes that occurred on conidia structures was evaluated using scanning electron microscopy (SEM) and transmission electron microscope (TEM).

Results

Thymus vulgaris was found to damage the conidia structures of this fungal pathogen, one of the key reproductive structures of *P. citricarpa*.

Conclusions

It was evident in this study that thyme oil has the capability to target the conidia structures. Moreover, the results propose that the selected essential oil can be a natural-based alternative to conventional synthetic fungicides currently being used to control CBS.

Effect of wintergreen essential oil and its solution in acute dermatitis *in vivo* model

Eszter Csikós[1], Sarah Shubail[1], Ágnes Kemény[2,3], Margita Szilágyi-Utczás[4], Györgyi Horváth[1*]

[1] Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Pécs, HUNGARY
[2] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, HUNGARY
[3] Department of Medical Biology, Medical School, University of Pécs, Pécs, HUNGARY
[4] Hungarian University of Sports Science, Center of Sports Nutrition Science, Budapest, HUNGARY

* Email: horvath.gyorgyi@gytk.pte.hu

Keywords: *Gaultheria fragrantissima*, *Nigella sativa*, GC-MS, acute dermatitis, *in vivo* mouse model

Objective

Essential oils can be used externally in massage oil to reduce pain or inflammation associated with rheuma. The essential oil of wintergreen (*Gaultheria fragrantissima* L.) is used for anti-inflammatory and joint diseases nowadays [1]. We realized that this essential oil is available in some Hungarian webshops and popular among lay people. The external application of essential oils may cause side effects, e.g. dermatitis. *Nigella sativa* L. (black seed fatty oil) has been used in Arab countries for centuries for food, cosmetic and medicinal purposes [2]. The objective of this work was to evaluate the chemical composition of *Gaultheria fragrantissima* leaf essential oil and to evaluate its effect in alone or in dilution with *Nigella sativa* fatty oil in acute dermatitis *in vivo* mouse model.

Methods

The chemical composition of the wintergreen essential oil was determined with GC-MS technique. In our research, oxazolone-induced contact dermatitis mouse model was used, which demonstrates the type IV hypersensitivity skin reaction. The first phase of the experiment was sensitization with 2% oxazolone on the skin, followed by elicitation, then the measurement of ear edema, blood perfusion, and body weight. Cytokine levels and myeloperoxidase (MPO) activity were measured from the collected samples.

Results

Methyl salicylate was the main component in the wintergreen oil. Oxazolone significantly increased edema and blood flow in the ear during 24 hours compared to the ethanol control, and this change continued until 72 hours. During 72 hours, both black seed fatty oil and 10% ethanol solution of wintergreen essential oil slightly reduced oxazolone-induced ear edema. 10% solution of wintergreen essential oil dissolved in black seed fatty oil significantly reduced this change after 48 hours. The combination of wintergreen essential oil and black seed fatty oil could reduce the blood perfusion on the treated skin area. Furthermore, this combination reduced the MPO activity and TNF-α level in the homogenized ear samples. We plan further histological evaluation from the collected samples.

Conclusions

Based on our results, both black seed fatty oil and wintergreen essential oil can be effective in the treatment of allergic dermatitis. Their combination is proved to be much more effective than the fatty oil and essential oil administered alone due to the possible synergistic effect. 10% of ethanol solution of wintergreen essential oil did not show dermatitis as a side effect.

REFERENCES

[1] Liu W. R., Qiao W. L., Liu Z. Z., Wang X. H., Jiang R., Li S. Y., Shi R. B., She G. M., (2013). Gaultheria: Phytochemical and pharmacological characteristics, Molecules, 18(10), 12071–12108.
[2] Dalli M., Bekkouch O., Azizi S. E., Azghar A., Gseyra N., Kim B., (2021). Nigella sativa L. Phytochemistry and Pharmacological Activities: A Review (2019-2021), Biomolecules, 12(1), 20.

Essential oil composition of *Lithraea molleoides* (Vell.) Engler (*Anacardiaceae*), a controversial medicinal, edible, and allergenic species from South America

Manuel Minteguiaga[1,2,*], Eduardo Dellacassa[2], Cecilia Rodríguez-Rego[2], Enzo Fagúndez[2], Fernando Ferreira[2], Marta Pavarino[1], Patrizia Rubiolo[1], Cecilia Cagliero[1], Barbara Sgorbini[1]

[1] Laboratory of Pharmaceutical Biology and Food Chemistry, University of Turin, Turin, Italy
[2] Chemical Science and Technology, and Aromas Biotechnology Laboratories, University of the Republic, Tacuarembó/Montevideo, Uruguay

* Email: manuel.minteguiaga@pedeciba.edu.uy

Keywords: *Lithraea molleoides*, Essential Oils, Medicinal/Edible/Allergenic Plants, GC-MS, Chiral Selectors

Objective

Lithraea molleoides (Vell.) Engler (Anacardiaceae family) is an evergreen tree species native from South America, considered as a medicinal and edible plant in Argentina [1]. The infusions and decoctions from the vegetative aerial parts are frequently used as medicines for respiratory and digestive illnesses [1]. Besides, the fruits are employed to elaborate fermented beverages [1]. However, in Uruguay this plant is not recognized as medicinal/edible, and it is considered highly allergenic, with frequently reported cases of contact dermatitis in the face and the arms occurring in sensibilized people [2]. Not volatile alk(en)yl-catechols (ACs) have been pointed out as responsible of such contact dermatitis [2]. Moreover, an orally transmitted tradition in Uruguay indicates that such affections are also possible when the people just approach to the trees, without being necessary the contact (that is, an eventual airborne allergy). This suggests the intervention of volatile allergens in the process, a fact that needs to be better investigated given the medicinal/edible utilization of this plant species. As a first step to validate such traditional information, the aim of this work was to characterize the chemical compositions of *L. molleoides* essential oils (LMEO) of Uruguayan origin using different GC-MS methods and stationary phases.

Methods

Aerial parts (i. leaves + small stems; ii. fruits) of *L. molleoides* were collected at Iporá (Tacuarembó, Uruguay), and their essential oils were obtained by both hydrodistillation at laboratory scale and steam distillation at pilot scale. The oils were dried and diluted properly in cyclohexane before the GC-MS analyses. Different stationary phases were employed to obtain more detailed information about the LMEOs composition: SE52-MS, MEGA-Wax-MS, SLB-IL60i, and 2,3-diethyl-6-tertbutyldimethylsilyl-β-cyclodextrin (CD). Mass spectra and linear retention index (LRI) comparisons with commercial/in-house libraries were performed to identify the components.

Results

The yields of LMEOs were around 0.2% (v/w) for both plant materials. Monoterpenes and sesquiterpenes were the main components of the LMEOs, some of which have been previously reported as contact allergy elicitors, among them: α- and β-pinene, δ-3-carene, myrcene (main component: almost, 40% of the oil), *p*-cymene, limonene, 1,8-cineole, α-terpinene, α-terpinolene, α-phellandrene, linalool, α-terpineol, α-terpinyl acetate, β-caryophyllene, aromadendrene, and caryophyllene oxide [3]. As expected, by their low volatility, ACs were not detected in the samples. In addition, and for genuineness purposes, the determination of the enantiomeric excesses of monoterpene chiral compounds was performed with a CD derivative as chiral selector.

Conclusions

The fact that at least 16 reported volatile allergens represented more than 50% of the chemical composition of LMEOs suggests the need of more detailed investigations to ensure the safe use of this plant species.

REFERENCES

[1] López P., Basile P., Wallace F., Olivaro C., Minteguiaga M., Ferreira F, (2021). Medicinal and Aromatic Plants from South America V.2, Springer Nature, Cham. pp. 339-356.
[2] Alé S., Ferreira F., González G., Epstein W., (1997). Allergic contact dermatitis caused by Lithraea molleoides and Lithraea brasiliensis: identification and characterization of the responsible allergens, Am. J. Contact Derm., 8, 144-149.
[3] de Groot A.C., Schmidt E, (2016). Essential Oils, Contact Allergy and Chemical Composition, CRC Press-Taylor and Francis, Boca Raton.

Evaluation of chemical composition and cytotoxic activity of essential oil isolated from Macedonian *Rosmarinus officinalis*

Bojana Kiprijanovska[1], Zoran Zivikj[2], Kristina Shutevska[2], Tanja Petreska Ivanovska[2], Iskra Davkova[1], Ana Trajkovska[1], Gjose Stefkov[1], Ivana Cvetkovikj Karanfilova[1], Marija Karapandzova[1*]

[1] Institute of Pharmacognosy, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia

[2] Institute of Applied Biochemistry, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia

* Email: marijakarapandzova@yahoo.com

Keywords: *Rosmarinus officinalis;* essential oil; chemical composition; GC/MS; brine shrimp lethality test.

Objective

Rosmarinus officinalis has been used since ancient times due to its medicinal and cosmetical properties. Its essential oil has bioactive components with various beneficial properties and is traditionally used as cholagogue, diaphoretic, digestant, diuretic, emmenagogue, laxative etc. Also, this essential oil is used in the treatment of headache, menstrual disorders, nervous complaints, tiredness, defective memory, sprains and bruises [1]. Due to records indicating its widespread use as well as concerning reported cases of essential oil exposure [2] the question regarding its appropriate and safe usage arises. Therefore, the aim of our study was to determine the essential oil composition of *Rosmarinus officinalis* originating from R.N. Macedonia and its cytotoxic potential.

Methods

The essential oil was obtained by hydrodistilation in Clevenger apparatus using the official Ph. Eur. method. The chemical composition was obtained with GC/MS method. The cytotoxic evaluation was conducted by brine shrimp lethality assay (BSLA) [3]. LC_{50} was determined from the 24 hours counts using the probit regression analysis method.

Results

Twenty components were identified, representing 92.99% of the total essential oil. The most abundant components were 1,8-cineol (27.42%), camphor (15.35%), α-pinene (14.49%), myrcene (5.49%), camphene (4.99%), β-pinene (3.46%), borneol (3.43%), verbenone (3.24%), linalool (3.05%), limonene (2.68%), E-caryophylene (2.14%), Δ3-carene (1.75%), bornyl acetate (1.66%), terpinolene (1.33%) and γ-terpinene (1.15%). The essential oil was categorized using the Meyer's [4] and Clarckson's [5] scale of toxicity. According to both scales of toxicity, this oil showed cytotoxic effects (LC_{50} of 13.85 $\mu g/mL$) and consequently is characterized as toxic according to Meyer's and highly toxic according to Clarkson's criteria.

Conclusions

This cytotoxic effects are probably due to the presence of the 1.8-cineole and camphor, which have confirmed toxic properties [6, 7]. However, further examinations should be done in order to define the proper mechanism of cytotoxic activity as well as possible synergistic effect with the other present constituents in order to establish the safe usage of the *Rosmarinus officinalis* essential oils.

REFERENCES

[1] WHO monographs on selected medicinal plants (World Health Organization. WHO Monographs on Selected Medicinal Plants (Vol. 4)

[2] Lee, K. A., Harnett, J. E., & Cairns, R., (2019). Essential oil exposures in Australia: analysis of cases reported to the NSW Poisons Information Centre. Med. J. Aust.

[3] McLaughlin, J.L., Rogers, L.L., Anderson, J.E., (1998). The use of biological assays to evaluate botanicals. Ther. Innov. Regul. Sci. 32(2), 513-524.

[4] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5), 31-34.

[5] Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., Folb, P.I., (200). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol. 92(2-3), 177-191.

[6] Ellenhorn, M.J., Barceloux, D.G., (eds): Camphor. In Medical Toxicology: Diagnosis and Treatment of Human Poisoning, Elsevier,New York, 1988: 505-507.

[7] Bhowal, M., Gopal, M. (2016). Eucalyptol: Safety and Pharmacological Profile. RGUHS J. Pharm. Sci. 5, 125–131.

Volatile constituents and cytotoxic screening using BSLA of commercially available *Thymi aetheroleum*

Natasa Draskovik[1], Zoran Zivikj[2], Ana Marija Bajatovska[2], Tatjana Kadifkova Panovska[2], Iskra Davkova[1], Ana Trajkovska[1], Ivana Cvetkovikj Karanfilova[1], Svetlana Kulevanova[1], Marija Karapandzova[1*]

[1] Institute of Pharmacognosy, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia

[2] Institute of Applied Biochemistry, Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia

* Email: marijakarapandzova@yahoo.com

Keywords: *Thymus vulgaris*; essential oil; chemical composition, GC/MS, cytotoxicity.

Objective

Thymus vulgaris is an herb that may provide a long list of health benefits. According to Ph. Eur.11, 0,1-2.5% of the plant material is composed of essential oil, which has unique chemical properties and pharmacological activity, making it valuable for therapeutic purposes and highly beneficial for health. The expectorant, bronchospasmolytic, and antiseptic effects [1] of the oil are due to the presence of terpene components, particularly monoterpenes and sesquiterpenes, which are known to act in synergy. Furthermore, *thymi aetheroleum* is used as a main component in lot of cosmetic preparations which are used in the treatment of acne and problematic skin. According to this, the aim of this study was to examine the qualitative and quantitative composition as well as the cytotoxic potential of a commercial sample of thyme essential oil available on the market in R.N. Macedonia.

Methods

An optimized GC/MS method was used to determine the chemical composition of the essential oil. *In vivo* model using brine shrimp lethality assay (BSLA) [2] was used to determine the cytotoxic potential of the tested oil.

Results

Fifteen terpenoid components were identified, representing 90% of the total essential oil composition. The most abundant components were o-cymene (38.60%), thymol (35.06%), α-pinene (6.03%), camphene (2.24%), α-terpineol (1.45%), 1,8-cineole (1.40%), terpinolene (1.33%), and linalool (1.07%).With regard to the evaluation of the cytotoxicity of the essential oil, the LC50 of 0.75 µg/mL is considered toxic according to the Meyer's scale [3] and highly toxic according to the Clarkson's scale [4] of toxicity.

Conclusions

Due to the positive correlation among the obtained results from the BSLA and antitumor properties of the tested essential oil, additional examinations should be done in order to define the relationship between the determined chemical composition and cytotoxic activity of thyme essential oil.

REFERENCES

[1] S. Kulevanova, G. Stefkov, M. Karapandzova, I. Cvetkovikj Karanfilova (2023) Pharmacognosy, 3rd edition (265-267)

[2] McLaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. Ther. Innov. Regul. Sci. 32(2), 513-524.

[3] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5), 31-34.

[4] Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., Folb, P. I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol. 92(2-3), 177-191

Biological activity and compositional analysis of essential oil of *Homalomena aromatica* Schott: A high value aromatic species from Northeast India

Raghu Tamang[1,2], Tanmita Gupta[1,2], Twahira Begum[2], Roktim Gogoi[1], Neelav Sarma[1], Vikash Kumar Choubey[1] and Mohan Lal[1*]

[1]Agro- technology and Rural Development Division, CSIR-North East Institute of Science and Technology (NEIST), Jorhat Assam 785006, INDIA
[2]AcSIR- Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India, 201002

*Email: drmohanlal80@gmail.com

Keywords: *Homalomen aaromatica*, essential oil, biological activity, Northeast India

Objective
Homalomena aromatica Schott. is a valuable aromatic and medicinal plant having a wide range of application in ethnobotany, pharmacology, perfume and flavor industries. The current study aimed to evaluate the compositional analysis of essential oil and its antioxidant, anti-inflammatory, anti-diabetic, tyrosinase inhibitory activity, acetylcholinesterase (AChE) activitiey and protease inhibitory activity of rhizome essential oil of *H. aromatica* from Northeast India.

Methods
Germplasm of *Homalomena aromatica* were collected from the experimental farm of Council of Scientific and Industrial Research - North East Institute of Science and Technology (CSIR- NEIST), Jorhat, Assam, India.The rhizome essential oil of *Homalomena aromatica* was extracted by Clevenger apparatus and analyzed through gas chromatography mass spectroscopy (GC/MS). DPPH free radical scavenging assay, ABTS assay, metal chelating activity, anti-inflammatory, anti-diabetic activity, acetylcholinesterase (AChE) activities, tyrosinae inhibitory assay and protease inhibitory activity were performed as per standard protocol.

Results
The yield of essential oil was found (1.20% w/w). GC/MS profiling of *H.aromatica* essential oil revealed linalool as a major compound (66.27%), followed by *tau*-muurolol, α-cadinol, linalool oxide, neointermedeol and 3-carene as other compounds. Antioxidant activity was evaluated using three different methods *viz*., DPPH, ABTS, and metal chelating activity. DPPH radical scavenging activity results IC_{50} value of 50.12µL/mL. ABTS activity results IC_{50} value of 33.05µL/mL. Again, metal chelating analysis revealed IC_{50} value of 35.23µL/mL. Protease inhibitory activity results IC_{50} value of 19.59µL/mL. Albumin denaturation assay depicted IC_{50} value of 32.16µL/mL. Again, α-amylase inhibitory activity results IC_{50} 29.84µL/mL. Tyrosinase inhibitory activity results IC_{50} value of 73.62µL/mL which is more than the standard kojik acid (23.36µL/mL). Strong AChE inhibitory activity was observed with an IC_{50} value of 38.13µL/mL.

Conclusions
The current research provides a detail information regarding the essential oil composition of *H. aromatica* and its various biological activity which can be used in numerous pharmacological applications as well as in drug development. Hence, the present research findings could be used in pharmaceutical sector after in vivo study. .

ACKNOWLEDGMENTS

We appreciate that the director of CSIR-NEIST Jorhat, Assam, India gave us access to the research laboratories as well as experimental farm. The research work was funded by Government of India, in the form of project CSIR-Aroma Mission, HCP-0007.

Kaempferia parviflora Wall., ex Baker (Black ginger) a high value ethnomedicinal plant endemic to Northeast region of India: Its rhizome essential oil chemical makeup & pharmacological potential evaluation

Twahira Begum[2], Roktim Gogoi[1,2], Neelav Sarma[1,2], Mohan Lal[2],*

[1] AcSIR-Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, 201002, INDIA
[2] Agrotechnology and Rural Development Division, CSIR-North East Institute of Science and Technology (NEIST), Jorhat, Assam 785006, INDIA

*Email: drmohanlal80@gmail.com

Keywords: Anti-cholinesterase, GC/MS, *Kaempferia parviflora*, Protease, Tyrosinase

---

Objective
Kaempferia parviflora Wall., ex Baker is a perennial plant with numerous medicinal properties, an endemic species, only found in Indian-Myanmar border region. The aim of the present study was to evaluate *K. parviflora* rhizome essential oil yield, chemical profile as well as biological activities.

Methods
The essential oils extracted by hydrodistillation unit by using Clevenger apparatus and were chemically profiled by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS).Essential oils were also analysed for antioxidant, anti-inflammatory, antimicrobial, anti-cholinesterase, tyrosinase, anti-diabetic, and genotoxicity properties.

Results
Essential oil extracted from rhizome contains 0.92% (w/w). GC and GC/MS analysis revealed linalool as the major compound with significantly higher linalool content 43.35%.A total of 30 compounds were identified in GC/MS analysis with an area percentage of 99.14%. The essential oil exhibited superior activities than the standards. The essential oil exhibited antioxidant potential with an IC_{50}value of 24.01in DPPH ppm; 7.14 ppm in ABTS; and 21.277 ppm in metal chelating assay. Anti-inflammatory potential with an IC_{50} value of28.16ppm in albumin denaturation; 119.91 ppm in protease inhibitory.Anti-cholinesterase activity with an IC_{50}value of 3.3249 ppm, tyrosinase activity with an IC_{50}value of 08.76 ppm, and anti-diabetic activity with an IC_{50}value of 06.77 ppm. However, essential oils possessed inferior antibacterial and weak antifungal properties towards 8 microbial strains, except against *Micrococcus luteus*(13 ± 1.52 mm at 500 ppm concentration) and *Fusarium keratoplasticum* (18 ± 1.08 mm at 500 ppm concentration). Considering the genotoxicity assay, the essential oils was found to be non-toxic with a chromosomal aberration rate of 13.00%.

Conclusion
From the results it can be concluded that *K. parviflora* rhizome essential oil composes with high concentration (43.35%) of linalool content which might be responsible for strong pharmacological activities. Further, the essential oils can be used for herbal as well as pharmaceutical drug formulations considering their higher biological activity as compared to standards.

Acknowledgements
The funding agency: CSIR, New Delhi, Project Grant- HCP-0007.

Identification of a Novel Myrcene and Methyl iso-Eugenol Rich Essential Oil Variant (Jor Lab L-11) of Lemongrass (*Cymbopogon flexuosus* L.)

Mohan Lal[*1], Twahira Begum[1], Sunita Munda[1]

[1]CSIR-North East Institute of Science and Technology, Jorhat, Assam -785006, INDIA

* Email: drmohanlal80@gmail.com

Keywords: essential oil, high yielding variety, lemongrass, methyl iso-eugenol, myrcene, varietal development

Objective

Cymbopogon flexuous of the family Poaceae is a highly valued grass known throughout the world for its aromatic properties. The objective of the study is to identify novel myrcene and methyl iso-eugenol rich essential oil variety of lemongrass. Myrcene is an earthy, spicy, clove fragrance commonly used in the culinary and perfume industry while methyl iso-eugenol has a typical sweet-warm, spicy- clove, woody, floral odour mostly used in perfume industries.

Methods

In the present study, a total of 230 lemongrass accessions were collected from different regions of North East India which were planted in Randomized Complete Block Design (RCBD) with three replications at experimental farms of CSIR-NEIST, Jorhat, during the year 2015. Morphological and essential oil quality data were recorded during the studied the year 2015-16 and 2016-17. Based on two years of selection trial data a high myrcene and methyl iso-eugenol rich essential oil line was identified and named as Jor Lab L-11. The identified line was planted along with two checked varieties at four different locations during the year 2017-18.

Results

The average essential oil yield of the Jor Lab L- 11 for the multi-location trial was 0.58 % and contained an average of 48.02 % and 39.11 % myrcene and methyl iso-eugenol respectively as major component in the essential oil. Myrcene and methyl iso-eugenol percentage, essential oil percentage, tillers/plant were significantly higher in the identified variety compared to check varieties. GC/MS data obtained from essential oil leaf Jor Lab L- 11 essential oil showed limonene, elemicin, β-pinene, citronellol, α-pinene, neryl acetate, linalool, methyl eugenol, geranyl acetate, geraniol, citral b, citronellal, citral a, α-terpineol, nerol, eucalyptol, caryophyllene oxide, 2-methylbicyclo[4.3.0]non-1(6)-ene, m-camphorene, camphor, diepi-cubenol, T-cadinol, anethole and epi-α-murulol as minor compounds.

Conclusions

The present study reveals the lemongrass variety with myrcene and methyl isoeugenol rich essential oil was identified based on a two-year selection trial with multilocation trials.The variety was based on clonal selection. The newly developed variant of lemongrass with myrcene and methyl isoeugenol rich essential oil can be highly beneficial for meeting the industrial demands.

ACKNOWLEDGMENTS

This research work is a part of Council of Scientific and Industrial Research New Delhi, Government of India CSIR-Phytopharmaceutical, Aroma Mission project (HCP-0010 and HCP007). The authors are grateful to the Director, CSIR NEIST, Jorhat, for his consistent support and encouragement during the investigation.

Chemical composition and bioactivities of Azorean *Cryptomeria japonica* essential oils extracted by two different methodologies

Alexandre Janeiro[1,2], Tanner Wortham[3*], Filipe Arruda[1,2], Ana Lima[1,4], Tânia Rodrigues[2], José Silvino Rosa[2] José Baptista[1,4], Elisabete Lima[1,4]

[1] Institute of Agricultural and Environmental Research and Technology, Azores University, Angra do Heroísmo, PORTUGAL
[2] Department of Biology, Faculty of Science and Technology, Azores University, Ponta Delgada, PORTUGAL
[3] The Perfumery, 621 Park East Blvd. New Albany, IN, USA
[4] Department of Physics, Chemistry and Engineering, Azores University, Ponta Delgada, PORTUGAL
[5] Biotechnology Centre of Azores, Azores University, Ponta Delgada, PORTUGAL

* Email: twortham@theperfumery.com

Keywords: Azorean forestry wastes valorisation, *Cryptomeria japonica*, essential oil, hydrodistillation, water-steam distillation, Essential oil chemical variability, antibacterial, antifungal

Objective

Cryptomeria japonica (Thunb. ex L.f.) D. Don (Cupressaceae) is the most important commercial tree species in the Azores archipelago, Portugal. Consequently, its industrial exploitation generates large amounts of biomass waste, such as leaves (CJL), which can be further utilized as a rich source of essential oils (EOs). Thus, this study sought to unravel and compare the chemical variability, antibacterial and antifungal properties in CJL EOs extracted by hydrodistillation (HD) and water-steam distillation (WSD), from trees grown in S. Miguel Island, Portugal.

Methods

CJL was colected in S. Miguel island during the summer of 2022, in Achada (37°48'28.59''N , 25°16'16.14'' W, altitude 780 m). The fresh material was subjected to both HD, using a Clevenger-type apparatus, and WSD for 3 h. The chemical composition of the EOs was analyzed via gas chromatography-mass spectrometry (GC-MS). Four bacterial strains were selected for this study, namely *Micrococcus luteus* DSM 20300 and *Staphylococcus aureus* DSM 1104, *Escherichia coli* DSM 491 and *Serratia marcescens* DSM 48 and two fungal species, *Penicillium digitatum* and *Penicillium italicum*. The bioactivity of the EOs was evaluated employing the Kirby-Bauer method with some modifications.

Results

The yield of the EOs was 0.62 ± 0.02% and 0.36 ± 0.14% (fresh weight v/w) for HD-EO and WSD-EO, respectively. Regarding the chemical composition, 93 components were identified in HD-EO and 94 in WSD-EO, comprising 98.68% and 99.29% of the total contents, respectively. Additionally, some noteworthy differences are the higher amounts of monoterpene hydrocarbons in WSD-EO (29.07%), while HD-EO is more abundant in oxygenated sesquiterpenes (OS) (26.52%). Both EOs were active against *S. aureus*, with a diameter inhibition zone of 10.00 ± 3.00 mm and 8.33 ± 2.08 mm for HD-EO and WSD-EO, respectively. Only HD-EO was active against *E. coli* (6.33 ± 0.58 mm) and *M. luteus* (9.00 ± 2.00 mm). None of the EOs proved to be active against *S. marcescens* nor the fungal species tested.

Conclusions

In this study we elucidated the impact of the distillation method in the chemical composition of Azorean CJL EOs. It is further supported by HD-EO exhibiting an overall higher bioactivity than WSD-EO against the microorganisms tested, which could be due to its higher content in OS. Further research is required to formally determine if the OS from HD-EO were responsible for the observed bioactivities.

ACKNOWLEDGMENTS

This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Filipe Arruda thanks FRCT for Doctoral Research Scholarship (refª M3.1.a/F/008/2021).

Variability in chemical composition and antibacterial activity of *Cryptomeria japonica* essential oil from different locations on São Miguel island, Azores

Filipe Arruda[1,2], Tanner Wortham[3*], Ana Lima[1,4], Alexandre Janeiro[2], Tânia Rodrigues[2], José S. Rosa[2,5], José Baptista[1,4], Elisabete Lima[1,4]

[1] Institute of Agricultural and Environmental Research and Technology, Azores University, Angra do Heroísmo, PORTUGAL
[2] Department of Biology, Faculty of Science and Technology, Azores University, Ponta Delgada, PORTUGAL
[3] The Perfumery, 621 Park East Blvd. New Albany, IN, USA
[4] Department of Physics, Chemistry and Engineering, Azores University, Ponta Delgada, PORTUGAL
[5] Biotechnology Centre of Azores, Azores University, Ponta Delgada, PORTUGAL

* Email: twortham@theperfumery.com

Keywords: Azorean forestry wastes valorisation, *Cryptomeria japonica*, essential oil, hydrodistillation, antibacterial

---

Objective

Cryptomeria japonica (Thunb. ex L.f.) D. Don, commonly known as japanese cedar and belonging to the Cupressaceae family, holds immense economic significance in the Azores archipelago, Portugal. The industrial processes associated with this tree species generates a substantial volume of biomass wastes, such as foliage, which constitute a valuable opportunity as a abundant source of essential oils (EOs). This study aimed to investigate the variability in the chemical composition and antibacterial activity of *C. japonica* leaves' EO grown in two different locations of S. Miguel island, Portugal.

Methods

C. japonica foliage was collected in S. Miguel Island in November 2022, at the locations Achada (ACH) (37°48'28.59''N , 25°16'16.14'' W, altitude 780 m) and Cerrado dos Bezerros (CB) (37°44'41.4''N , 25°21'52.3'' W, altitude 460 m). The fresh plant material was subjected to hydrodistillation (HD) for 3 h, using a Clevenger-type apparatus. The chemical composition of the EOs was analysed by gas chromatography–mass spectrometry (GC–MS). Antibacterial activity was assessed by agar disc diffusion method against Gram+ (*Micrococcus luteus* DSM 20300 and *Staphylococcus aureus* DSM 1104) and Gram- (*Escherichia coli* DSM 491 and *Serratia marcescens* DSM 48) bacteria, according to Kirby-Bauer method.

Results

EOs yield was 0.95% (*v/w*; F.W.) and 0.66% (*v/w*; F.W.) for ACH and CB, respectively. Concerning the EOs chemical composition, 89 components were identified in the ACH-EO and CB-EO, accounting for 98.51% and 99.25% of the total content of the EOs components, respectively. Both EOs displayed similar compositions, being CB-EO richer in monoterpene hydrocarbons, particularly in limonene content (9% vs 1%) and in diterpene hydrocarbons (24% vs 20%), but poorer content in oxygenated diterpenes, specifically in nezukol (1.2% vs 4.1%). Regarding antimicrobial activity, both EOs revealed weak activity (< 10 mm) against Gram-positive bacteria and no activity against Gram-negative ones.

Conclusions

In this study, we revealed significant differences in the yields and chemical composition of *C. japonica* leaf EOs, collected from two locations on S. Miguel island, Portugal. Our results indicated that the geographic location of the collection site and the growing conditions could affect the yield and chemical components of *C. japonica* EO, but without significant effect on antibacterial activity.

ACKNOWLEDGMENTS

This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Filipe Arruda thanks FRCT for Doctoral Research Scholarship (refª M3.1.a/F/008/2021).

Chemical composition and antioxidant activity of *Juniperus* species found in the Indian Himalayan Region

Abhishek Gupta[1,3 *], Baleshwar Meena[2], Abhishek K. Dwivedy[3], Tikam Singh Rana[1]

[1] CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India
[2] CSIR-Traditional Knowledge Digital Library, New Delhi-110 067, India
[3] Department of Botany, Banaras Hindu University-221005, India

* Email: abhigupta962@gmail.com

Keywords: Essential oil, Indian Himalayan Region, Juniper, Terpenes

Objective

Genus *Juniperus* L. are aromatic shrubs and trees growing throughout Indian Himalayan Region (IHR) from Union Territory of Jammu & Kashmir to Manipur at altitudes 2500-4700 m msl [1]. Junipers are primarily used as food, spice, medicine, flavour, fragrance, timber, and ornamentals since ancient times. This study was aimed to determine comparative yield of needle essential oils (EO) of six *Juniperus* species viz., flaky juniper (*Juniperus squamata* Buch.-Ham. ex D.Don), pencil juniper (*Juniperus semiglobosa* Regel.), common juniper (*Juniperus communis* L.), black juniper (*Juniperus indica* Bertol.), drooping juniper (*Juniperus recurva* Buch.-Ham. ex D.Don) and weeping juniper (*Juniperus fargesii* Kom.) sampled from wild in IHR, their chemical composition and antioxidant potential for prospective healthcare interventions.

Methods

Representative sampling of six *Juniperus* species to ascertain morphological identity as well as EO extraction was undertaken in five Indian states (Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim & Nagaland). The EOs were obtained by steam distillation from shade dried chopped terminal twigs and were characterized by GC-MS. The antioxidant activity was determined using 1,1-diphenyl-2-picrylhydrazyl radical method (DPPH) and EC50 value was defined as the dose of sample which reduced the initial DPPH of 50%.

Results

The minimum and maximum EO yield (v/w) was observed in samples of *J. fargesii* (2.0%) and *J. indica* (5.8%). A total 101 chemical compounds were identified by GC/MS representing 93.56%, 96.30%, 98.27%, 95%, 97.03% and 97.83% of *J. squamata*, *J. semiglobosa*, *J. communis*, *J. indica*, *J. recurva* and *J. fargesii* EOs, respectively. The terpenoids found in EOs were classified into monoterpenes (29.34-61.47%), oxygen containing monoterpenes (4.66-23.37%), sesquiterpenes (5.37-19.4%), oxygen containing sesquiterpenes (13.53-44.66%) and diterpenes hydrocarbons (0.64-1.84%). The most abudant constituent of EOs, α−Pinene ranged from 2.49-31.53% followed by Sabinene (0.25-23.27%), α−Elemol (0.22-19.85%), D−Limonene (1.45-16.78%), Bornyl acetate (to 16.87%), 4−Terpineol (0.49-10.55%), δ−3 Carene (to 8.96), β−Myrcene (2.15-5.89%) and δ−Cadinene (to 5.11). Principal component analysis (PCA) indicated that α−Pinene and Sabinene might be utilized as potential markers to delimit *Juniperus* species in IHR. DPPH based antioxidant assay revealed comparable EC_{50} value for the six juniper EOs ranging from 23.95 µg/ml (*J. indica*) to 53.17 µg/ml (*J. semiglobosa*) with a mean (34.71 µg/ml).

Conclusions

Among the investigated species *J. indica* resulted maximum EO (v/w) yield. The EOs showed predominance of monoterpenes hydrocarbons followed by other terpenes. The exhibition higher antioxidant potential by *J. indica* EO than others might be due to higher content of sabinene and α−Elemol. These findings on Junipers substantiate their historical use for food, medicine, spice, beverages as well as other industrial applications and suggests for developing conservation strategy in IHR.

REFERENCES

[1] Adams R. P., 'Junipers of the World: The Genus Juniperus', Trafford. Publ. 2014, 4th edn.

Do not waste the thyme! Essential oils and distillation by-products of different *Thymus* taxa

Zsuzsanna Pluhár[1,*], Karim Etri[1], Klára Ruttner[1], Dorisz Boros[1], Beáta Gosztola[1]

[1] Department of medicinal and Aromatic Plants, Institute of Horticultural Science, Hungarian University of Agriculture and Life Sciences, Budapest, HUNGARY

* Email: zsuzsanna.pluhar@uni-mate.hu

Keywords: garden thyme varieties, residual herb, residual water, total polyphenol content, antioxidant capacity

Objective

By-products of hydrodistillation can be considered as rich sources of biologically active compounds (polyphenols, triterpenic acids, etc.), which are also detected in *Thymi herba*. Beyond thymol type essential oil, distillation water and solid residues may also have various possible applications due to their aromatic and antimicrobial properties. The purpose of this experiment was to determine the content and composition of thyme (*Thymus vulgaris* L., T. *pannonicus* All.) essential oils (EO) as well as total polyphenol content (TPC) and antioxidant capacity (AC) of by-products (residual water, residual herb) derived from hydro-distillation of dried drug samples originating from different varieties. Moreover, this study aimed to point out the possible differences in polyphenol supplying ability among thyme taxa involved.

Methods

Flowering aerial parts of 4-year-old populations belonging to 6 *Thymus vulgaris* L. varieties with thymol type essential oil ('Deutscher Winter', 'Varico 3', 'Standard Winter', 'French Summer', 'Sloneczko', 'Pannon Timol') and to 1 *Thymus pannonicus* All. (Ceglédbercel) as well as to 4 *Thymus vulgaris* L. clones with different major EO compounds (TV115: geraniol; TV121: linalool; TV135: thymol; TV143: α-terpineol) were collected in May 2022, by 5 replications. According to the requirements of Pharmacopoeia Hungarica [1], samples processed to obtain *Thymi herba* by natural air-drying and crumbling. Dried original samples (OS) were hydro-distilled by a Clevenger type apparatus for 2 h. The volatile oil samples were subjected to GC/MS analysis using an Agilent Technologies 6890 N GC equipped with an Agilent Technologies MS 5975 inert mass selective detector. After the distillation procedure, we collected the residual water (RW) and the residual herb (RH) of each samples as by-products. Total phenolic content (TPC) and antioxidant capacity (AC) of all the original samples (OS), liquid (RW) and solid (RH) distillation waste products were then determined. Based on a modified method of Singleton and Rossi (1965), TPC assessment results expressed as a gallic acid equivalent (mg GAE g^{-1} DW). The antioxidant capacity was determined by the FRAP assay according to Benzie and Strain (1996), where AC values of samples were calculated from a standard curve equation and expressed as ascorbic acid equivalent based on the dry weight (mg AAE g^{-1} DW).

Results

Significant differences were found in the essential oil content of taxa involved, where *T. vulgaris* 'Deutscher Winter', TV121 and TV143 represented the highest values (>2.0 ml/100 g DW), followed by 'Varico 3', 'Sloneczko' and TV115 (>1.5 ml/100 g DW). In general, original powdered samples (OS) prior to distillation resulted in the best antioxidant capacity (AC) values (250-300 mg AAE g^{-1} DW) in comparison with those of the distillation by-products (RW, RH: 100-200 mg AAE g^{-1} DW) There were not significant differences in AC data neither among thyme taxa involved, nor between residual water and residual herb of the same variety. On the contrary, total polyphenol content (TPC) varied according to the extract type and thyme varieties, with peak values in the residual water (174.93-271.91 mg GAE g^{-1} DW) and with the lowest ones in the residual herb (47.60-153.45 mg GAE g^{-1} DW). Outstanding TPC levels (>250 mg GAE g^{-1} DW) detected at 'Deutscher Winter' and 'French Summer', while 'Varico 3', TV135, TV143 and *T. pannonicus* were also valuable belonging to the group with >200 mg GAE g^{-1} DW.

Conclusions

We can conclude that both distillation by-products (RW, RH) still possess by considerable AC, while excellent TPC values can be found mainly in RW of thyme. The highest AC values of the original drug samples was probably due to the presence of monoterpene phenols, thymol carvacrol and derivative compounds prior to distillation.

REFERENCES

[1] Pharmacopoeia Hungarica, 8th ed. (2004). Medicina Publisher, Budapest, Hungary

Comparison of the two EO extraction methods and its influence on yield and composition of *Mentha×piperita* cv. Kristinka

Ivan Kron[1,*], Saroj Kumar Chaudhary[2], Jozef Fejér[3], Daniela Gruľová[3]

[1] Training & Consulting Ltd., Žilina, SLOVAKIA
[2] Central Department of Chemistry, Tribhuvan University, Kritipur, Kathmandu, NEPAL
[3] Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, SLOVAKIA

* Email: kron.ivan@gmail.com

Keywords: peppermint, hydrodistillation, ultrasonic assisted extraction, menthol, monoterpene

Objective

Ultrasound-assisted extraction is a promising technique to obtain active compounds from plants with high efficiency [1]. Ultrasound-assisted extraction (UAE) comes to the forefront with several advantages. The UAE method present simple and clean process, but also ultrasound effect improves the extraction efficiency by increasing the penetration of solvent into the plant cells via cavitation.

Mentha×piperita L. is one of the most studied aromatic and medicinal plant in the world. The quality of the plant material depends on cultivar as well as on the environmental condition where the plants are growing. *Mentha×piperita* L. cv. Kristinka was bred at University of Presov (Slovakia) and it is known for the high content of the menthol (about 70%)[2]. UAE method was used for this cultivar for the first time to increase the EO yield in comparison to the coventional method of hydrodistillation without UAE. The influence on the quantity of the main components as menthol and menthone were also evaluated.

Methods

M. piperita cv. Kristinka was grown in the experimental field belongs to University of Presov, Slovakia. Plant material was harvested in 2022 and dried in room temperature for few days. Dried aboveground parts were then ground. For UAE, 25 g of sample was taken inside a polyester bag, placed into a sonicator containing 500 mL of distilled water and sonicated at 40 °C for 30 minutes. After that, hydrodistillation of the UAE was performed using Clevenger apparatus for 3 hours. Likewise, another 25 g of sample was taken into a round bottom flask and hydrodistilled conventionally for 3 hours without the aid of Ultrasonic. All experiments were performed in duplicate. After the completion of distillation, essential oils were collected separately. The yield and composition of the essential oils were compared. Dominant components were identified by using GC-MS.

Conclusions

Several methods are applied for the acquisition of essential oils, the most frequently used is steam or hydro-distillation. That method is used to obtain 93% of essential oils, the remaining 7% being acquired with other methods. Based on the previous study and statistical analysis of the results show that the oil efficiency was significantly higher for treatments in which ultrasound-assisted maceration was applied, relative to the control sample.

ACKNOWLEDGMENTS

Presented research was supported by the grants VEGA 1/0087/20 and VEGA 2/0018/20.

REFERENCES

[1] Tekin K., Akalın M. K., Şeker M. G. (2015) Ultrasound bath-assisted extraction of essential oils from clove using central composite design, Industrial Crops and Products, 77, 954–960
[2] Fejér J., Gruľová D., De Feo V. (2017) Biomass production and essential oil in a new bred cultivar of peppermint (Mentha × piperita L.), Industrial Crops and Products, 109,812–817

Development of a green pesticide based on essential oils for grain crops in Brazil.

Nilson Borlina Maia[1*], José Roberto Gonçalves[1*], Sergio Pierobom Borlina[1*]

[1] Linax Óleos Essenciais, Votuporanga, BRAZIL

* Email: linax@uol.com.br

Keywords: Green Pesticides, essential oil, organic fungicide, organic inseticede, natural pesticide.

Objective

The objective of this work was to develop a commercial pesticide with active principles based on essential oils, environmentally friendly, has low toxicity, and causes less damage to human and animal health, in order to replace the insecticides and fungicides traditionally used in extensive grain crops. So, based on an extensive bibliographic review, 23 essential oils that had already been tested were chosen for the control of 22 pests of importance to Brazilian crops. Only essential oils that can be produced locally and have the possibility of having a stable supply chain of raw materials were chosen to be used in the formulations to be developed.

Mixtures in different concentrations of oils were tested for physical and chemical stability, shelf life, and the viability of mixing with other products for application in the field. Three prototypes of insecticides and three fungicides were tested and compared with conventional products in the laboratory and in field trials located in different producing regions. One fungicide prototype was economically viable. The product is on registration, and it is expected to be commercially launched next year. The brand trend has the potential to reach up to 10% of the defensive market in Brazil.

Methods

Essential oils extracted from plants of the families *Meliaceae, Lamiaceceae, Poaceae, Rutaceae, Rubiaceae, Caryophyllaceae,* and *Myrtaceae* with scientifically proven insect and fungus control were blended and formulated with dispersants for proper field pulverization. The six more stable mixtures were evaluated in the entomology laboratory both with spraying in a Portter camera and in specific diets, to define the LD50 and minimum dose for *Bemisia tabaci. Brevicoryne brassicae, Diaphorina citri, Helicoverpa armigera,* and *Tetranychus urticae in vitro* tests were performed with mycelia of Colletrotrichum truncatum (isolated from soy and cotton), Sclerotinia sclerotiorum, and Sclerotium rolfsii. Twenty field trials were carried out on crops of corn (*Zea mays)*, soybean (*Glycine max),* and coffee (*Coffea arabica).* The phytotoxic effects of the formulations were evaluated, and the safe dosages to be applied for the control of insects and fungal diseases were defined. The field trials showed greater efficiency were selected for commercial release.

Results

When applied in fields trials, formulations developed for insecticidal action showed lower phytoxidity than fungicide prototypes. Nevertheless, both could be used with high safety in dosages of up to 1Lha-1. The product developed for fungicide showed high efficiency in controlling Colletrotrichum truncatum in vitro. The developed product achieved the same productivity as the conventional product. The aging of the formulation reduced the disease control effectiveness and productivity, indicating that the product has a longer shelf life than the conventional product. The association of half the dose of the product based on essential oil allowed the reduction of half the dose of the conventional product, maintaining the same productivity, showing great potential for reducing environmental impacts and increasing profitability for farmers. Insect control with the essential oil-based product in the field was not as effective as the fungicide product.

Conclusions

The fungicide developed based on essential oils developed in this work, showed great potential to replace conventional fungicides, with economic competitiveness and low environmental impact.

Effects of chopping and different preservation methods on the volatiles and organoleptic properties of Parsley (*Petroselinum crispum* (Mill) Nym. var. *neapolitanum)* leaves

Beáta Gosztola[1*], Zoltán Kovács[2], Zsanett Bodor[2], Urbashi Hazarika[1]

[1] Department of Medicinal and Aromatic Plants, Institute of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences (MATE), Budapest, HUNGARY

[2] Department of Measurements and Process Control, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences (MATE), Budapest, HUNGARY

* Email: gosztola.beata@uni-mate.hu

Keywords: chopping, essential oil, organoleptic properties, parsley, preservation

Objective

Flat-leaf (or Italian) parsley is a well known biennial plant belonging to the *Apiaceae* plant family with significant importance in culinary and herbal medicine. Its aromatic leaves are generally used in dried and cut form as a spice or phytotherapeutic ingredient, but the order of processing and the method of preservation can significantly affect the quality of final product. The aim of this study was to evaluate the effect of chopping of fresh leaves before drying, furthermore to find the best preservation methods in order to conserve the active substance content and organoleptic properties (color, taste) of parsley leaves.

Methods

A selected flat-leaf parsley (*P. crispum* var. *neapolitanum*) strain was used for the examinations. The plantation was established in Fajsz, Hungary. Approximately 8 kg of fresh leaves were harvested at the end of June, before flowering. The homogenised plant material was divided into two parts: one part was chopped immediately after harvest, while the other half remained in whole. Then in both cases different preservation methods were applied (sun drying, shade drying, oven drying at 40 and 60ºC, lyophilization, microwave drying at 250 and 700W, freezing at -18ºC), and compared to the fresh (whole and chopped) plant materials.

Results

In raw, whole parsley leaves 0.71 ml/100 g of essential oil (EO) content was measured, which was not significantly reduced by freezing, drying at 40ºC or drying in shade. However, microwave drying methods and sun drying already resulted in a significant decrease in the amount of volatiles (0.39-0.55 ml/100 g), and in case of drying at 60ºC further decrease was detected (0.29 ml/100 g). Among the examined preservation techniques, lyophilization proved to be the least efficient method, because during its process the EO content almost completely lost (0.07 ml/100 g).

In our experiment, chopping before drying reduced the EO content of fresh leaves by 41%, and that of preserved leaves by 31-51%, depending on the preservation method. The preservation processes, however, affected the EO content of chopped leaves in exactly the same way as for the whole leaves.

According to GC-MS analysis, Apiole (38.3%), 1,3,8-p-Menthatriene (24.6%), p-Cymenene (7.9%), β-Phellandrene (8.7%), Myristicin (6.3%) and Myrcene (4.9%) were identified as main compounds in the EO of fresh, whole parsley leaves. Examining the EO composition of preserved samples, we found the same constituents, in similar proportion, except lyophilized parsley leaves, in which the ratio of Apiole and Myristicin increased (70.3% and 12.4%, respectively) but the proportion of other compounds significantly decreased.

In all chopped parsley samples the proportion of Apiole in the EO was higher than in whole leaves, especially for drying methods with higher volatile loss (drying in the sun, at 60ºC, in case of microwave drying and lyophilization). According to the results, it can be assumed that the loss of EO at chopping and during preservation is mainly due to the higher evaporation of monoterpenes, which is much more spectacular for cut leaves.

Freezing could preserve the fresh sample's original color the best, but microwave dried, lyophilized and oven-dried leaves at 40ºC also had very similar appearance properties. Pre-chopping of parsley leaves did not affect the color changes significantly during the applied preservation techniques. Based on e-tongue measurements we found that the taste of each sample (fresh, preserved, whole, chopped) was distinct, but in most cases not remarkably.

Conclusions

Freezing and convective drying at 40ºC are the best methods to preserve parsley leaves. Chopping before drying shortens the drying time to a small extent, but it results in too much loss of EO, so it cannot be recommended.

Does essential oil from invasive *Solidago canadensis* L. have herbicidal influence on the selected weeds?

Beata Baranová[1], Marta Kotuła[2], Saroj Kumar Chaudhary[3], Joanna Kapusta-Duch[2], Barbara Borczak[2], Daniela Gruľová[1,*]

[1] Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, SLOVAKIA
[2] Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Kraków, POLAND
[3] Central Department of Chemistry, Tribhuvan University, Kritipur, Kathmandu, Nepal

* Email: daniela.grulova@unipo.sk

Keywords: bioherbicide, canadian goldenrod, model plants, essential oils, phytotoxic effect,

Objective

Plants invasions are receiving more attention due to their increasing ecological and economic impacts. The mechanisms of successful plant invasions are not well known. *Solidago canadensis* L. is perennial rhizomatous plant native to North America and was introduced as ornamental plant all over the world where become invasive [1]. Invasive species do not suffer from herbivory and show increased vigour and competitive ability than native populations [2]. They suppress the original indigenous plants and are now most aggressive plant invaders in Europe [3]. Chemicals produced by alien species are allopathic to native plants. Essential oils (EOs) are natural secondary metabolites identified as allelochemical and are able to exert their phytotoxic effects as they limit the growth of competing plants in the surrounding environment. Present study evaluated potential phytotoxic effect of the essential oil from the above ground parts of invasive species *Solidago canadensis* L. (Asteraceae) against an selected weeds, naturally growing within agrosystems in Slovakia.

Methods

Aboveground parts of *Solidago canadensis L.* plants, including stems, leaves and inflorescences, were collected from three geographically different localities of Eastern Slovakia (Hankovce, Gemerska hôrka and Plaveč). The collected biomass was left to freely air dried in dark place. Thirty grams of grounded plant material were mixed with 300 mL of distilled water in the 1000 mL round bottom flask. Content was hydrodistilled for an 3 hours using clevenger apparatus. Essential oils were obtained as distillate with yellow colour and stored in glass vials at 5°C till time of further analysis. GC-MS was used for the determination of the main components in each EO sample. To test their phytotoxicity, EOs were diluted to four different concentrations, ranged from 0.625 –5.0 µg/mL. 7 ml of dilution was applied into the Petri dishes with 10 seeds of selected weed model organisms: *Lolium perenne* L., *Sinapis alba* L., *Portulaca oleracea* L. and *Barbarea vulgaris* W. T. Aiton, 1812. Petri dishes were incubated in the growing chamber for 5 days at ±20.00 °C and under the light conditions day 14 h/night 8 h. After five days, we evaluated the number of germinated seeds and the length of the roots. Obtained results were compared to control.

Conclusions

Dominant chemical groups identified in the *S. canadensis* essential oils from different localities were monoterpene hydrocarbons and sesquiterpene hydrocarbons, depending on harvest locality. Herbicidal effect was dependent on the concentrations of EO applied as well as on the weed model organisms used. Our results indicate, that essential oil from an invasive neophyte Solidago canadensis can be used for development of bio-based ecological herbicide.

ACKNOWLEDGMENTS

Presented research was supported by the grants VEGA 1/0087/20 and VEGA 2/0018/20 and CEEPUS M-RS-1607-2223-168622.

REFERENCES

[1] Smart A.J., Larson G.E. and Bauman P.J. (2013). Grass and Canada goldenrod (*Solidago canadensis*) competition and implications for management in the northern tallgrass Prairie. *The Prairie Naturalist* 45, 4-12.
[2] Jakobs G., Weber E. and Edwards P.J. (2004). Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than cospecifics in the native range. *Diversity and Distribution*, **10,** 11-19.
[3] Amtmann M. (2010). The chemical relationship between the scent features of goldenrod (*Solidago canadensis* L.) flower and its unifloral honey. *Journal of Food Composition and Analysis,* **23**, 122-129.

Testing of the new potential method for the evaluation of the essential oil antifeedant activity using yellow worm larvae.

Beáta Baranová, Daniela Gruľová*

Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, SLOVAKIA

*Emails: beata.baranova@unipo.sk, daniela.grulova@unipo.sk

Keywords: essential oils, yellow mealworm larvae, antifeedant effect, method

Objective

Production of aromatic oils is a part of the defence system which protects plants against herbivores, plant juice sucking insect, viruses, microbes or fungi. Essential oils can affect metabolic, biochemical, physiological or behavioural functions of the insect representatives. These features make essential oils to be possible tools for crops and stored products protection against insect pest, as the plant-based, health and ecologically safely alternative approaches in agriculture [1,2]. Larvae of the yellow mealworm, *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tene-brionidae) are one of the most common as well as the most harmful grain pests because of damaging different fractured grains of maize, wheat or soybean, their metabolic products cause grains contamination [3]. Simultaneously, *T. molitor* is a good model organism due to its short life cycle and being easy to grow. The objective of this work was to test the new potential method for the evaluation of antifeedant activity of essential oils using yellow mealworm larvae. The method is based on the determination of the weight loss of the feeding substrate treated with essential oil. In the study, ten different essential oils were used.

Methods

A mixture of semi-coarse wheat flour, breadcrumbs and wheat semolina in a weight ratio of 1:1:1 was used as a feeding substrate. The prepared mixture was weighed and placed in closable glass bottles. Mixture without an essential oil served as a control. The appropriate essential oil was added to the mixture in a volume corresponding to the following weight concentrations: A – 0.01% = 10 µl per 100 grams of feeding substrate; B – 0.1% = 100 µl per 100 grams of feeding substrate; C – 1% = 1000 µl per 100 grams of feeding substrate. The mixture was prepared 24 hours before starting the experiment and left in a dark place. Following essential oils prepared by steam distillation in Calendula a.s., Nová Ľubovňa, Slovakia were used in the experiment: *Origanum vulgare* L., *Foeniculum vulgare* Miller, *Citrus* × *sinensis* L., *Lavandula angustifolia* Miller, *Pimpinella anisum* L., *Carum carvi* L., *Pinus sylvestris* L., *Eucalyptus* sp., *Mentha* × *piperita* L. and *Salvia officinalis* L. Tvelwe grams of feeding substrate treated with essential oil were weighed into plastic cups and 3 or 5 larvae were added. Cups were covered with parafilm. Ten replicates were prepared for each concentration/number of larvae, the control as well and placed in a phytochamber with a constant temperature, humidity and light regime for 3 or 6 days. After the exposure time, the larvae were removed from the cups and the cup with the substrate was weighed. Antifeedant coefficient was determined according to the formula A = ((C-T/C+T))*100, where C = weight loss of the control, T = weight loss of the experiment. Index values were expressed on a scale from 0 to 200 (150-200 ++++; 100-150 +++; 50-100 ++, 0-50 +), where the value 0 corresponded to an inactive substrate with no antifeedant effect and the value 200 responded to the substrate with maximum effect.

Results

Based on the results, orange and lavender essential oil showed the most significant antifeedant effect, followed by cumin and anise essential oils. The antifeedant effect of eucalyptus oil was the least significant. The marjoram, fennel and pine essential oil did not show any antifeedant effect against the larvae of the yellow mealworm larvae based on the antifeedant coefficient.

Conclusions

Based on the evaluation of the obtained results, we conclude that the larvae of the *Tenebrio molitor* are suitable as a model organism for laboratory testing of the essential oil´s antifeedant effect evaluated on the basis of the weight loss of the treated substrate. Suggested method seems to be suitable for the easy, quick and costless preliminary assessment of such a type of essential oils biological activity. On the basis of the obtained data, the method variant with exposure of the substrate to 5 model organisms for 6 days and a 0.1% weight concentration of essential oil and under constant incubation conditions (20 ° C, humidity 55%, light mode 0/ 24 D/L) seems to be the most sufficient and suitable.

ACKNOWLEDGMENTS

Presented research was supported by the grants VEGA 1/0087/20 and VEGA 2/0018/20.

REFERENCES

[1] Raguso R.A. and Pichersky E. (1999). New perspectives in pollination biology: Floral Fragrances. A day in the life of a linalool molecule: Chemical communication in a plant pollinator-system. Part 1: Linalool biosynthesis in floweringplants. *Plant Species Biology* **14**, 95-120.
[2] Das A., Lee S.H., Hyun T.K., Kim S.W. and Kim J.Y. (2013). Plant volatiles as method ofcommunication. *Plant Biotechnology Reports* **7**, 9-26.
[3] Martynov V.O., Hladkyi O.Y., Kolombar T.M. and Brygadyrenko V.V. (2019). Impact of essential oil from plants onmigratory activity of *Sitophilus granarius* and *Tenebrio molitor*. *Regulatory Mechanisms in Biosystems* **10(4)**, 359-371.

Application of Machine Learning algorithms to essential oils to develop quantitative composition-activity relationships (QCAR) classification models.

R. Astolfi[1*], A. Oliva[2], F.U. Sapienza[1], R. Ragno[1]

[1] Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University, Rome, Italy;
[2] Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy;

* E.mail: astolfi.1766291@studenti.uniroma1.it

Keywords: Machine Learning, QCAR, Carbapenem-resistant Acinetobacter baumannii, Essential oils design

Objective

In this study, the relationship between essential oils (EOs) chemical composition and their antibacterial activity against carbapenemase-resistant Acinetobacter baumannii (CRAB) was investigated, exploiting the capabilities of machine learning (ML) algorithms to identify the chemical components most responsible for the experimentally observed biological profile. The models were generated and used to predict the antibacterial activity of experimentally extracted essential oils.

Methods

Two different training sets were compiled, and two separate calculations were performed for each dataset, one for Minimum Inhibitory Concentration (MIC) and the other for Minimum Bactericidal Concentration (MBC) values. The data were extracted from the PyEO database (eo.3d-qsar.com or soon www.ai4essoil.com) and then processed through python in a Jupyter Notebook platform. The datasets were subjected to optimization using a Monte Carlo approach. The final models were analyzed through the Skater library, first determining the features importance (FI) for each component and then the partial dependence (PD). The PD emphasizes the positive or negative influence of each component within the essential oil against the associated biological activity. The four generated models were experimentally validated using an external test set of 11 essential oils.

Results

For both datasets, models with higher accuracy and internal predictivity were obtained by classifying essential oils into active and inactive using a threshold value of 0.03% v/v (foe either MIC or MBC data). The FI analysis defined that carvacrol, eugenol, thymol, limonene, and eucalyptol were found to be the most influential on antibacterial activity, while for PD analysis carvacrol, eugenol, and thymol revealed positive influence according to their concentration within the essential oils. Limonene and eucalyptol, on the other hand, were assessed to negatively influence antibacterial activity. A mixed result was observed in the case of α-pinene, probably related to a synergistic or anti-synergistic effect depending on the percentage present. External validation of these four optimized models on the 11 essential oils with unknown activity toward CRAB predicted that only the essential oil extracted from Thymus vulgaris would have an activity less than 0.03% v/v. Microbiological tests determined MIC values in a range from 1.25% v/v to >5% v/v. By PD analysis a series of chemical components were selected, and their antibacterial activity was experimentally verified. In agreement with the PD data, the data confirmed an excellent MIC and MBC value for thymol (0.3% v/v) and carvacrol (0.3% v/v) and higher values for compounds such as eucalyptol and α-pinene, 2.5% v/v and 5% v/v, respectively.

Conclusions

Of the eleven newly tested EOs, the models correctly classified as non active ten samples (91%). In addition, the PD analysis was confirmed by experimentally obtained data on some chemical components. In light of these results further studies are in due course to develop more robust and predictive ML models that can be used in the future to design "ad hoc" blended EOs containing mainly the most important components. The application of ML to essential oils thus represents an innovative perspective to address the threat of A. baumannii, paving the way for new natural and sustainable therapies in the fight against multidrug-resistant bacteria.

Investigating the Antioxidant Potential of Essential Oils Using PALMSENS-Assisted Electrochemical Analysis

Filippo U. Sapienza[1,*], Danilo Dini[2] and Rino Ragno[1]

[1] Department of Drug Chemistry and Technology Biological, Sapienza University of Rome, Rome, ITALY

* Email: filippo.sapienza@uniroma1.it

Keywords: essential oil, redox, antioxidant, palmsens.

Objective:

Antioxidants activity play a vital role in neutralizing harmful free radicals, and natural compounds such as essential oils have shown promise in this regard. Using a new experimental methods based on Palmsens technology, we conducted a series of electrochemical analyses to evaluate the antioxidant potential of various essential oils. PalmSens is a company specialized in the development and manufacturing of potentiostats, which are instruments used in electrochemical research and analysis. They provide software solutions for controlling and analyzing data obtained from their potentiostats.

In this study, we aimed to explore the antioxidant properties of essential oils using an innovative approach based on a Palmsens potentiostat using specific modified procedure: due to the strong degradable effects that essential oils have on the chip the screen printed electrodes had to be modified using multi-walled carbon nanotubes (MWCNT) and titanium oxide nanoparticles (TiO2), with a bio-based ionic liquid (RTIL) as a drop-casting medium. The modified electrodes allowing us to immobilizing nanomaterials and measure the redox reactions associated with antioxidant activity. The analyses of the oils were done by means of cyclic voltammetry measures.The combination of PALMSENS software and a modified electrode proved to be a powerful tool in investigating the redox reactions associated with antioxidant activity.

Methods

Screen printed electrodes (SPE) have been used and modified with: Carbon nanotubes (MWCNT) (O.D. = (10 ± 1) nm; I.D. = (4.5 ± 0.5) nmL =3-6 μm) supplied by Sigma-Aldrich (Buchs, Switzerland), anatase titanium dioxide (99% purity; 5 nm) and 2-Hydroxy-N,N,N trimethylethan-1-aminium hydroxide (choline hydroxide).

The carbon nanotubes were mixed with nanoparticles of TiO2 and choline. The solution was placed in a sonic bath for 15 minutes in order to minimize the formation of aggregates and the modified electrodes were placed in a oven at T 35 C° with maximum ventilation for 15 hours.

Conclusions

These findings contribute to the growing body of knowledge on the antioxidant properties of essential oils and provide a novel perspective on their potential applications. Overall, this study underscores the importance of assessing the antioxidant potential of essential oils using state-of-the-art techniques.

The outcomes of this research have implications for diverse fields, including natural product development, pharmaceuticals, and nutraceuticals, where the utilization of essential oils as antioxidants could lead to the development of innovative and sustainable products.

REFERENCES

[1] Zappi D., Sadun C., Gontrani L., Dini D., (2019). A new electrochemical sensor for extra-virgin olive oils classification, Food control, 109(1):106903

[2] Sinha A., Stavrakis K. A., Simic M., Kojic S., Stojanovic M. G. (2022). Gold Leaf-Based Microfluidic Platform for Detection of Essential Oils Using Impedance Spectroscopy, Bionseser, 12:1169

Antimicrobial evaluation of *Mentha piperita & Thymus serpyllum & Pelargonium graveolens* essential oil combinations with chlorhexidine for mouthwash application

Ayşe Esra Karadağ[1], Sümeyye Elif Kahya[1*], Ayşegül Çaşkurlu[1], Betül Demirci[2], Fatih Demirci[2,3]

[1] Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Beykoz, Istanbul, TÜRKİYE
[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, TÜRKİYE
[3] Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, N. Cyprus, Mersin 10, TÜRKİYE

* Email: sumeyye.kahya@medipol.edu.tr

Keywords: Chlorhexidine, essential oil, synergism

Objective
In this study, the potential antimicrobial effects of *Mentha piperita & Thymus serpyllum & Pelargonium graveolens* essential oils were evaluated. The plant preparations are known and used ethnobotanically against throat infections.

Methods
In vitro antimicrobial evaluation of the commercial essential against methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans*, were performed using a microdilution assay. Binary combinations of chlorhexidine and individual essential oils were evaluated using the checkerboard method to determine the potential synergistic effect. Active samples were then prepared for mouthwash formulations and the effects were challenged and compared also using the agar-well diffusion method.

Results
The calculated fractional inhibition concentration index (FICI) value of chlorhexidine-*T. serpyllum* essential oil combinations against *S. mutans* was synergic, while the FICI value of the chlorhexidine-*P. graveolens* essential oil combination was found antagonistic. The synergic combinations were effective against both *S. mutans* and MRSA pathogens. To the best of our knowledge, combinations of the selected oils with chlorhexidine were observed for the first time for the potential as mouthwash formulation.

Conclusions
In addition to the mouthwash and oral care, the new systematically designed chlorhexidine combinations with *T. serpyllum* essential oil can also be used against throat pathogens.

ACKNOWLEDGMENTS
This work was supported by TÜBİTAK project number 1139B412102271. A patent application was made with the number 2022/016513.

Subcritical extraction of *Rosa alba* L. with mode influence

Ana Dobreva[1,*], Daniela Nedelcheva-Antonova[2], Nenko Nenov[3]

[1] Institute for Roses and Aromatic Plants, Kazanlak, BULGARIA
[2] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, BULGARIA 3 InnoSolv Ltd, Plovdiv, BULGARIA

* Email: anadobreva@abv.bg

Keywords: *Rosa alba* L., low pressure extraction, freon R134a, chemical profile, GC-MS

Objective

The objective of this work was to evaluate the aromatic products of *Rosa alba*, extracted with liquified 1,1,1,2-tetrafluoroethane (R134a) in static or dynamic mode. The white oil-bearing rose is a second of importance for the Bulgarian roseproduction. Its essential oil, hydrosol and aromatic products are valued and wellcome by the perfumery, cosmetics and food industry. There is some data concerning it's subcritical extraction [1], but the process can be improved and made more efficient. The previous study with *R.damascena* showed that the low pressure static extraction with 0.5-0.6 MPa was the most successful [2]. Now we investigated ~~if~~ the dynamic mode, knowing that circulation of the solvent is useful tool for the yielding. As addition, the two-stage periodical extraction was studied.

Fresh rose flowers of *R.alba* L., picked up from the experimental field of the Institute for Roses and Aromatic Plants, Kazanlak, Bulgaria, was used as raw material. The used solvent, 1,1,1,2-tetrafluoroethane (freon R134a, CAS number 811-97-2), was food grade, purchased from the Frigo Chem Ltd.

Methods

Extraction of the raw material was performed on a pilot apparatus. The process was conducted as static (Variant 1), dynamic (Variant 2) and static-staged (Variant 3) mode. The yield was calculated as percentage, on the basis of weights of product and the raw material. The individual compounds of the extracts were identified using GC/MS and their quantitative content was determined using GC-FID.

Results

The yield data showed values 0.039%, 0.048% and 0.042% for the V1, V2 and V3 respectively. As a result of the analysis, more than 80 compounds with concentrations higher than 0.01% were detected in the extracts, representing 92.7, 88.03 and 88.37% of the total content. The study revealed that the scent phase of the products consists mainly of 2-phenylethanol ranging 12.57÷14.97%, followed by geraniol (12.09÷14.82%), nerol (5.90÷6.39%), benzyl alcohol (3.63÷5.34%) and citronellol (3.21÷4.04%). The main compounds of the solid phase were nonadecane+nonadecene with 15.21÷6.85%, heneicosane (11.81÷13.78%) and tricosane (2.46÷2.96 %).

Conclusions

After complex evaluation of the quantitative and quality features of the extracts, the experiment with static , one-stage mode (Variant 1) was proven as most appropriate for the subcritical extraction of *R.alba* blossoms with freon R134a.

ACKNOWLEDGMENTS

The financial support of the project KP-06-OPR-01/5 “BG ROSEnsing: Traditional and e-sensing of Bulgarian Rose aromatic products: towards fast authenticity and quality control”, funded by the National Science Fund (Ministry of Education and Science) of Bulgaria is gratefully acknowledged.

REFERENCES

[1] Dobreva A., Nedeltcheva-Antonova D., Nenov N., Getchovska K., Antonov L. (2021). Subcritical Extracts from Major Species of Oil-Bearing Roses—A Comparative Chemical Profiling. Molecules 2021, 26, 4991. https://doi.org/10.3390/molecules26164991
[2] Dobreva A., Dincheva I. & Nenov N. (2021). On the subcritical extraction of Rosa damascena Mill. Bulg. J.Agric. Sci., 27 (4), 785– 791.

Yield and Composition of Rose Oil and Hydrosol from the Industrial Oil-Bearing Roses in Bulgaria

Ana Dobreva[1,*], Daniela Nedeltcheva-Antonova[2]

[1] Institute of Roses and Aromatic Plants, Agriculture Academy, Kazanlak, BULGARIA
[2] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, BULGARIA

* Email: anadobreva@abv.bg

Keywords: Rose valley, oil-bearing roses genotype, essential oil, hydrolates, GC/MS

Objective

The objective of this work was to establish the genotypes of the oil-bearing roses in the industrial plantations in the Rose valley, Bulgaria, the yield and chemical composition of their main products - essential oil and hydrosol. Although the R. damascena Mill. is spread as the main genotype [1], the recent survey in 2021 revealed that farmers in some areas grough also other pure species as *R. alba*, *R. gallica* or *R centifolia*, but also hybrids (Raduga) or different genotypes mixture, some of them of unknown origin. The samples of these gardens was used to parallel chemical study of the essential oil and aromatic water from all the genotypes. The same geographical and soil-climatic conditions remove the influence of abiotic factors and give a clear picture of the quantitative and qualitative differences of the rose products.

Methods

The essential oil content in the blossoms was established after water-vapour distillation in Clevenger type apparatus. The hydrolates were proceeded after double distillation of fresh flowers and water according to proven technology. The oil content of the hydrosols was measurement after exhaustive triple extraction with diethyl ether, collection and subsequent evaporation of the solvent. The chemical composition of the rose products was performed using the GC-FID/GC-MS techniques.

Results

The yields of rose oil were: 0.050 % (*R.damascena*); 0,045 % (mixture field); 0.033 % (Raduga); 0.030 % (*R.alba*) and 0.017 % (*R.centifolia*). The content of the rose oil in the hydrosols was higher than the in the blossoms and ranged from 0.025 % to 0.071 %. The chemical composition of the essential oils revealed the typical profiles of the genotypes with geraniol (18.71 - 33.25 %), citronellol (9.07 - 30.71 %) and nerol (5.90 - 12.81 %) terpenes in different ratio. The solid phase was represented by saturated aliphatic homologues with an odd number of carbon atoms, the main ones being: nonadecane (7.95 - 23.07 %), heneicosane (5.25 - 11.20 %), heptadecane (1.27 - 3,07 %) and tricosane (0.90 - 5.85 %). In contrast, the chemical profile of the hydrosols was performed by phenylethyl alcohol (30.45 - 70.81 %), geraniol (14.04 - 29.06 %) and citronelol+nerol (5.51 - 16.96 %). We grouped the chemical components and after comparing the essential oil and hydrosols it was found that they have different model.

Conclusions

The survey showed that the industrial fields with oil-bearing roses in Bulgaria have areas with different genotypes. Their essential oil and hydrosol have different chemical profile.

ACKNOWLEDGMENTS

The financial support of the project KP-06-OPR-01/5 “BG ROSEnsing: Traditional and e-sensing of Bulgarian Rose aromatic products: towards fast authenticity and quality control”, funded by the National Science Fund (Ministry of Education and Science) of Bulgaria is gratefully acknowledged.

REFERENCES

[1] Rusanov R., Kovacheva N., Rusanova M., Atanassov, I. (2012). Low variability of flower volatiles of Rosa damascena Mill. plants from rose plantations along the Rose Valley, Bulgaria. *Ind Crops Prod*., 37(1), 6-10. https://doi.org/10.1016/j.indcrop.2011.11.010

A comparative chemical profiling of essential oils from oil-bearing rose varieties, grown in Bulgaria and Crimea

Daniela Nedeltcheva-Antonova[1*], Kamelia Gechovska[1], Viktor Zolotilov[2], Natalya Nevkrytaya[2], Liudmil Antonov[3]

[1] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Research Institute of Agriculture of Crimea, Simferopol, Russia
[3] Institute of Electronics, Bulgarian Academy of Sciences, Sofia, Bulgaria

* Email: daniela.nedeltcheva@gmail.com

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

Objective

The chemical composition of the rose essential oil is strongly affected by the botanical and geographical origin of the raw plant material, environmental conditions, the production method, etc. Therefore, eleven rose oil samples, nine of them from rose varieties, grown in Crimea (four Bulgarian and five owned by Research Institute of Agriculture of Crimea (RIAC)) and two – grown in Bulgaria, were studied by means of gas chromatography-mass spectrometry (GC/MS) and gas chromatography with flame-ionization detection (GC-FID), in order to reveal the differences in chemical composition and aroma profile due to their geographical origin.

Methods

The rose oil samples, studied in this work, are derived by hydro-distillation in laboratory-scale equipment (Ginsberg and Clevenger-type aparatus). GC/MS and GC-FID were applied for the chemical profiling. The identification of the compounds was performed using commercial mass spectral libraries and retention times/indices.

Results

Rose oil is a very complex mixture containing compounds with high structural diversity. The main factor determining the quantitative and qualitative characteristics of rose essential oil is its botanical origin (i.e., rose species genotype), although there are many other factors affecting the chemical compositions and quality of the final product: geographical origin (climatic and soil conditions), flower processing technology (storage conditions and production methods). GC/MS and GC-FID analysis of the aroma constituents of the studied samples reveals, that the rose essential oils, derived from the rose varieties grown in Crimea (including Bulgarian cultivars) are, in general, not consistent with the International standard ISO 9842:2003 for the rose oil. The most abundant class of aroma compounds presented in the Crimea rose oil samples are monoterpene alcohols: geraniol (20.79-47.79%) and β-citronellol+nerol (13.36-29.58%). Phenyl ethyl alcohol, which is responsible for the characteristic rose-like odor of Rosaceae plants shows relatively high variability (0.27-1.73%). The same was observed for eugenol (0.13-1.94%) and its methyl ether – methyl eugenol (0.06-0.98%).

Conclusions

Comparative chemical profiling revealed different chemical composition and aroma profiles of the rose oils from the same varieties, depending of their geographical origin. The essential oils, derived from rose species, grown in Bulgaria show much higher concentration of characteristic aroma constituents. In addition, the chemical profiles of the rose oils from the rose varieties, grown in Crimea, differ significantly from the requirements of the International Standard for the rose oil (ISO 9842:2003).

ACKNOWLEDGMENTS

The financial support of the project KP-06-OPR-01/5 “BG ROSEnsing: Traditional and e-sensing of Bulgarian Rose aromatic products: towards fast authenticity and quality control”, funded by the National Science Fund (Ministry of Education and Science) of Bulgaria is gratefully acknowledged.

REFERENCES

[1] Dobreva, A.; Nedeltcheva-Antonova, D. Comparative Chemical Profiling and Citronellol Enantiomers Distribution of Industrial-Type Rose Oils Produced in China, Molecules 2023, 28, 1281
[2] Zolotilov, V.; Nevkrytaya, N.; Zolotilova, O.; Seitadzhieva, S.; Myagkikh, E.; Pashtetskiy, V.; Karpukhin, M. The Essential-Oil-Bearing Rose Collection Variability Study in Terms of Biochemical Parameters. Agronomy, 2022, 12, 529

Alternative for perfume analysis: GC×GC-TOFMS with hydrogen carrier gas

Lena M. Dubois[1], Nick Jones[2], Dmitrii Rakov[1], Sebastiano Panto[1, *]

[1] LECO European Application & Technology Center, Berlin, GERMANY

* *Email:* sebastiano_panto@leco.com

Keywords: perfume, GC-MS, hydrogen, GCxGC, TOFMS

Objective

Commercial perfumes as well as their ingredients such as essential oils, are quite often complex mixtures of synthetic or natural organic compounds. Besides the olfactive investigation by perfumers, it also requires advanced analytical tools to determine the composition of such samples. Gas chromatography (GC) coupled to mass spectrometry (MS) is widely utilized to separate the individual components and identify them. Due to the complexity of the mass spectral information, especially in case of chromatographic co-elutions, the use of time-of-flight mass spectrometry (TOFMS) and deconvolution algorithms is particularly helpful to identify all constituents correctly. The use of comprehensive two-dimensional gas chromatography (GC×GC) is gaining popularity in many laboratories as the technique offers unsurpassed resolution and sensitivity. The principle relies on the coupling of two GC columns with different stationary phases with a modulator in between, allowing continuous heart-cuts from the first column to be analyzed on the second column. In addition, the coupling with TOFMS detection and deconvolution software provides one of the most powerful tools available today in the field of separation sciences.

The current shortage of helium (He), used as carrier gas in GC and GC×GC, concerns perfume but also all related industries. One alternative to He is the use of hydrogen (H_2) instead. Though, switching carries gases requires the transfer and adaption of existing methods, which was until today probably one of the major impediments. Fortunately, there are several online tools which can be accessed freely to aid this transition.

The objetives of this study include I) to demonstrate the ease of transition when switching carrier gasses and II) the evaluation of the analysis of a perfume by means of GC×GC.

Methods

The analysis of a perfume sample with He and H_2 as carrier gas was performed, using GC×GC, a unique TOFMS technology design and deconvolution software.

Results

Comparisons of mass spectral fragmentations, dynamic range, sensitivity, robustness, and chromatographic resolution obtained with both He and H_2, were examined. The results demonstrated both carrier gases gave very similar mass spectral fragmentation, similar sensitivity, and dynamic range.

Conclusions

The transition from a He- to a H_2-based GC×GC-TOFMS method for perfume analysis results in comparable results with regards to chromatographic resolution and mass spectral quality.

Effects of Inhalation of Essential Oils (Phytincide) extracted from *Pinus densiflora* and *Chamaecyparis obtuse* on the Physiological and Psychological Stability of College Students

Bum-Jin Park[1*], Hye-Jung Gho[1], Dawou Jung[2]

[1] Department of Environment and Forest Resources College of Agriculture and Life Sciences, Chungnam National University, Republic of Korea
[2] Institute of Agricultural Science College of Agriculture and Life Sciences, Chungnam National University, Republic of Korea

* Email: bjpark@cnu.ac.kr

Keywords: Olfactory Arousal, Aroma, Comfortable, Concentration, Relaxation, Therapeutic Environments

Objective

To provide a more therapeutic environment for hikers on trails in the forest, this study was conducted to clarify the physiological and psychological effects on the human body when hikers inhale pine (*Pinus densiflora*) and cypress (*Chamaecyparis obtusa*) essential oils, which are major species in the temperate central climate.

Method

The subjects of the experiment were 46 university students (22.43±3.17 years old) in Daejeon city, consisting of the pine oil inhalation group and the cypress oil inhalation group to observe changes in tree oil inhalation compared to odorless air. The experiment was conducted in an artificial climate room, and the subjects closed their eyes for 1 minute, stabilized, and inhaled odorless air and tree oil for 2 minutes each. During the experiment, the autonomic nervous system response was evaluated by continuously measuring the heart rate variation, and the psychological state was evaluated through a mood condition test and a semantic discrimination survey after each stimulus.

Results

Physiological changes used the average HF change representing the activity of the parasympathetic nervous system. As a result of the measurement, it decreased significantly when inhaling pine oil and increased significantly when inhaling cypress oil ($p<0.05$). As a result of measuring psychological changes through mood condition tests, the pine oil inhalation group showed a significant effect on reducing comprehensive emotional disorders, and the cypress oil inhalation group showed a significant effect on reducing tension and depression ($p<0.05$). As a result of measuring psychological changes through semantic discrimination, the comfort and sedation of the pine oil suction group and the natural feeling of the cypress oil suction group significantly increased ($p<0.05$).

Conclusions

Based on the above results, it is judged that pine oil is effective in "comfortable awakening" by inducing positive sympathetic nerve activity in a psychologically stable state, and cypress oil is effective in "comfortable relaxation" by improving physiological and psychological stability. These findings are expected to serve as the basis for subsequent studies to verify the effectiveness of oil refining by a tree and to be widely used in the fragrance industry.

ACKNOWLEDGMENTS

The current study received the “R&D Program for Forest Science Technology (Project No. 202138500-2123-0101)” by the Korea Forest Service (Korea Forestry Promotion Institute).

Hydrodistillation wastewaters: a source of biologically active compounds

Edoardo Napoli[1,*], Giuseppe Ruberto[1], Alessandra Carrubba[2], Mauro Sarno[2], Claudia Muscarà[3], Antonio Speciale[3], Mariateresa Cristani[3], Francesco Cimino[3], Antonella Saija[3]

[1] Institute of Biomolecular Chemistry – National Research Council (ICB-CNR), Catania, ITALY
[2] Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Build 4, Entr. L, 90128 Palermo, Italy.
[3] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: edoardo.napoli@icb.cnr.it

Keywords: distillation wastes; phenolic profile; rosmarinic acid; intestinal inflammation; anti- inflammatory; radical scavenging; antioxidant

Objective

Essential oils (EOs) from plant material are obtained mainly by distillation (either hydrodistillation or steam distillation). The biomass used in the process is returned as a solid reside together with variable amounts of wastewaters rich in water-soluble compounds, which are not addressed to any further application. The scope of our work is to evaluate the phenolic composition of hydrodistillation wastewaters (DWWs) of aerial parts of five aromatic plants (*Origanum vulgare*, *Thymus vulgaris*, *Salvia officinalis*, *Rosmarinus officinalis* and *Origanum majorana*) and asses their biological activities for future application in the cosmetic, pharmaceutical, nutraceutical and food fields.

Methods

Aerial parts of each species (100 g of air-dried material) were hydrodistillated by means of a Clavenger-type apparatus for about 3 h. After cooling, the essential oil and hydrolates were removed, and the wastewater was separated from the solid biomass through a filter paper filtration process. Then, it was frozen and freeze-dried and stored at room temperature in sealed plastic Falcon tubes until analyses.
The phenolic profiles of the DWWs were determined by HPLC-DAD and HPLC-ESI/MS. Free radical scavenging ability, oxygen radical antioxidant capacity and superoxide dismutase mimetic activity of the samples under study were measured. Moreover, to investigate the anti-inflammatory activity of the DWWs, an in vitro experimental model of intestinal inflammation was used.

Results

The DWW samples' phytochemical analysis allowed the identification of 37 phenolic compounds, all exhibiting good antioxidant and anti-inflammatory activity. The analysis of DWWs showed that it contains, among others, 8–12% of organic acids, of which only 5–7% of rosmarinic acid and up to 20% of dihydroxyphenylacetic acid.
Antioxidant power expressed as DPPH values gave the highest response in *T. vulgaris* and O. vulgare, whereas the measurements in terms of ORAC values stressed, besides these two species, the outstanding response of S. officinalis. All the investigated wastewaters induced a significantly good anti-inflammatory effect. The treatment with TNF-α induced a significant nuclear accumulation of the transcription factor NF-κB and consequently an increased expression of the inducible COX-2. Both effects were prevented by the treatment with the anti-inflammatory steroid dexamethasone used as a positive control.

Conclusions

Our study contributes to the knowledge on the polyphenolic composition of the DWWs of five aromatic plants of the Lamiaceae family. Although further studies are necessary to complete the picture of the chemical composition of these residues by extending the analyses to the non-polyphenolic components of the matrix, our results highlight the presence of high quantities of compounds with proven biological activity (especially rosmarinic acid) and therefore of great interest in the pharmaceutical and nutraceutical fields.

ACKNOWLEDGMENTS
The authors wish to thank the Azienda Agricola Rinoldo, Aragona (AG) - Italy, for the gift of plant materials.

Inula chrithmoides and *Chrithmum maritimum* – GC/MS analyses of essential oil and hydrolates

Olivera Politeo[1,*], Andrea Bakaric[2], Pavao Curlin[2], Marijana Popovic[3]

[1] Department of Biochemistry, Faculty of Chemistry and Technology, University of Split, Split, CROATIA
[2] Faculty of Chemistry and Technology, University of Split, Split, CROATIA
[3] Department of Applied Sciences, Institute for Adriatic Crops and Karst Reclamation, Split, CROATIA

* Email: olivera@ktf-split.hr

Keywords: *Inula chrithmoides*, *Chithmum maritimum*, GC/MS, essential oil, hydrolate

Abstract

Halophytes are plants that naturally occur in saline environments such as coastal regions, salt marshes, inland deserts, and others. They have evolved a number of adaptive mechanisms to tolerate seawater and higher salt concentrations [1], and are widely distributed in the Mediterranean region [2]. The most abundant species include *Crithmum maritimum* and *Inula crithmoides*. The inhabitants of the coastal region and Croatian islands have been using these plant for nutrition since ancient times. This study evaluates the potential of halophytes as a source of valuable and biologically active compounds. The objective of this work was to determine the chemical composition of essential oils and hydrolates of air-dried aerial plant material of two halophytes from Croatia. The essential oils were isolated by hydrodistillation, and the hydrolates were obtained after removal of the essential oils. Headspace solid-phase headspace microextraction (HS-SPME) was used to isolate volatile components from the hydrolate.Chemical analysis of the essential oils and hydrolates were performed by gas chromatography/mass spectrometry (GC/MS). The main components of the essential oil of *Inula chrithmoides* were *p*-cymene, 8,9-dehydrothymol methyl ether and *p*-cymene-7-ol, while the main components of the hydrolates were 8,9-dehydrothymol methyl ether, 8,9-dehydrothymol and 8,9-dehydro-4-hydroxythymol dimethyl ether. The major constituents of the essential oil of *Crithmum maritimum* were limonene and sabinene, while the major hydrolate compounds were terpinen-4-ol and limonene.

ACKNOWLEDGMENTS

This work is part of the PRIMA programme supported by the European Union. Project title *Innovative sustainable organic sea fennel (Crithmum maritimum L.)-based cropping systems to boostagrobiodiversity, profitability, circularity, and resilience to climate changes in Mediterranean small farms*, acronym: SEAFENNEL4MED. The authors are also thankful for the scientific research equipment financed by the EU grant *Functional integration of theUniversity of Split, PMFST, PFST and KTFST through the development of the scientific and research infrastructure* (KK.01.1.1.02.0018).

REFERENCES

[1] Mohammed H. A., Emwas A.-H., Khan R. A., (2023). Salt-Tolerant Plants, Halophytes, as Renewable Natural Resources for Cancer Prevention and Treatment: Roles of Phenolics and Flavonoids in Immunomodulation and Suppression of Oxidative Stress towards Cancer Management. International Journal of Molecular Sciences, 24, 5171. https://doi.org/10.3390/ijms24065171
[2] Petropoulos S. A., Karkanis A., Martins N., Ferreira I.C.F.R., (2023). Edible halophytes of the Mediterranean basin: Potential candidates for novel food products. Trends in Food Science & Technology, 114, 155-169. https://doi.org/10.1016/j.tifs.2018.02.006.

Volatile constituents of two Corsican liverworts: *Diplophyllum albicans and Scapania undulata.* Evaluation of their allelopathic effect.

Manal El Ali [1,*], Anais Pannequin [1], Aura Tintaru [2], Alain Muselli [1]

[1] Université de Corse, UMR CNRS 6134, Laboratoire Chimie des Produits Naturels, BP 52, 20250 Corte, France
[2] Centre Interdisciplinaire de Nanoscience de Marseille, Aix-Marseille Université, CNRS, UMR7325, 13288 Marseille, France

[*] Email: EL-ALI_M@univ-corse.fr, muselli_a@univ-corse.fr

Keywords: *Diplophyllum albicans*; *Scapania undulata*; essential oils; allelopathy.

Bryophytes are about 25000 species in the world, they estimated at 1800 in Europe and among 1400-1300 species in France. On Corsica Island, 547 species are distributed over all vegetation floors, among which mosses, liverworts and hornworts can be distinguished [1]. These species constitute an important plant biomass available throughout the year, and which to date, is not valued. Hepatics are very diverse plants, complicated to identify and poorly studied at phytochemical level, yet known to contain very specific secondary metabolites. To our knowledge, the bryophytes of Corsica have been the subject of only few phytochemical studies carried out earlier by our group [2,3,4]. Liverworts are highly diverse plants that have not been extensively studied at the phytochemical level. However, they are known to harbor unique secondary metabolites with specific characteristics.

The primary challenge encountered by phytochemists interested in bryophytes is the collection of plant material. Harvesting presents a highly sensitive operation due to the small size of bryophytes, making on-site identification a delicate task, particularly since multiple species of bryophytes often grow together in a mixture.

The present work aims to demonstrate the contribution of chemical profiling and confirm the crucial importance of mastering the sampling step in phytochemistry. Furthermore, it provides information on the phytotoxic potential of two liverwort species.

After harvesting *Scapania undulata*, the plant material was prepared through hydrodistillation, and the resulting essential oil was subjected to chemical profiling using chromatographic techniques (GC-FID, GC-MS).

To our surprise, the main constituents of the essential oil were *epi*-cubenol, a sesquiterpene alcohol characteristic of *S. undulata* [5], accompanied by diplophyllin, a sesquiterpene lactone known as a taxonomic marker of *Diplophyllum albicans* [6]. This finding indicated that the sampling included a mixture of the two species. To resolve this, a comprehensive supplementary collection of the two closely related species, *Scapania undulata* and *Diplophyllum albicans*, was conducted, followed by analysis of their respective essential oils.

The study of *S. undulata* allowed the identification of 49 metabolites, which accounted for 96.8% of the essential oil. The dominant terpene constituents were cadinane-type sesquiterpenes, including *epi*-cubenol, as well as tricyclic sesquiterpenes with longipinane, longifolane, and longibornane skeletons.

The analysis of the essential oil from *D. albicans* resulted in the identification of 20 compounds, representing 84.9% of the total essential oil. Among them were a mixture of sesquiterpene hydrocarbons and oxygenated molecules, with a majority of sesquiterpene lactones. The allelopathic tests revealed the phytotoxic potential of these metabolites on the development of radish, leek, rice, and watercress seeds. Liverworts could represent new natural sources of high-potential molecules for agriculture, serving as biocides or biostimulants.

REFERENCES

[1] Sotiaux. A., Pioli. A., Royaud. A., Schumacker. R., *Vanderpoorten*. A., (2007). A checklist of the bryophytes of Corsica (France): new records and a review of the literature, *Bryology*, 41–53.
[2] Pannequin. A., Tintaru. A., Desjobert.J.-M., Costa. J., Muselli. A., (2017). New advances in the volatile metabolites of *Frullania tamarisci*. *Flavour Fragr. J.*, *32*, 409–418.
[3] Pannequin. A., Quetin-Leclercq. J., Costa. J., Tintaru. A., Muselli. A., (2023) First Phytochemical Profiling and In-Vitro Antiprotozoal Activity of Essential Oil and Extract of *Plagiochila porelloides*, Molecules, 2023, 28, 616.
[4] Pannequin. A., Laurini. E., Giordano.L. , Muselli. A., Pricl. S., Tintaru. A., (2020). Caution: Chemical Instability of Natural Biomolecules During Routine Analysis, *Molecules*, 25, 3292
[5] Naghashima.F., Suda.K., Asakawa.Y., (1994). Cadinane-type Sesquiterpenoids from the Liverwort Scapania undulata, *Phytochemistry* ,37, 1323.
[6] Asakawa. Y., Toyota. M., Takemoto. T., Suire. C., (1979). PUNGENT SESQUITERPENE LACTONES OF THE EUROPEAN LIVERWORTS CHILOSCYPHUS POLYANTHUS AND DIPLOPHYLLUM ALBICANS, *Phytochemistry*, 18, 1007-1009.

Phytotoxic essential oils, sesquiterpene lactones from the roots of the invasives *X. italicum* and *X. spinosum*

Sylvain Baldi[1], Manal El Ali[1,*], Pascale Bradesi[1], Alain Muselli[1]

[1] Laboratoire Chimie des Produits Naturels, UMR CNRS 6134 SPE, Université de Corse, 20250 Corte, France

* Email: EL-ALI_M@univ-corse.fr

Keywords: invasive plant, essential oil, sesquiterpene lactone, phytotoxicity

Invasive alien species represent one of the main environmental issues. In Corsica, more than thirty introduced plant species are considered as invasive. Among them, *Xanthium orientale subsp. italicum* and *Xanthium spinosum*, commonly called cockleburs, are highly invasive herbaceous weeds in the genus *Xanthium* (*Asteraceae*) which include 25 species native from America and now widely distributed throughout the world [1]. Cockleburs are known for their various pharmaceutical activities, imputed to sesquiterpene lactones which are commonly found in the Xanthium genus, and have been the object of a large number of chemical and biological studies [2].

Objective

The objective was to investigate the not yet investigated chemical composition and allelopathic activity of *X. italicum* and *X. spinosum* root essential oil and hydrosol.

Methods

Wild-growing *X. italicum* and *X. spinosum* roots were harvested in Corsica near Corte. EOs and hydrosol were obtained by hydrodistillation of dried roots using a Clevenger-Type and chemical composition of both extracts were investigated using GC-FID, GC/MS, NMR (1D and 2D) and hemi-synthesis. Extracts allelopathic activities were assessed on the seed germination and seedling growth of two plants: *Allium porrum* (*Alliaceae*) as monocotyledon model and *Raphanus sativus* (*Brassicaceae*) as the dicotyledon one.

Results

38 and 69 components were reported in *X. italicum* and *X. spinosum* extracts, accounting for 82,6 to 93,8% of the total amount. Compositions of all extracts were dominated by oxygenated sesquiterpene and especially sesquiterpenes lactones. Our results deal with the first reports of β-xanthanène and 11β-dehydroziniolide occurrence as naturals products.

Allelopathic study revealed an inhibitory effect for *X. italicum* and *X. spinosum* root metabolites. Strongest effect was observed with the hydrosol and especially on the monocotyledon specie. Results suggested that lactones were strongly involved in phytotoxicity however global effect is probably due to a synergy between different inhibition mechanisms from several oxygenated compounds.

Conclusions

EOs and hydrosols from *X. italicum* and *X. spinosum* showed high concentration of sesquiterpene lactones and extracts demonstrated a strong and promising phytotoxic potential, especially on monocotyledon specie. Sesquiterpene lactones seem to be strongly involved in allelopathic interactions and could be the key to understanding the invasive mechanisms of weeds.

REFERENCES

[1] D. Jeanmonod, A. Schlüssel, J. Gamisans (2011). Status and trends in the alien flora of Corsica, EPPO Bulletin, 41, 85-99
[2] J. Cumanda, G. Marinoni, M. Bernardi, G. Vidari, P.V. Finzi (1994). New sesquiterpenes from Xanthium catharticum, Journal of natural products, 54, 460-65

Chemical composition and chemical variability of *Senecio cineraria* essential oil.

Stéphane Andreani[1], Manal El Ali[2*], Pascale Bradesi[2] Alain Muselli[2].

[1]Office de Développement Agricole et Rural de la Corse, Avenue Paul Giacobbi, 20600 Bastia, France
[2]Université de Corse, UMR CNRS 6134 SPE, Laboratoire Chimie des Produits Naturels, BP 52, 20250, Corte, France.

*Email: el-ali_m@univ-corse.fr

Keywords: Senecio cineraria, essential oil, chemical variability; Gas chromatography; Mass spectrometry

Senecio genus is one of the biggest of the Asteraceae family and includes more than 1500 species worldwide [1]. In Corsica 10 species were reported which including 8 natives (3 endemic species) and 2 exogenous species [2].
Senecio cineria syn. Jacobea cineraria is a schrub of 1-1,5 m with silver stems and leaves powder to the touch. It blooms from may to august and exhibits florale stalks of 10-30 flowerhead arranged in highbush [2]. S. cineraria is commonly widespread in the whole littorals of Corsican Island. It is a serpentine and halotolerant plant which roots grow directly implanted on the rock exposed to the sea spray [3].
The aim of this work was the study of the chemical biodiversity of S. cineraria from Corsica. For this, the chemical compositions of S. cineraria essential oils from 40 Corsican locations were investigated using gas chromatography (GC-FID), gas chromatography-mass spectrometry (GC-MS). One hundred and fourteen components which accounted for 95.3% to 98.2% of the total amount were identified. Among them, nonene (0.3-12.7%), α-pinene (1.3-19.4%), (E)-β-ocimene (2.5-19.6%), albene (0.2-14.9%), hexyl hexanoate (4.3-19.8%) and germacrene-D (1.5-10.2%) have been described as main components.
In addition, statistical analysis using PCA and CA analysis of the chemical set of data provide the discrimation of three clusters called Sci 1-3. Cluster Sci1 included 14 samples dominated by germacrene-D (6.3-10.2%) and nonene (5.3-6.3%). Cluster Sci2 included 19 samples dominated by hexyl hexanoate (11.2-19.8%) and albene (11.2-19.8%). Then Sci3 included 7 samples with the highest rate of α-pinene (10.7-19.9 %) and (E)-β-ocimene (12.0-19.6%).
Our results seem to expose a correlation between secondary metabolites production and the composition of soils. Cluster Sci1 grouped the whole samples growing in shale soil. Cluster Sci2 included plant specimens implanted in granite rock and Cluster Sci3 the whole specimen living on limestone soil. Our results have gained more knowledge about the secondary metabolite production that occurs during the plant life, they could be interesting in order to manage the future commercialization of S. cineraria essential oil.

REFERENCES

[1] Tutin T. G., Löve D., Hansen A. (1976). Flora Europea, Cambridge University press, Cambridge,
[2] Jeanmonod D., Gamisans J. (2007). Flora Corsica, Edisud, Aix en Provence
[3] Jeanmonod D., Schlüssel A., Gamisans J. (2004). Compléments au prodrome de la flore corse Asteraceae II, Conservatoire et Jardins botaniques Ville de Genève.

Natural plant extracts as antibacterial agents against periodontal pathogens

Zuzanna Bacińska[1,*], Daniel J. Strub[2], Lucyna Balcerzak[2]

[1] Department of Engineering and Technology of Chemical Processes, Wrocław University of Science and Technology, Wyb. Wyspiańskiego 27, 50-370 Wrocław, POLAND
[2] Department of Biological Chemistry and Bioimaging, Wrocław University of Science and Technology, Wyb. Wyspiańskiego 27, 50-370 Wrocław, POLAND

* Email: zuzanna.bacinska@pwr.edu.pl

Keywords: natural fragrance materials, plant extracts**,** oral pathogens, periodontal disease, antibacterial activity

Objective

The aim of this study was to evaluate the antibacterial activity of selected natural fragrance materials and plant extracts against pathogenic microorganisms such as *Streptococcus mutans* (ATCC 25175) (MediMark) and *Porphyromonas gingivalis* (ATCC 33277) (Thermo Scientific). These species are known as plaque bacteria, which not only contribute to the progression of caries but also to the development of systemic diseases [1]. Commonly used oral hygiene products contain chemical preservatives such as chlorhexidine or cetylpyridinium chloride. Numerous studies have shown that despite their effectiveness, long-term use of these preservatives is associated with some undesirable side effects, such as a change in the perception of taste and the appearance of yellow-brown staining on the enamel [2,3,4]. Natural extracts offer a promising alternative to these preservatives, due to their numerous biocidal and antibacterial properties. Natural fragrance materials could play multiple roles in oral hygiene formulations. Apart from fighting pathogenic bacteria responsible for tartar, they could act as good preservatives and give a unique taste, smell, and color.

Methods

Preliminary screening tests enabled the selection of substances from among 750 natural extracts that showed an inhibitory effect on the growth of microorganisms. Minimum inhibitory concentration (MIC) values were determined for the most active materials using a twofold dilution method. MIC tests were also performed for selected chemical preservatives to compare their effectiveness with natural extracts. The tests were carried out in 96-well plates using the AlamarBlue® reagent.

Results

Twelve natural fragrance materials with MIC values ≤400 μg/ml inhibited *Porphyromonas gingivalis*, while 92 natural extracts with MIC values ≤400 μg/ml inhibited *Streptococcus mutans*. Four chemical preservatives with MIC values ≤200 μg/ml inhibited both species.

Conclusions

The study has demonstrated that natural fragrance materials exhibit suppressive activity against periodontal pathogens. The difference in the number of materials that inhibited the growth of the studied species may be attributed to differences in their genome and cellular structures. Some natural extracts showed a comparable or even better inhibitory effect than the tested chemical preservatives such as chlorhexidine. Therefore, there is significant potential for the use of natural fragrance materials as antibacterial agents in oral hygiene formulations that effectively combat tartar.

ACKNOWLEDGMENTS

We would like to thank representatives of the essential oils industry and International Federation of Essential Oils and Aroma Trades (IFEAT) Members: A.Fakhry & Co., Albert Vieille, Amigo & Arditi, Ashna Samai, Berje Inc., Bordas S.A., BoreA Canada, Brüder Unterweger, Christodoulou Bros S.A., Citrus and Allied Essences Ltd, D.V. Deo Industries, Dutjahn Sandalwood Oils, Essential Oils and Herbs, Essential Oils of Tasmania, Eucaforest, Hierbas Patagónicas, S.R.L, Kallin ltd, Jacarandas International, Mane Kancor Ingredients Private Limited, Lebermuth, Les Arômes du Maroc, Lluch Essence, Metainy, Nelixia, New Zealand Mānuka Group, Payan Bertrand, Plant's Power, Van Aroma, Robertet S.A., Ultra International, and Vessel Essential Oils for the donation of most of the samples of natural flavor and fragrance materials for this study.

REFERENCES

[1] Peng, X., Cheng, L., You, Y. et al. (2022). Oral microbiota in human systematic diseases. Int J Oral Sci 14, 14, 1-11.
[2] Polizzi, E., TetÃ¨, G., Bova, F., Pantaleo, G., Gastaldi, G., CapparÃ¨, P., Gherlone, E. (2019). Antibacterial properties and side effects of chlorhexidine-based mouthwashes. A prospective, randomized clinical study. J Osseointegration, 12, 1, 2–7.
[3] Balagopal S., Arjunkumar R. (2013). Chlorhexidine: The Gold Standard Antiplaque Agent. J Pharm Sci Res, 5, 12, 270-274.
[4] Karpiński, T. M., Szkaradkiewicz A. K. (2015) Chlorhexidine – pharmaco-biological activity and application. Eur Rev Med Pharmacol Sci 19, 7, 1321-1326.

Transformation and antimicrobial activity of natural and volatile phenolic compounds and their derivatives

Kinga Baberowska[1,*], Alicja K. Surowiak[2], Daniel J. Strub[3]

[1] Department of Analytical Chemistry and Chemical Metallurgy, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, POLAND
[2,3] Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, POLAND

* Email: kinga.baberowska@pwr.edu.pl

Keywords: phenolic compounds, volatile compounds, antimicrobial activity, MIC, lily alcohol

Objective

The aim of this study was to investigate the inhibitory effects of natural volatile phenolic compounds and their derivatives on the growth of *Staphylococcus aureus* (ATCC 6538, Sterbios), *Kocuria rhizophila* (ATCC 9342, Sterbios), and *Enterobacter gergoviae* (ATCC 33028, Sterbios) strains.

Methods

In the experiment, eight natural phenolic compounds and seven of their carboxylic derivatives, synthesized by the Bargellini's reaction, were tested to evaluate their antimicrobial activity. The viability and proliferation capacity of the cell cultures were assessed using the alamarBlue™ assay and the minimum inhibitory concentration (MIC) was determined by microdilution method on 96-well plates.

Results

The results obtained showed that the *p*-Cresol derivative, 2-methyl-2-(4-methylphenoxy)propanoic acid, and α-(3-methylphenoxy)isobutyric acid, exhibited the best inhibitory effect on the *S. aureus* strain, with a MIC of 300 µg/mL, which was 2 times lower than the standard, a m-Cresol derivative (600 µg/mL). The 4-ethylphenol derivative, α-(4-Ethyl-phenoxy)-isobutyric acid, exhibited a four times lower antimicrobial activity than the standard (300 µg/mL and 1200 µg/mL) for *S. aureus*. For the *K. rhizophila* strain, the lowest MIC value of 1200 µg/mL was obtained for two synthetic volatile compounds, *i.e.*, 2-(3-fluorophenoxy)-2-methylpropanoic acid (a derivative of 3-fluorophenol) and α-(4-Ethyl-phenoxy)-isobutyric acid (a 4-ethylphenol derivative).
For *E. gergoviae*, α-(4-Ethyl-phenoxy)-isobutyric acid (a 4-ethylphenol derivative) showed the lowest MIC value of 600 µg/mL. In general, among the seven synthesized compounds, the best results for all tested strains were obtained for the 4-ethylphenol derivative, α-(4-Ethyl-phenoxy)-isobutyric acid.

Conclusions

The global market for fragrance compounds is expected to grow from approximately $14 billion in 2020 to approximately $20 billion in 2026 [1]. This market is dominated by synthetic fragrances (57%) due to the transparency of the production process, functionality, and safety of use. Therefore, it is reasonable to search for new fragrance compounds with the desired characteristics odor, which may be difficult to achieve with unprocessed raw materials or their metabolites. The synthesis of carboxylic acids in the Bargellini's reaction, followed by their transformation into derivatives with potential aroma properties, will allow the discovery of completely new compounds that can be used in the future as preservatives in cosmetics due to their ability to inhibit the development of pathogens that may develop in them.

REFERENCES

[1] Frost & Sullivan report: Global Fragrances Market Growth Opportunities, 2021

Phytochemical composition, analgesic and anti-inflammatory properties of *Pelargonium peltatum* (L.) L'Hérit. essential oils from Eastern Cape, South Africa

P. Rungqu[1], A.O. Oyedeji[2*], M. Gondwe[3], O.O. Oyedeji[1]

[1] Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, SOUTH AFRICA
[2] Department of Chemistry, Faculty of Natural Sciences, Walter Sisulu University, Mthatha, SOUTH AFRICA
[3] Department of Human Biology, Faculty of Health Science, Walter Sisulu University, Mthatha, SOUTH AFRICA

* Email: aoyedeji@wsu.ac.za

Keywords: *Pelargonium peltatum*, essential oils, chemical composition, analgesic, anti-inflammatory

Objective

This is the first study to look at the chemical composition, analgesic and anti-inflammatory properties of *Pelargonium peltatum* (L.) L'Hérit. essential oils from the Eastern Cape, South Africa. The objective of this work was to study the chemical composition of *Pelargonium peltatum* (L.) L'Hérit. leaf and twig essential oils and to assess the analgesic and anti-inflammatory activity of the oils. The study aimed at extracting essential oils from leaf and twig of *Pelargonium peltatum* (L.) L'Hérit., to determine the chemical composition of the essential oils and the assess the biological potential of the essential oils as analgesic and anti-inflammatory agents.

Methods

P. peltatum essential oils were obtained by hydro-distilling fresh or dry (leaf and twig) of *P. peltatum*, and were analysed by GC-FID and GC-MS. The oils were evaluated for acute toxicity test by using Lorke's method in mice, the animals were grouped into two phases, the first phase of the test consisted of three sub-groups (n = 3) for each dose level of 10, 100 and 1000 mg/kg. The second phase employed 4 subgroups (n = 1) per dose level of 1000, 1600, 2900 and 5000 mg/kg, respectively. The analgesic activity was determined by using a tail immersion method in rats. Whereas, the anti-inflammatory effect was assessed using right hind paw oedema induced by egg albumin;100, 200 and 400 mg/kg were the 3 doses selected for analgesic and anti-inflammatory experiments.

Results

According to the GC-FID and GC-MS results a total of fifty-three compounds were identified in the essential oils of *P. peltatum*, with camphene (3.6-33.4%), α-terpineol (4.8-19.1%), α-thujone (1.5-15.6%), piperitone (0.9-12.2%), linalool (1.6-11.7%), myrcene (5.2-10.7%), germacrene d (3.7-10.4%), β-caryophyllene (1.2-9.5%), β-cadinene (3.4-6.7%), β-bourbonene (4.2-6.2%), caryophyllene oxide (1.9-6.1%), β-pinene (1.4-6.0%), β-cubenene (5.6%), α-caryophyllene (0.8-5.5%), α-armophene (1.2-5.2%), phytone (0.4-5.0%) and β-phellandrene (4.6%) as the major compounds identified in the essential oils. For the bioactivities, the essential oils from *P. peltatum* revealed that even at the highest dosage of 5000 mg/kg caused no death for acute toxicity. While, an analgesic effect was shown by increasing the pain latency in hot water, and in an inflammation test the essential oils reduced the egg albumin induced paw oedema in both the first and second phase.

Conclusions

The present results indicate that fresh or dry (leaf and twig) essential oils of *P. peltatum* possess analgesic and anti-inflammatory activities; thus, endorsing the use of the plant in pain management and arthritic inflammatory conditions.

Chemical composition, analgesic and anti-inflammatory activities of *Hypoxis hemerocallidea* Fisch. & C.A. Mey from Eastern Cape, South Africa

P. Rungqu[1], A.O. Oyedeji[2], M. Gondwe[3], O.O. Oyedeji[1*]

[1] Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, SOUTH AFRICA
[2] Department of Chemistry, Faculty of Natural Sciences, Walter Sisulu University, Mthatha, SOUTH AFRICA
[3] Department of Human Biology, Faculty of Health Science, Walter Sisulu University, Mthatha, SOUTH AFRICA

* Email: ooyedeji@ufh.ac.za

Keywords: *Hypoxis hemerocallidea*, essential oils, chemical composition, analgesic, anti-inflammatory

Objective

This is the first report on the chemical composition, analgesic and anti-inflammatory activities of *H. Hemerocallidea* essential oils from Eastern Cape, South Africa. The objective of this work was to investigate the chemical composition of leaf and corm essential oils of *Hypoxis hemerocallidea* Fisch. & C.A. Mey from South Africa, and to evaluate the analgesic and anti-inflammatory effect of the essential oils. The study was aimed at extracting the essential oils from both fresh and dry (leaf and corm) of *Hypoxis hemerocallidea* Fisch. & C.A. Mey, determine the chemical composition, and evaluate their biological potential as analgesic and anti-inflammatory agents.

Methods

Hydro-distilling fresh or dry (leaf and corm) was used to obtain the essential oils of *H. hemerocallidea*, which were then analyzed by GC-FID and GC-MS. Lorke's method was used to examine the acute toxicity of the oils in mice. The mice were divided into two phases, with the first phase consisting of three subgroups (n = 3) for each dose level of 10, 100, and 1000 mg/kg. The second phase included four subgroups (n = 1), each receiving a dosage of 1000, 1600, 2900, or 5000 mg/kg. The analgesic activity was assessed by using a tail immersion method in rats. While, egg albumin-induced right hind paw oedema was used to test the anti-inflammatory effect of the essential oils; 100, 200 and 400 mg/kg were the 3 doses selected for analgesic and anti-inflammatory experiments.

Results

The essential oils of *H. hemerocallidea* comprised of fifty-one components with sabinene (0.9-27.6%), linalool (15.3-25.4%), α-terpineol (3.5-13.8%), β-caryophyllene (2.2-11.5%), α-terpinolene (0.4-9.8%), β-terpineol (2.1-9.2%), terpinene-4-ol (6.6-8.6%), hexadecane (2.7-8.1%), *cis*-nerolidol (6.8-7.7%), myrcene (4.1-7.5%), β-phellandrene (1.3-7.5%), *n*-hexadecanoic acid (6.6-6.9%), γ-terpinene (2.6-6.5%), linoleic acid (3.2-6.5%), *trans*-β-ocimene (0.4-6.4%), δ-3-carene (0.5-6.4%), octadecane (2.0-6.4%), β-bourbonene (3.0-6.2%), α-ionone (1.5-5.3%), β-selinene (2.4-5.2%), α-caryophyllene (1.8-4.8%), *trans*-isolimonene (0.1-4.6%), limonene (1.1-4.3%), ethyl linoleate (1.5-4.3%) and δ-cadinene (3.2-4.2%) being the most prevalent components of the oil. Pharmacological investigation the essential oils of *H. hemerocallidea* revealed that even the highest dose of 5000 mg/kg showed no acute toxicity-related deaths. While, in analgesic test the oils increased the reaction time substantially in all 100, 200 and 400 mg/kg doses. Moreover, the anti-inflammatory test of essentials oils effectively inhibited ($p<0.01$-0.001) the paw oedema induced by egg albumin at all the doses 100, 200 and 400 mg/kg.

Conclusions

These results confirmed the great potential of *H. hemerocallidea* essential oils and their use in traditional medicine. Therefore, fresh, or dry (leaf and corm) essential oils of *H. hemerocallidea* could be used as analgesic and anti-inflammatory agents with a high potential in the cosmetic and pharmaceutical fields.

Antibacterial activity of leather enriched with oregano essential oil under dynamic contact conditions

Elżbieta Bielak[1,*], Justyna Syguła-Cholewińska[2]

[1] Department of Non-food Product Quality and Safety, [2] Department of Microbiology, Institute of Quality Sciences and Product Management, Cracow University of Economics, Krakow, POLAND

* Email: bielake@uek.krakow.pl

Keywords: oregano essential oil, antimicrobial activity, dynamic contact conditions, lining leather, leather quality

Objective

The aim of the study was to determine the antibacterial activity of bovine lining leather finished with the addition of Portuguese oregano oil produced from the *Origanum vulgare* herb. This oil was applied into the leather at a concentration of 3%$_{wt}$ together with a fatliquoring mixture during the finishing step. The described experiment is one the part of broader research carried out to develop the light industry materials with the increased hygiene propieries, due to the bacteriostatic and fungistatic properties.

Methods

The antibacterial activity of lining leather treated with oregano oil 3%$_{wt}$ was tested in dynamic culture conditions, after half a year of its storage. The activity was assessed against two strains of gram-positive bacteria: non-sporulating *Staphylococcus aureus* (ATCC 25923) and spore-forming *Bacillus licheniformis* (environmental isolate), in accordance with ASTM E2149:2020 standard [1]. Leather samples, dimeter of 25 ± 5 mm, fatliquored with and without the addition of essential oil (control samples), were placed in the liquid broths with bacteria, under dynamic conditions. Then, the decrease in the number of bacteria after 1h and 24h of contact of the samples with microorganisms was assessed and compared to the concentration of bacteria in liquid broths at the beginning of experiment. The number of bacteria was calculated on the basis of the number of colonies which grown on the TSA medium during incubation at 35±1˚C, after 48h cultivation.

Results

The initial concertation of *S. aureus* suspension was 1.04 - 3.89 x 10^6 cfu/ml in the cultures. No significant changes in the number of bacteria were observed after 1h of the bacteria contact with bovine leather fatliquored with addition of oregano essential oil and without its addition. Additionally, after 24h of incubation, an increase in the number of staphylococci to the level of 2.40 - 7.50 x 10^9 cfu/ml (3 log) was noted, both in cultures with leather samples (with and without the addition of oil) and in bacteria culture itself. Results obtained for *B. licheniformis* were similar. Only in this case for 24-hour incubation, a smaller increase in the number of bacteria was observed, when compared to *S. aureus* (3.13 x 10^7 - 1.20 x 10^9 cfu/ml), in the bacteria culture without the sample and with the leather which was not enriched with essential oil. Although no increase in the number of bacteria was noted in the culture with the presence of the sample with addition of oregano oil.

Conclusions

As the result of the microbial analysis carried out with the dynamic contact conditions tests, no reduction in the number of bacteria was observed, so the antibacterial effect of leather fatliquored with 3%$_{wt}$ of oregano oil has not been proven. However, the strong antimicrobial activity for the same leather has been confirmed in other authors' studies [2] when their antibacterial activity was assessed with ISO 22196:2011 and ISO 20645:2005 standards. The conclusion is that the dynamic method is not recommended for verifying the antimicrobial properties of leather enriched with essential oils.

ACKNOWLEDGMENTS

The publication was financed from the funds of the Excellence in Research Programme, contract no. 011/ZJB/2022/DOS, being part of the subsidy granted to the Cracow University of Economics.

REFERENCES

[1] ASTM E2149 (2020). Standard test method for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions.
[2] Bielak E., Marcinkowska E., Syguła-Cholewińska,(2017). The durability of antimicrobial effect of leathers finished with oregano oil, Journal of the American Leather Chemists Association, vol. 112, no. 11, p. 377-386.

In vitro and *in situ* efficacy of a nanobiopesticide formulated by combining essential oils of *Syzygium aromaticum* and *Zingiber officinale* (70/30) against pathogenic *Lasiodiplodia theobromae* associated with post-harvest *Carica papaya* L rot in Cameroon

Davy Moussango[1,2*], Sameza Modeste[2], Mabou Christelle[3], Ampére Bédine[3], Jazet Dongmo[2], Nabin Kumar[4].

[1]Department of Energy Engineering, University Institute of Technology, University of Douala, Douala, Cameroon.

[2]Department of Biochemistry, Faculty of Sciences, University of Douala, Douala, Cameroon.

[3]Department of Biochemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon.

[4]Environment and Sustainability Department Institute of Minerals and Materials Technology Bhubaneswar-751013, India.

*E-mail: davy.moussango@yahoo.com

Keywords : Nanobiopesticide, Essential oils, antifungal activity, *Carica papaya*.

Objective

Papaya (*Carica papaya L.*) is one of the most consumed fruits in tropical and subtropical countries, such as Cameroon. It is very interesting to meet the dietary needs of humans because it is rich in glucose, minerals, vitamins and fiber [1]. However, the benefits of this fruit cannot be consumed exclusively because nowadays papaya is the breeding ground for many pests, the leader of which is *Lasiodiplodia theobromae*. It infects fruits in the field and the damage is manifested by significant yield losses (65–80%), devaluation of their organoleptic quality and a reduction in market value. To fight against this pathogen, farmers most often use chemical fungicides. These, although effective, have presented a number of limitations, including environmental pollution, the presence of residues on the fruits affecting the health of consumers [2]. To overcome these limitations, many researchers have turned to a control alternative based on essential oils and their formulation, like nanobiopesticides.

Methods

The flower buds of *S. aromaticum* (SA) and the rhizomes of *Z. officinale* (ZO) were collected in coastal Cameroon. The essential oils of the plants were extracted by hydrodistillation. The nanobiopesticide was formulated was formulated by nanoemulsion using the pressure homogenization technique by mixing tween 80, Mono Propylene Glycol, Glycerol, the EO combination (SA/ZO: 70/30) and distilled water under stirring for 05 hours. The antifungal potential *in vitro* on mycelial growth was done by the method of incorporation into agar and *in situ* on the development of necrosis by a preventive/curative treatment at different concentrations (from 125 ppm to 500 ppm for the *in vitro* tests and from 500 ppm to 5000 ppm for *in situ* tests).

Results

The nanobiopesticide based based on the combination of EO of *S. aromaticum* and the rhizomes of *Z. officinale* (70/30) to present homogeneity and stability over time. For *in vitro* activity, mycelial growth of *Lasiodiplodia theobromae* was completely reduced at 375 ppm. Regarding the *in situ* test, the nanobiopesticide completely inhibited the development of necrosis induced by Lasiodiplodia theobromae on the fruits of *Carica papaya L* at 2250 ppm for the preventive test and 3000 ppm for the curative test.

Conclusion

The formulated nanobiopesticide showed in vitro and in situ efficacy against the rot of *Carica papaya* induced by *Lasiodiplodia theobromae*. And therefore could be used as an alternative to chemical fungicides in the protection of papayas.

REFERENCES

[1] Balogun A., Ariahu C. (2020). Quality evaluation of fresh papaya fruits stored in evaporative coolers. Asian Food Sciences Journal, 15 : 6-16.
[2] Sarkhosh A., Schaffer B., Vargas A., Palmateer P., Lopez A., Soleymani A., Farzaneh M.. (2018). Antifungal activity of five plant-extracted essential oils against anthracnose in papaya fruit, Biological Agriculture & Horticulture, 34 (1) :18-26.

Fast and Reliable Identification of Non-Volatile Residue of Citrus Essential Oils by Supercritical Fluid Chromatography Coupled with Electron Ionization Mass Spectrometry

Roberta La Tella[1*], Francesca Rigano[1], Paola Dugo[1,2], Luigi Mondello[1,2,3]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[3] Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Rome, ITALY

* Email: roblatella@unime.it

Keywords: supercritical fluid chromatography, electron ionization mass spectrometry, SFC-EI-MS, citrus essential oils, spectral libraries

Objective

Supercritical fluid chromatography (SFC) is considered a very versatile technique, mainly due to the possibility of coupling with both gas and liquid chromatography detectors. Within this context, the present research focuses on the development of a prototypal instrumental setup, where SFC is serially hyphenated to UV detection and Electron Ionization Mass Spectrometry (EI-MS). A systematic study was carried out for a deep investigation of the influence of each SFC and EI-MS parameter on the performance of the new SFC-EI-MS instrumental setup, while the UV chromatogram is used as reference profile in order to monitor how the processes occurring at the SFC-EI-MS interface and into the ion source can affect the separation.

Methods

In this study seven citrus essential oils were analyzed: lime (*Citrus aurantifolia*), bergamot (*Citrus bergamia*), sweet orange (*Citrus sinensin*), bitter orange (*Citrus aurantium*), pink grapefruit (*Citrus paradise*), mandarin (*Citrus deliciosa*), and lemon (*Citrus limon*). Following treatment in a water bath at a temperature of around 100 °C to remove the volatile fraction, 25 mg of the residue were dissolved in 1 mL of ethanol and examined.

The chromatographic separation was achieved with a Ascentis Express F5 (2.7 µm, 150 mm x 4.6 mm). The binary mobile phase was composed of carbon dioxide (A) and methanol (B).

The SFC-EI-MS interface consists of 10 µm fused silica capillary tubing, heated at 200 °C, connected to a Tee Valve which splits the flow rate between the backpressure regulator (BPR) and MS: major is the pressure set at the BPR, major will be the eluent amount entering into the MS. The interface heating contrasts the CO_2 expansion and allows the fast transfer of the analytes into the ion source. Finally, setting the maximum ionization source temperature (300 °C) led to a considerable increase in signal intensity, because of very fast and efficient vaporization/desolvation/ionization phenomena.

Results

The SFC-EI-MS prototype was then employed for a fast, automatic and reliable identification of semi/non-volatile compounds, by the comparison with thousands of spectra present in the commercially available libraries. Specifically, the new system was employed for a fast screening of the non-volatile residue of citrus essential oils achieving a spectral similarity higher than 90% for coumarins, furocoumarins and polymethoxyflavones successfully detected in these samples.

Conclusions

The benefits of a SFC-EI-MS prototype system were briefly discussed. Despite the higher identification power arising from the highly informative EI-MS detector, the additional advantages were exploited: high speed (few minutes of total analys time), low cost and environmental-friendly (less than 6% of modifier at 2 ml/min flow rate) analysis were carried out.

Comprehensive Chemical Characterization of Unconventional Cold Pressed Seed Oils

Cinzia Cafarella[1,*], Francesca Rigano[1], Emanuela Trovato[1], Paola Dugo[1,2] , Luigi Mondello[1,2]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont SRL, c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: cinzia.cafarella@unime.it

Keywords: cold pressed, seed oils, chemical characterization

Objective

Edible seed oils have been extracted and used as a source of food ingredients since ancient times. As the body of evidence that links health benefits to the consumption of vegetable oils continues to grow, as well as the diffusion of vegetarian/vegan diet, many consumers prefer vegetable oils instead of animal fats. Due for that, the demand for high quality seed oils has greatly increased. Next to the rising consumption of conventional vegetable oils (olive, soybean, peanut, etc.), unconventional seed oils are appearing on the market (chia, hemp, etc.). Within this context, the aim of the present work was to investigate some unconventional cold-pressed seed oils with an attractive profile.

Methods

Carrot, raspberry, strawberry, blackcurrant, radish, pomegranate, rosehip and plum seed oils were investigated in terms of their chemical composition, including fatty acids, triacylglycerols (TAGs), tocopherols (vitamin E), phenols and the interesting volatile profile. Indeed, the peculiar feature of such seed oils is related to their aroma and color, *viz.* their organoleptic characteristics. The fatty acid composition and volatile fraction were elucidated by Gas Chromatography coupled to Flame Ionization Detector and Mass Spectrometry (MS). Intact lipids, in the form of TAGs, were reliably identified through liquid chromatography (LC) coupled to MS, with an automatic dual-filter identification strategy, which exploits the complementarity between Linear Retention Index and a home-made MS spectral library [1]. Vitamin E was fast determined without sample preparation procedures by means of a simple and cost-effective instrumentation, based on a sensitive and selective fluorimetric detection, coupled to LC. The phenolic extracts were analyzed by LC coupled to Photodiode Array Detector and MS in order to achieve complementary information usable for their reliable identification.

Results

Triglycerides are the main components of vegetable oils. Despite trilinolein and triolein were detected as the most abundant TAGs in most of the analyzed samples, alpha-linolenic acid was highly represented in all the samples with significant amounts in strawberry, raspberry and rosehip, while pomegranate seed oil showed trilinolenin as main peak. Quali-quantitative differences in the content of tocopherols and polyphenols were registered among different samples. Morover, differences in volatile compounds of the analized samples were observed and statistically evaluated.

Conclusions

The qualitative and quantitative data would represent the basis of future applications or selection of those seed oils for health purposes as a source of bioactive compounds.

REFERENCES

[1] L. Mondello, F. Rigano (2017).Identification of unknown compounds by using a novel retention index system in liquid chromatography, *PCT patent.*

A fast and eco-friendly HPLC-MS/MS method to determine oxygen heterocyclic compounds in *Citrus* essential oils

Giovanna Cafeo[1,*], Marina Russo[1], Paola Donato[1], Paola Dugo[1, 2], Luigi Mondello[1, 2]

[1] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Chromaleont S.r.l., c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: giovanna.cafeo@studenti.unime.it

Keywords: *Citrus* essential oil, furocoumarins, HPLC-MS/MS, coumarin, fast analysis

Objective

Citrus essential oils (EOs) are often employed both in food and cosmetic fields. The non-volatile fraction of cold-pressed *Citrus* EOs (2-15%) contains, among other class of molecules, Coumarins (Cs), Furocoumarins (FCs) and Polymethoxyflavones (PMFs), known as Oxygen Heterocyclic Compounds (OHCs). Unfortunately, negative effects have been reported both for coumarin and FCs, that can cause liver damage, and phototoxic reactions to skin respectively. Coumarin content is regulated both in food and cosmetics [1,2] whilst FCs content is regulated only in cosmetics [3,4]. Therefore, the evaluation of OHCs profile is important to quality control. For these reasons, over the years, differents analytical approaches (like GC-FID, NP-HPLC, RP-HPLC coupled with UV, fluorescence or mass detectors) were applied for identification and quantification of OHCs in *Citrus* oils, foods and cosmetic products containing *Citrus* derivates. This presentation will focus on a recently developed liquid chromatography tandem mass spectrometry method (HPLC-MS/MS) for the detemination of OHCs in *Citrus* essential oils and finished products.

Methods

The analyses were carried out on a liquid chromatographic Nexera X2 system coupled with a triple quadrupole mass spectrometer LCMS-8060 (Shimadzu, Duisburg, Germany) via an APCI interface set in positive ionization mode. Water and Ethanol were employed as mobile phase A and B respectively; the column was an Ascentis Express C18 (5 cm X 2.1 mm, 2.7 µm) (Merck). The target analytes (Cs, FCs, PMFs) were detected in Multiple Reaction Monitoring (MRM) acquisition mode.

Results

The HPLC-MS/MS method here proposed allowed a correct identification and quantification of the target compounds. Method validation was performed in terms of limit of detection (LoDs), limit of quantification (LoQs), linearity range, reproducibility and repeatability were calculated. LoQ and LoD values were in the ppb levels, making the method suitable to detect analytes at trace level.

Conclusions

This method is useful to quantify, in less of 5 minutes, OHCs contained also at trace levels in *Citrus* essential oils, food and cosmetic products in order to give informative data for new opinions and regulations in these fields.

ACKNOWLEDGMENTS

The authors acknowledge Merck Life Science and Shimadzu Corporation for their continuous support.

REFERENCES

[1] European Commission. Regulation (EC) No 1334/2008 on flavorings and certain food ingredients. Off. J. Eur. Union, 2008.
[2] Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the European Commission on Coumarin in flavourings and other food ingredients with flavouring properties. The EFSA J. 93, 1-15. 2008.
[3] European regulation (EC) No 1223/2009, Off. J. Eur. Union. 2009.
[4] IFRA Standard, 50th Amendment, 2021.

Development of an environmental-friendly SFC/PDA for the quantification of oxygen heterocyclic compounds in cold-pressed *Citrus* essential oils

Antonella Satira[1,*], Marina Russo[2], Paola Dugo[2], Luigi Mondello[1,2]

[1]Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

[2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: antonella.satira@chomaleont.it

Keywords: Oxygen heterocyclic compounds, *Citrus* essential oils, supercritical fluid chromatography, furocoumarins

Objective

In a context where consumers claim natural and environmentally friendly products, the chemical industry must adapt and move towards greener technologies, both in production and analysis [1]. Therefore, the analysis of natural products is increasingly carried out using "green" analytical methodologies.

Alternative approaches for the analysis of oxygen heterocyclic compounds in *Citrus* essential oils (EOs) are of primary importance for quality control departments in the perfume and flavor industry and other industrial fields [2]. In particular, the non-volatile fraction of *Citrus* EOs is composed for 10-20% of coumarins, furocoumarins and polymethoxyflavones.

A large number of analytical techniques to characterize the non-volatile composition of *Citrus* EOs were introduced in the literature. However, the reports of *Citrus* EOs analysis by supercritical fluid chromatography (SFC) with UV or mass spectrometry detectors for the analysis of non-volatile polar compounds are still few.

This study describes a new approach using SFC with a green mobile phase for the analysis of such compounds in cold-pressed *Citrus* EOs, to confirm the potential use of SFC-UV for oil classification in the context of quality control of raw materials in cosmetics. Gradient conditions are determined to achieve a satisfactory separation in 10 minutes.

Methods

Briefly, analyses were carried out by testing several columns. Mobile phase was composed of CO_2 (solvent A) and EtOH (solvent B). Analyses were carried out under gradient conditions. The oven temperature and BPR were set at 44°C and 120 bar, respectively.

Results

This method can be applied for the rapid analysis of cold-pressed *Citrus* EOs using the SFC-UV system, without the need for expensive instrumentation to determine target compounds. To satisfy the requirements of the most important organizations focused on cosmetics, however, a new sensitive SFC-UV method for the analysis of oxygen heterocyclic compounds at trace levels in finished products is currently being studied.

Conclusions

The separation of oxygen heterocyclic compounds in cold-pressed *Citrus* EOs is a challenge due to the wide variety of compounds and due to small structural differences between the compounds. The results obtained show that SFC-UV is a perfectly suited method to investigate the essential oil composition, because of the great number of compounds separated in a reduced analysis time (around 10 min), and with a very short time for re-equilibration of the system at the end of the gradient analysis.

REFERENCES

[1] Santerre C., Delannay E., Franco P., Vallet N., Touboul D., (2022). Comparison of Supercritical Fluid Chromatography Hyphenated to an Ultraviolet Detector and Gas Chromatography Hyphenated to a Flame Ionization Detector for Qualitative and Quantitative Analysis of Citrus Essential Oils, Separations, 2022, 9, 183

[2] Gad H. A., El Hassab M. A., Elhady S. S., Fahmy N. M., (2023). Insights on Citrus clementina essential oil as a potential antiaging candidate with a comparative chemometric study on different cultivars, Industrial Crops and Products, 194, 116349

Development of Quality Standards for Rose Essential Oil Growing in Saudi Arabia by Using Metabolomics Fingerprinting

Mohamed Fahad AlAjmi[1,*] and Dhiab AlDosari[1]

[1] Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

* Email: malajmii@ksu.edu.sa

Keywords: Rose oil, Jasmin oil, Peppermint oil, Quality standard, Metabolomics.

Abstract

Essential oils have long been used in traditional medicine due to their medicinal and preservative properties and also to impart aroma and flavor, these are also used as antioxidants and antimicrobial agents. Essential oils are aromatic, odorous product, highly sensitive to light and heat, soluble in alcohol, inflammable volatile liquids obtained from plant material such as flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants. Essential oils vary greatly in their composition, due to genetic causes, climate, rainfall, or geographic origin. Currently, the assessment of essential oils quality relies on the identification and quantification of single or few biomarkers known to be specific to each oil, unfortunately, this method could be easily cheated by spiking low quality oils with adulterants. Metabolomics fingerprinting is becoming widely appreciated as difficult to be cheated because of multivariate quality control method. In the present work, metabolomics fingerprinting for Damask rose essential oils were attempted.

Objective

The aim of this study is to develop new multivariate analysis-based quality standard using high resolution detection instruments and metabolomics.

Method

Samples were collected from different geographical regions. The essential oils of these aromatic plant samples were extracted by distillation (Rose and Peppermint), organic solvent extraction (Jasmin). The essential oils were analyzed by GC-MS, GC-MS analysis of the acquired raw data were subjected to data extraction including peaks alignment, “extraction blanks” subtraction and finally features extraction were performed using Compound Discoverer software, then the metabolomics fingerprinting were developed using “R” studio software of these essential chromatogram.

Conclusion

The method is sensitive and is able to distinguish the samples taken from different regions in Taif town.

Essential Oils Diversity of Zingiberaceae Plants from Vietnam

Le Thi Huong[1], Do Ngoc Dai[2,3], Isiaka Ajani Ogunwande[4*]

[1] School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, VIETNAM
[2] Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, VIETNAM
[3] Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City, Nghe An Province, VIETNAM
[4] Foresight Institute of Research and Translation, Ibadan, NIGERIA

* Email: isiakaogunwande@gmail.com

Keywords: essential oils, hydrodistillation, GC/MS, terpenes, biological activities

Objective

In the search for natural compounds for pharmacological and industrial uses it is eminent to predict plant materials as a suitable source of these compounds. Vietnam is a country where theer are immense flora that are source of compounds iiincluding essential oils. A wide arraay of molecules or essential oils and their activities are yet to be discovered from these several medicnal plants which are yet to be exploited for their chemical and biological studies.

Methods

We collected several plants belonging to the Zingiberaceae family from different natural habitats in Vietnam. In the search for essential oils composition from the Zingiberaceae forest plants, several species from *Alpinia*, *Amomum*, *Beosenbergia*, *Curcuma*, *Distichochlamys*, *Elettariopsis*, *Etlingera*, *Hedychium*, *Kaempferia*, *Meistera*, *Newmania*, *Siliquamomum*, *Stahlianthus* and *Zingiber* were studied. Different parts of the plants' parts were collected, analysed and reported. Esential oils were isolated by hydrodistillation, analysed by GC-FID and GC-MS [-4]. Antimicrobial, antioxidant, anticholinestrase, cytotoxicity and larvicidal activities were evlauated according to established procedures.

Results

Studies showed the essential oils were rich source of monoterpenoids, sesquiterpenoids, phenylpropanoids, amides, fatty acids and aromatic compounds. Some of these compounds are considered as chemotaxonomic markers of the different genus in the Zingiberaceae family. Pharmacological studies indicated that these essential oils exhibited antibacterial, antifungal, antioxidant, anticholinestrase, and anticancer activities. Hence, these results clearly provide relavant information on the status of phytochemical features of all studied species, with empahsis on the essential oils, with a view to provide leadway for selection of species with important chemical profiles. The outcome of these studies further provide support for the therapeutic potential of the studied species aiming for future clinical application.

Conclusions

Potential antibacterial antifungal, antioxidant and larvicidal compounds were isolated and confirmed from the studied of Vietnamese flora. The effect helps in full exploration of the potential of these aromatic plants as a source of natural sustainable products.

ACKNOWLEDGMENTS

The authors acknowledged financial support from the Ministry of Education and Training, Vietnam, Vietnam Academy of Science and Technology, among others.

REFERENCES

[1] Viet, N.T., Sam, L.N., Huong, L.T., Dai, D.N., Ogunwande, I.A. (2023). Antimicrobial activities and compositions of essential oils from the leaves and rhizomes of *Meistera cristatissima* (N.S.Lý & Škorničk.) Škorničk. from Vietnam, *Chemistry of Natural Compounds.* 59, 396-399.
[2] Huong, L.T., Huong, T.T., Bich, N.T., Viet, N.T., Ogunwande, I.A. (2022) Chemical compositions, larvicidal and antimicrobial activities of *Zingiber castaneum* (Škorničk. & Q.B. Nguyễn) and *Zingiber nitens* (M.F. Newman) essential oils. *Brazilain Journal of Pharmaceutical Sciences*, 58: 1-17.
[3] Huong, T.H., Linh, L.D., Dai, D.N., Ogunwande, I.A. (2022) Essential oil compositions and antimicrobial activity of the leaves and rhizomes of *Alpinia calcicola* Q. B. Nguyen & M.F. Newman from Vietnam. *Chemistry of Natural Compounds*, 58, 939-942.
[4] Dai, D.N., Huong, L.T., Sam, L.Y. and Ogunwande, I.A. (2022) Chemical compositions and antimicrobial activity of essential oil from the rhizomes of *Curcuma pambrosima* Škorničk. & N.S. Lý growing in Vietnam. *Chemistry of Natural Compounds*, 58, 148-150.
[5] Huong, L.T., Huong, T.T., Dai, D.N., Ogunwande, I.A. (2022) Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils of *Hedychium stenopetalum* Lodd. and *Hedychium villosum* Wall. of Zingiberaceae family from Vietnam. *Journal of Essential Oil Bearing Plants*, 25, 924-938.

Chemical characterization of Finger Lime (*Citrus australasica* L.) essential oil and hexane extract

Filippo Alibrando[1,*], Giovanna Cafeo[2], Marina Russo[2], Paola Dugo[1, 2], Luigi Mondello[1, 2]

[1] Chromaleont S.r.l., c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: filippo.alibrando@chromaleont.it

Keywords: Citrus essential oil, Finger lime, *Citrus autralasica*, furocoumarins, GC-MS, GC-FID, Chiral, HPLC-MS/MS

Objective

Finger lime (*Citrus australasica L.*) is a *Citrus* species endemic to Australia. One of the most distinctive traits of this fruit, also known as "citrus caviar", is the pulp with a caviar-like appearance for the presence of spherical vescicles. The aim of the present study was to investigate both the volatile and non-volatile profiles of an essential oil of a finger lime cultivar grown in Calabria (southern Italy). The volatile fraction was investigated by Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Flame Ionization Detection (GC-FID). Furthermore, a chiral study was conducted on the samples by using a GC-FID equipped with two different capillary columns and two FID detectors (parallel detection). The oxygen heterociclic fraction (Coumarins, Cs, Furocoumarins, FCs and Polymethoxyflavones, PMFs) was analysed by liquid chromatography coupled with a triple quadrupole as spectrometer (HPLC-MS/MS).

Methods

The qualitative analysis of the volatile fraction was carried out on a GCMS-QP2020 NX system (Shimadzu Europa GmbH, Germany) equipped with a SLB-5ms fused-silica capillary column (30m x 0.25mm i.d. x 0.25 μm df) (Merck Life Science, Merck KGaA, Darmstadt, Germany), while quantitative analysis was performed on a GC-2030 (Shimadzu Europa GmbH, Germany) and the same previous capillary column. Chiral analysis was performed on the same previous GC-FID, equipped with β-DEX™ 120, (30m x 0.25mm i.d. x 0.25μm df) (Merck Life Science) and Mega-DEX DET-Beta (25m x 0.25mm id x 0.25μm df) (Mega, Legnano, Italy) capillary columns.

The analyses of non-volatile fraction were carried out on a liquid chromatographic Nexera X2 system coupled with a triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Europa GmbH, Germany) via an APCI interface set in positive ionization mode. For the separation, an Ascentis Express C18 column (5cm x 4.6mm, 2.7μm) was selected; water/methanol/THF (85:10:5 *v/v/v*) and methanol/THF (95:5 *v/v*) were used as solvent A and B, respectively. The 37 target analytes (Cs, FCs, PMFs) were detected in Multiple Reaction Monitoring (MRM) acquisition mode.

Results

The cold-pressed essential oil resulted richest than the hexane extract (both in volatile and non-volatile fractions). The identification of the 75 volatile compounds was carried out by using Linear Retention Index (LRI), previously calculated by injection of C7-C40 n-alkane reference mix. 12 oxygen heterocyclic compounds were detected in the samples, with a total amount of OHCs of 46995.6 mg L^{-1}. The most abundant compounds were bergamottin, 8-geranyloxypsoralen and 8-methoxypsoralen for both samples.

Conclusions

The obtained results were compared with those present in the literature, showing how the extraction technique affects the composition of the volatile and non-volatile fraction. The volatile fraction of the two types of finger lime extracts showed qualitative and quantitative differences, while the oxygen heterociclic profile presented only quantitative differences.

ACKNOWLEDGMENTS

The authors acknowledge Merck Life Science and Shimadzu Corporation for their continuous support, Azienda Agricola "Biore" and Baller S.r.l. for providing samples

Bergamot essential oil: characterization of volatile fraction, Oxygen Heterocyclic Compounds and enantiomeric distribution of volatile components.

Filippo Alibrando[1*], Federica Vento[2], Giuseppe Micalizzi[2], Marina Russo[2], Ivana Bonaccorsi[2], Micaela Galletta[2], Paola Dugo[1, 2], Luigi Mondello[1, 2]

[1] Chromaleont S.r.l., c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: filippo.alibrando@chromaleont.it

Keywords: Bergamot oil, Citrus EO, GC-MS, GC-FID, chiral analysis, oxygen heterocyclic compounds

Objective

Bergamot (*Citrus Bergamia, Risso*) is a fragrant citrus fruit and it is cultivated mainly in the south of Italy, especially along the Tyrrhenian and Ionian coasts in the southern part of Calabria, thanks to particular climatic and environmental conditions. Bergamot essential oil is extracted by using "pelatrice" machine and it is a valuable product, widely requested in the perfume industry. Three cultivars are used for its production: "Fantastico", "Castagnaro" and "Femminello".

Like other citrus oils, Bergamot essential oil is a mixture of monoterpene and sesquiterpene hydrocarbons, oxygenated derivatives and non-volatile residue.

The main aim of this research is the evaluation of the volatile and non-volatile fraction, that is to say those molecules commonly used to evaluate the quality and authenticity of the oils. Furthermore, a chiral study was conducted on the samples by using a GC-FID equipped with two different capillary columns and two FID detectors (parallel detection).

Methods

The qualitative analysis of the volatile fraction was carried out on a GCMS-QP2020 NX system (Shimadzu Europa, Germany) equipped with a SLB-5ms fused-silica capillary column (Merck , Darmstadt, Germany), while quantitative analysis was performed on a GC-2030 (Shimadzu Europa, Germany) and the same previous capillary column. Chiral analysis was performed on the same previous GC-FID, equipped with β-DEX™ 120, (Merck , Darmstadt, Germany) and Mega-DEX DET-Beta (Mega, Legnano, Italy) capillary columns. The analysis of the non-volatile fraction was performed by using a LC-2040C 3D Nexera-i PDA integrated UHPLC System (Shimadzu Europa, Germany). For the separation, an Ascentis Express HPLC (Merck , Darmstadt, Germany) column was selected, water/methanol/THF (85:10:5 v/v) and methanol/THF (95:5 v/v) were used as solvent A and B, respectively.

Results

Qualitative and quantitative results have been compared with those previously obtained by our research group, highlighting the differences due to pedo-climatic conditions or genetic variations. Furthermore, the results showed how the composition of the volatile fraction is influenced by the Bergamot collection period (early or late production season).

Conclusions

The analysed samples were collected in the years 2020-2023, by using "pelatrice" machine. The obtained results allowed us to have a clear picture about the composition of the volatile and non-volatile fractions of Bergamot essential oil.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corp. and Merck KGaA for their support and Simone Gatto S.r.l. for providing the samples.

Lemon essential oil: characterization of volatile fraction, Oxygen Heterocyclic Compounds and enantiomeric distribution of volatile components.

Filippo Alibrando[1*], Federica Vento[2], Giuseppe Micalizzi[2], Marina Russo[2], Ivana Bonaccorsi[2], Valentina Chiaia[2], Paola Dugo[1, 2], Luigi Mondello[1, 2]

[1] Chromaleont S.r.l., c/o Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY
[2] Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, Messina, ITALY

* Email: filippo.alibrando@chromaleont.it

Keywords: Lemon oil, Citrus EO, GC-MS, GC-FID**,** chiral analysis, oxygen heterocyclic compounds

Objective

Lemon (Citrus limon) has oriental origins and it is commonly cultivated in the Mediterranean area.
The extracted essential oil is characterized by the presence of monoterpene and sesquiterpene hydrocarbons, oxygenated derivatives, non-volatile residue and the color varies according to the production period. The extraction techniques are based on cold processes, such as “Pelatrice”, “Sfumatrice”, “FMC in-line” and the composition can be influenced by the employed technique.
The unique fragrance, together with beneficial propertier for human health, make this essential oil in high demand in food, perfume, pharmaceutical and cosmetic industry.
The main aim of this research is the evaluation of the volatile and non-volatile fraction, that is to say those molecules commonly used to evaluate the quality and authenticity of the oils, in order to ensure its genuineness. Furthermore, a chiral study was conducted on the samples by using a GC-FID equipped with two different capillary columns and two FID detectors (parallel detection).

Methods

The qualitative analysis of the volatile fraction was carried out on a GCMS-QP2020 NX system (Shimadzu Europa, Germany) equipped with a SLB-5ms fused-silica capillary column (Merck , Darmstadt, Germany), while quantitative analysis was performed on a GC-2030 (Shimadzu Europa, Germany) and the same previous capillary column. Chiral analysis was performed on the same previous GC-FID, equipped with β-DEX™ 120, (Merck , Darmstadt, Germany) and Mega-DEX DET-Beta (Mega, Legnano, Italy) capillary columns. The analysis of the non-volatile fraction was performed by using a LC-2040C 3D Nexera-i PDA integrated UHPLC System (Shimadzu Europa, Germany). For the separation, an Ascentis Express HPLC (Merck , Darmstadt, Germany) column was selected, water/methanol/THF (85:10:5 v/v) and methanol/THF (95:5 v/v) were used as solvent A and B, respectively.

Results

Qualitative and quantitative analyses were carried out on lemon essential oils extracted by using “Pelatrice”, “Sfumatrice” and “FMC in-line” techniques. The obtained results have been compared with those previously published in the literature, highlighting the differences due to pedo-climatic conditions or genetic variations. Furthermore, the results showed how the composition of the volatile fraction is influenced by the Lemon collection period (early or late production season).

Conclusions

The analysed samples were collected in the years 2020-2023, by using cold pressing techniques. The obtained results allowed us to have a clear picture about the composition of the volatile and non-volatile fractions of lemon essential oil, a useful tool for the quality assessment of this industrial product.

ACKNOWLEDGMENTS

The authors thank Shimadzu Corp. and Merck KGaA for their support and Simone Gatto S.r.l and F.lli Branca S.p.a. for providing the samples.

AUTHOR INDEX

Acknowledgments

Platinum Sponsor

Gold Sponsor

Silver Sponsor

Media Partner

Other Sponsor

Chromaleont s.r.l., Messina, Italy