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In Collaboration with



Chairmen:

Prof. Dr. Giovanni Dugo, Prof. Dr. Luigi Mondello, University of Messina, Italy

Scientific Program by type

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Plenary Lectures



Plenary Lectures

PL.01) Hyphenated Techniques in Essential Oil Analysis. Quo Vadis?

P. Sandra¹ and F. David²

¹ Ghent University, Department Organic Chemistry, Krijgslaan, Belgium.

² Research Institute for Chromatography, Kortrijk, Belgium.

PL.02) The Use of New Technologies to Develop Environmentally Benign Processes: Acid-Catalyzed Hydrolysis of Pinene in Aqueous Media

T. S. Chamblee¹, R. R. Weikel², S. A. Nolen², C. L. Liotta² and C. A. Eckert²

¹ The Coca-Cola Company, Atlanta, Georgia

² Schools of Chemical and Biochemical Engineering and Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia

PL.03) Recent advances in biochemistry and molecular biology of plant terpenoids

M. E. Maffei and C. M. Berteà

Department of Plant Biology and Centre of Excellence for Plant and Microbial Biosensing – University of Turin, Italy

PL.04) Analysis of allergens in fragrance: An analytical challenge

A. Chaintreau

Firmenich S.A., Geneva, Switzerland

PL.05) Different Pharmacological Activity of Essential Oils

S. Cuzzocrea, L. Dugo

University of Messina, Italy.

Hyphenated Techniques in Essential Oil Analysis. Quo Vadis?

P. Sandra¹ and F. David²

¹Ghent University, Department Organic Chemistry, Krijgslaan 281-S4, B-9000 Ghent, Belgium.

²Research Institute for Chromatography, Kennedypark 20, B-8500 Kortrijk, Belgium.

Abstract

In the last two decades, several hyphenated analytical techniques have been introduced as panaceas for our analytical work. The potential of most of them have been illustrated with essential oil analysis, however, only few of them are presently applied in R&D and QA/QC laboratories. What can be the reasons? ... are the hyphenated systems lacking robustness? ... too difficult to work with? ... can they not keep their promises? ... or is there simply no need for?

In this contribution, an overview will be presented on pre-separation, in-separation and post-separation hyphenated systems applied in our laboratories for the analysis of complex samples like essential oils. Emphasis will not only be on their performance but also on their robustness and validation.

Pre-separation methods are inherently connected to sample preparation and, more specifically to clean-up and/or fractionation. Key word is selectivity in broad sense and for some applications, selectivity can be high enough to bypass separation (MS-based chemical sensors). In multidimensional (GC-GC, LC-LC, etc) and multimodal (LC-GC) chromatographic techniques, applied as pre-separation technique, orthogonality is of utmost importance.

In-separation hyphenation nowadays receives a lot of attention through the introduction of the comprehensive approaches GC x GC and LC x LC producing indeed overwhelming profiles and more is to come (SFC x GC, SFC x LC). What can we expect from the comprehensive separation methods ... will they recognise a breakthrough? In principle, they should as no other methods can give the same degree of peak capacity and orthogonality. The potential of the comprehensive techniques for essential oil analysis will be illustrated and compared to the more classical multidimensional methods.

Post-separation hyphenation with spectrometers is currently routinely applied. New systems are introduced (MS-MS, LR-TOFMS, HR-TOFMS, ICPMS, FT-ICRMS, NMR) while others disappear (IR, AED). Present selection of post-separation hyphenation is mostly dictated by the aim of the analysis i.e. R&D or QA/QC.

Hyphenation also results in new software and imaging programs like the Deconvolution Reporting Software combining RTL and MS data. Deconvolution should, however, be critically evaluated.

The Use of New Technologies to Develop Environmentally Benign Processes: Acid-Catalyzed Hydrolysis of Pinene in Aqueous Media

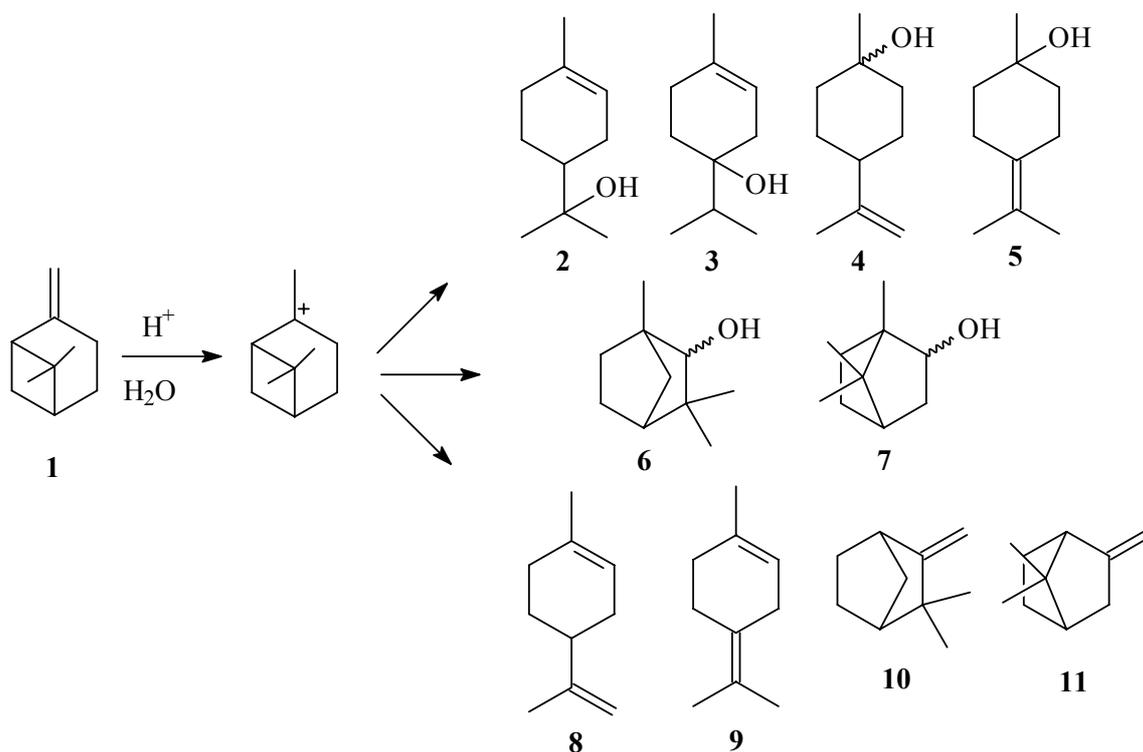
T. S. Chamblee¹, R. R. Weikel², S. A. Nolen², C. L. Liotta² and C. A. Eckert²

¹ The Coca-Cola Company, Atlanta, Georgia

² Schools of Chemical and Biochemical Engineering and Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia

Abstract

The formation of α -terpineol (**2**) from β -pinene (**1**) is a reaction of commercial significance to the flavor and fragrance industry. The process uses relatively large quantities of strong acid. We demonstrate that the reaction can be performed under more environmentally benign conditions using the *in situ* acid formation capabilities of two different green technologies: hot water reaction (200°C) and CO₂ gas expanded liquids. Both methods offer the advantage of *in situ* acid formation and self-neutralization, thus eliminating waste and offering advantages for product recovery. In addition, the cost of starting materials is reduced since no acid is added in the reaction. This work presents an example of the application of these methods to a reaction that has commercial significance and adds to our knowledge about the benefits and effect of co-solvents. The relative rates and product distributions achieved in each system will be discussed.



Recent advances in biochemistry and molecular biology of plant terpenoids

M. E. Maffei and C. M. Bertea

Department of Plant Biology and Centre of Excellence for Plant and Microbial Biosensing –
University of Turin, Italy

Abstract

Terpenoids are a structurally diverse group of secondary plant metabolites. About 30,000 representatives with a variety of biological functions, including medically important compounds such as vitamins, hormones, cytosolic agents and with a plethora of biological functions have been reported in the plant kingdom. The ability of plants to prosper throughout their long evolution has been strongly dependent on the constant development of new strategies to counteract, poison and lure insects and, in general, attacking herbivores. This has led to the creation of elaborate biosynthetic pathways, leading to the formation of both general defence and target-oriented molecules. To give just a few examples, carotenoids serve as light-harvesting and light-protecting pigments, sterols play important roles as modulators of membrane properties, the phytol side chain of chlorophyll is of terpenoid origin, and a wide variety of plant terpenoids function as insect attractants or repellents. Terpenoids have also attracted commercial interest as pharmaceuticals, cosmetics and, recently as nutraceuticals.

From a biochemical point of view, all terpenoids are biosynthesized from just two C₅ precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). During a period of several decades, the mevalonate pathway was considered as the universal source of biosynthetic DMAPP and IPP. Experimental data demonstrated that pyruvate and a triose phosphate can serve as precursors for the formation of IPP and DMAPP by an alternative pathway. Arigoni and co-workers showed that 1-deoxy-D-xylulose, can be diverted very efficiently to terpenoids. The deoxyxylulose pathway is involved in higher plants in the biosynthesis of monoterpenes, essential oils, diterpenes, chlorophyll and carotenoids, phytol and isoprene.

Biotechnological and molecular biological applications in the field of terpenoid production are of paramount importance. Conventional biotechnologies such as cell and tissue culture and biofermentation have been thoroughly studied and the new developments in gene manipulation techniques offer many potential attractive targets for several applications: from biomedicine to functional food production.

Terpenoids are powerful weapons in plant-environment interactions. By using terpenoids plants can actively respond to biotic and abiotic stress, arranging the response according to the stimulus perceived. Moreover, when attacked by herbivores many plants use terpenoids as a cry for help, attracting predators of the attacking herbivores, in a complex interaction called tritrophic interaction.

The lecture will give highlights on recent advances in terpenoid molecular biology, biochemistry and physiology, by covering several aspects from enzyme activity to gene activation up to signalling cascades upon perception of stress.

Analysis of allergens in fragrance: An analytical challenge

A. Chaintreau

Firmenich S.A., Route des Jeunes, 1, Geneva - 1211 GE 8, Switzerland

Abstract

Over the past few years, the determination of 24 suspected allergens in fragrances has become an analytical challenge, because European legislation now requires labeling finished product packages when these substances occur above a given limit. This presentation covers papers published up-to-now and some on-going developments concerning their quantification. First, a routine GC/MS method has been developed within the IFRA¹ framework. A suitable data treatment strategy allows solving almost all complex cases (co-elution, peak shifts). Tests in a sample spiked with all allergens suggest a limit of quantification down to 10-20 ppm in fragrance concentrates. Alternatively, a GC/CI-MS method allows the selective determination of allergens when their peaks are co-eluted with other perfume ingredients. As comprehensive two-dimensional gas chromatography (GCxGC) recently emerged as a state-of-the-art technique for the analysis of volatile compounds, this approach has also been investigated. It shows similar capabilities to GC/MS when a flame ionization detector is used. To go beyond this limit, a GCxGC/quadrupole MS (q-MS) prototype was built and showed a much higher performance than GC/MS and GCxGC/FID. Finally, coupling GCxGC with a time-of-flight MS offers unrivalled capabilities of peak separation and identification.

¹ IFRA: International Fragrance Association.

Different Pharmacological Activity of Essential Oils

S. Cuzzocrea, L. Dugo

Dipartimento Clinico e Sperimentale di Medicina e Farmacologia, Torre Biologica, Policlinico Universitario, 98123 – Messina, Italy

Abstract

Current interest in the various areas of inflammation, oxidative stress and cancer has led to development of many remedies linked to traditional medicine. Many bioactive volatile oils, with different structure-activity relationships, exhibit interesting and promising activities in various *in vivo* and *in vitro* studies.

Reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals, and hydrogen peroxide as well as peroxynitrite contribute to pathological situation associated with inflammation, shock and ischemia and reperfusion. The generation of ROS during these pathological situations has been directly demonstrated also in human using different analytic assays such as lipid peroxidation, electron paramagnetic resonance spectroscopy, chemiluminescence. In principle, ROS may injure cells by causing peroxidation of membrane lipids, denaturation of proteins including enzymes and ion channels, and by causing strand breaks in DNA. The hypothesis that the generation of ROS contributes to tissue damage is supported by many experimental studies demonstrating beneficial effects of interventions, which either attenuate the generation, or reduce the effects, of ROS. These therapeutic strategies include antioxidant enzymes such as superoxide dismutase (SOD) and catalase, radical scavengers such as mannitol and α -tocopherol, and agents, which prevent the generation of radicals such as allopurinol and deferoxamine. Therefore, recent evidence have suggested that antioxidants like vitamin E may have a role in the prevention of various pathological situation like atherosclerosis through inhibition of oxidative modifications of LDLs. In addition recent observational studies suggest an important correlation between the intake of antioxidants, such as vitamin E and β -carotene, and the incidence of coronary heart disease in contrast clinical trials of antioxidant supplementation in patients with ischemic heart disease have yielded apparently conflicting results. Current research into development of new free radical scavengers has confirmed that food rich in antioxidants plays an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases, the most well-known of which are Parkinson's and Alzheimer's diseases, as well as inflammation and problems caused by cell and cutaneous ageing. Active principles contained in several essential oils have proven to be effective in reducing ROS-induced damage in a various number of pathological situations ranging from acute and chronic inflammation to cancer and neurodegenerative diseases. Many of these active principles, such as the polyphenols in green tea (*Melaleuca alternifolia*) oil, or active components of other essential oils like eucalyptus, rosemary or citrus oil, are now used in complementary and alternative medicines, alone or in mixed formulations. The well documented cancer chemopreventive activities of green tea, for example, have been attributed, at least in part, to the antioxidant and free radical scavenging activities of tea polyphenols.

L.01) Comprehensive GCxGC: a new tool for essential oil analysis?

C. Bicchi, C. Cordero and P. Rubiolo

Dipartimento di Scienza e Tecnologia del Farmaco – Università di Torino, Torino, Italy

L.02) A "Chiral Pool" of Monoterpenes from Aromatic Plants

U. Ravid and O. Larkov

Department of Aromatic Plants, Agricultural Research Organization, Neve Ya'ar, Ramat Yishay Israel

L.03) The value of having a multidisciplinary approach to the study of essential oils – examples from the South African flora

A. Viljoen¹ and K. H. C. Başer²

¹Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

²Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Turkey

L.04) On the Stress Relieving Properties of Chiral Essential Oil Constituents: A New Assessment Method

M. Höferl and G. Buchbauer

Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria

L.05) High-speed GC Essential Oil Analysis with 50 and 100 µm ID Micro-bore Columns

P. Q. Tranchida¹, A. Casilli¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy

²Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, Messina, Italy

³Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

L.06 Application of Raman Spectroscopy for Non-Destructive Analysis of Essential Oil Plants

H. Schulz, M. Baranska

Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute for Plant Analysis, Quedlinburg, Germany

L.07) Comprehensive Two-dimensional NP (Adsorption)-RP Liquid Chromatography for Essential Oil Analysis

M. Lo Presti¹, R. Luppino², P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy

²Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, Messina, Italy

³Avantech S.r.l., Via Crocefisso, 75, 84012 - Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, Via Crocefisso, 75, 84012 - Angri (SA), Italy

L.08) A Novel Type of Membranes based on Cholesteryl Phosphate

S. Sedaghat¹, S. Streiff², N. Ribeiro², L. Désaubry², Y. Nakatani² and G. Ourisson²

¹Department of Chemistry, Islamic Azad University, North Tehran Branch,

²Centre de Neurochimie, Strasbourg, France

L.09) Homogeneity and quality of the drug in wild growing *Achillea* populations

E. Nemeth, J. Bernáth, E. Héthelyi and K. Demeter

Dept. of Medicinal and Aromatic Plants (Medicinal Plant Group of the Hungarian Academy of Sciences), BKA University, Budapest, Hungary

L.10) Aromatic production of Morocco

S. Zrira and B. Benjlali

Département des Sciences Alimentaires et Nutritionnelles, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco

L.11) Determination of Antimicrobial Effects of Essential Oil Obtained from Some Natural Plants In Northern Cyprus

M. Akin and A. Uslu

Department of Biology, Science Faculty, University of Selcuk

L.12) Biological effects of Corsican Everlasting Essential Oil (*Immortelle*) *Helichrysum italicum* (Roth) G. Don ssp. *serotinum* (Boiss.) on human skin in –vitro and ex-vivo assays

L. Decomme¹, G. Gueneva², D. Davenne³

¹Laboratoire de biogénotoxicologie Faculté de Pharmacie 27 Boulevard Jean Moulin

13385 Marseille France cedex 5

²Laboratoire de biochimie cellulaire Faculté de Pharmacie 27 Boulevard Jean Moulin

13385 Marseille France cedex 5

³Laboratoire Rosier Davenne 50 Rue Henri Bessemer 13854 Aix en Provence, France cedex 3

L.13) Phytochemical Investigation of *Arnica montana* (L.) and *A. chamissonis* (Lessing) Grown in Scotland

K. Svoboda¹, J. Brooker², G. Ruzickova¹, D. Taylor³ and R. Greenaway¹

¹SAC Auchincruive, Dpt of Life Sciences Scotland UK

²Avian Science Research Centre, Auchincruive campus, SAC, Edinburgh Scotland UK

³CPL Aromas Ltd, Barrington Hall, Hatfield Broad Oak, Stortford, UK

L.14) Effect of Mycorrhization on Amount and Composition of Essential Oils of Marjoram, Thyme, Sage and Caraway

J. Richter, D.Franz and I. Schellenberg

Department of Bioanalysis, Anhalt University of Applied Sciences, Bernburg, Germany

L.15) Practicing Aromatherapy in Elsalvador - Use of essential oils in basic health; Care in developing countries

J-V. Pineda Monge

Sanlúcar La Mayor, Sevilla, España

L.16) New compounds from *Pimpinella* oils

K.H.C. Baser¹, N. Tabanca^{1,3}, N. Kirimer¹, E. Bedir^{2,3}, I. A. Khan³

¹Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

² Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, Turkey

³National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS, USA

L.17) Determination of pesticide and plasticizers residues in bergamot essential oils

D. Giuffrida¹, G. Di Bella², A. Bruzzese¹, L. Serrao¹, Giacomo Dugo¹

¹Dipartimento di Chimica Organica e Biologica, University of Messina, Messina, Italy

²Dipartimento Farmaco-chimico, University of Messina, Messina, Italy

L.18) Herbs and Essential Oils as Additives in Stock Farming and Pet Food - Actual Situation, Experimental Results and Outlook

R. Thomann¹, U. Bauermann¹, I. Halle², F. Bauer³

¹IGV Institut für Getreideverarbeitung GmbH, Bergholz-Rehbrücke, Germany,

²Institut für Tierernährung, Bundesforschungsanstalt für Landwirtschaft (FAL), Braunschweig, Germany

³Veterinärwissenschaftliche Universität Wien, Wien, Austria

L.19) Derivative stripping chronopotentiometry for heavy metals analysis in citrus essential oils

L. La Pera, D. Pollicino, M. Saitta, R. B. Lo Curto and Giacomo Dugo

Department of Organic and Biological Chemistry, University of Messina, Messina, Italy

L.20) Forum Essenzia – Essential oil application and Aromatherapy in the European Context

K. Duerbeck

Forum Essenzia e.V., Munich, Germany

L.21) GCxGC AND GCxGC/qMS for Complex Sample Analysis

A. Casilli¹, P. Q. Tranchida¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy

²Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, Messina, Italy

³Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

L.22) ESSENTIAL OILS- VISION 2007 (India Emerging as Major producer of many bulk Essential Oils)

R. Harlalka

Nishant Aromas, Milan Ind. Estate, Mumbai, India

L.23) A Comparison of Microwave-Assisted Hydro Distillation, Hydro Distillation and Supercritical Fluid Techniques for the Preparation of Essential Oils

M. Lo Presti¹, A. Trozzi³, S. Ragusa³, P. Dugo², G. Colnaghi⁴, F. Visinoni⁴, Giovanni Dugo¹ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, Facoltà di Farmacia, University of Messina, Messina, Italy

²Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.NN.FF., University of Messina, Messina, Italy

³Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università di Catanzaro, Catanzaro, Italy

⁴Milestones S.r.l., Sorisole (BG), Italy

L.24) Essential Oils Composition of the Six Populations of *Foeniculum vulgare* Mill. ssp. *capillaceum* from Iran

M.M. Barazandeh, M.B. Rezaei and K. Jaimand

Research Institute of Forests and Rangelands, Tehran, Iran

L.25) New Sesquiterpenoids from three southern hemispheric liverworts: *Lepidozia spinosissima*, *Chiloscyphus subporosus* and *Mastigophora diclados*

F. Nagashima, L. Harinantenaina and Y. Asakawa

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan

L.26) New Potent Aldehydes and Related Alcohols in Fresh Lemon Peel

R. Naef and A. Jaquier

Firmenich SA, Corporate R&D Division, Geneva 8, Switzerland

Comprehensive GCxGC: a new tool for essential oil analysis?

C. Bicchi, C. Cordero, P. Rubiolo

Dipartimento di Scienza e Tecnologia del Farmaco – Università di Torino, Via Pietro Giuria 9 – I-10125 Torino

Abstract

Comprehensive GCxGC is the most recent and sophisticated gas chromatographic technique for the separation of very complex mixtures. Essential oils are generally medium to high complexity mixtures of medium to low polarity compounds that are often very difficult to be separated: this is particularly true for sesquiterpenoids.

This communication aims to discuss critically advantages, disadvantages and present limits of comprehensive GCxGC when essential oils or fractions of them have to be analysed. Each topic will be illustrated through some examples concerning the analysis of complex essential oils or fractions of them, the quantitative determination of trace components, and direct enantiomer recognition of marker compounds characteristic of commercial essential oils.

Homogeneity and quality of the drug in wild growing *Achillea* populations

E. Nemeth¹, J. Bernáth, E. Héthelyi and K. Demeter

¹ Dept. of Medicinal and Aromatic Plants (Medicinal Plant Group of the Hungarian Academy of Sciences), BKA University, 1118 Budapest, Hungary

Abstract

Hungary is one of the greatest exporter of *Achillea* drugs, however, their chemical and pharmaceutical value are extremely variable. Our intention was to clear up the level and possible causes of variability concerning of productional and chemical characteristics of *A. collina* both hat the level of populations and individuals.

19 populations were tested during the vegetation period in 2003, evaluating the homogeneity of the stands, the vegetative as well as the generative reproduction capacity, biomass production, essential oil content and the main components of the oil.

A considerable variability among and inside the populations could be established concerning several morphological and productional characteristics. The average content of essential oil in the populations reached 0,02-0,71% with considerable individual plant differences (marginal values 0,01- 1,77% d.w.). Chamazulene proved to be the main component of nine populations, while there were other constituents registered as main component in four cases (mainly sesquiterpenes). In the remaining populations chamazulene together with another terpenoid (e.g. borneole) represented the major components. In the best individuals chamazule accumulated as high as 80% of the oil.

The correlation among morphological and chemical homogeneity as well as the form of natural multiplication will be evaluated.

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Application of Raman Spectroscopy for Non-Destructive Analysis of Essential Oil Plants

H. Schulz¹, M. Baranska^{1,2}

¹ Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute for Plant Analysis, Neuer Weg 22-23, D-06484 Quedlinburg, Germany. E-mail: H.Schulz@bafz.de.

² Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

Abstract

In order to ensure the quality of essential oils used in fragrances, cosmetic materials and aroma preparations, usually GC combined with FID or MS detection is applied. Since these methods are time-consuming and expensive, some attempts have been made to find alternative analytical options. In this context several new vibrational spectroscopy methods in combination with sophisticated chemometric algorithms were successfully introduced (e.g. using NIR- and ATR-IR-techniques) for an efficient determination of individual essential oil components [1,2]. Whereas NIRS data can be interpreted only by application of statistical methods, Raman spectra in most cases present characteristic key bands of the individual volatile fraction and therefore in principle allow easily the discrimination of different plant species or chemotypes. Raman spectroscopy has also proven to be a fast and reliable method for quality control of various isolated essential oils requiring both minimal sample preparation and minimum amount of analyte. Furthermore, this technique principally allows to identify non-destructively various components in fresh plant material if characteristic key bands of the individual analyt molecules can be found in the spectrum [2,3]. In this context, especially NIR FT Raman spectroscopy has been described as a valuable tool for in vivo investigations because fluorescence and thermal decomposition of the plant tissue can be reduced to a minimum. Several examples are presented to demonstrate the special potential of vibrational Raman microspectroscopy for the study of essential oil plants. This technique allows to obtain 2-dimensional spectroscopic images of the investigated samples which can be directly compared to the corresponding visual images. Furthermore, mapping provides detailed information regarding the distribution of specific plant substances occurring in the surface layer. Based on these data not only qualitative but also semi-quantitative interpretation of the spectroscopic measurements is possible. Contrary to IR imaging, Raman mapping does not need any sample preparation and can be also performed at intact living plant material. The results obtained in this study prove that principally the distribution of secondary metabolites can be determined directly in the individual essential oil glands.

Acknowledgment

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1. H. Schulz (2004) Application in analysis of coffee, tea, cocoa, tobacco, spices, medicinal and aromatic plants, and related products. In: Roberts, C.; Workman, J.; Reeves, J. (Eds.): Near-infrared Spectroscopy in Agriculture, American Society of Agronomy - Crop Science of America - Soil Science Society of America, Madison, USA, Agronomy Monograph, No. 44, 1-32
2. H. Schulz, R. Quilitzsch, H. Krüger (2003) Rapid evaluation and quantitative analysis of thyme, origano and chamomile essential oils by ATR-IR and NIR spectroscopy; *J. Mol. Struct.* **661-662**, 299-306
3. H. Schulz, M. Baranska, H.-H. Belz, P. Rösch, M. Strehle, J. Popp (2004) Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements, *Vib. Spectrosc.* **35**, 81-86

A "Chiral Pool" of Monoterpenes from Aromatic Plants

U. Ravid and O. Larkov

Department of Aromatic Plants, Agricultural Research Organization, Neve Ya'ar, P.O. Box 1021, Ramat Yishay 30095, Israel

Abstract

Chiral molecules are currently of great relevance to the chemical industry. A valuable approach to the production of bioactive compounds consists in using a "chiral pool" as a large reservoir of optically pure building blocks, mainly derived from natural sources. Optically pure natural compounds, such as monoterpenes, constitute the building blocks of optically pure synthetic flavors, fragrances, pharmaceuticals, pheromones and others.

Many wild aromatic plants may serve as a source of optically pure monoterpenes. Systematic breeding efforts and modern agronomic techniques have enabled us to develop cultivated aromatic plants with high yields of essential oils and their derived optically pure monoterpenes.

The combination of Automatic Headspace-Solid Phase MicroExtraction with chiral gas chromatography-mass spectrometry (Auto HS-SPME-Chiral GC-MS) has given us valuable information about the enantiomeric purity of chiral monoterpenes in fresh aromatic plants as well as in dried plants, essential oils and oleoresins. We used different β - and γ -cyclodextrin derivatives as chiral stationary phases to broaden the scope of enantiomer separation by GC.

Screening aromatic plants for chiral economic monoterpenes may extend the reservoir of optically pure natural building blocks for the chemical industry.

The value of having a multidisciplinary approach to the study of essential oils – examples from the South African flora

A. Viljoen¹ and K. H. C. Başer²

¹ Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York rd, Parktown 2193, Johannesburg, South Africa

² Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

Abstract

Essential oils may be studied for the following reason:

1. from an analytical perspective only,
2. to identify favorable chemotypes for commercial development,
3. the research may be directed to the biological activity or essential oil
4. essential oil data may be applied to resolve taxonomic problems.

Often the above approaches are mutually exclusive. Most of the effort in generating an essential oil profile or a dataset involves the tedious collection of the plant material and the researching opportunity is not maximized to extract as much as possible from the essential oil sample.

Using indigenous aromatic South African plants, examples will be presented to illustrate the informative value of essential oils. A thorough investigation of *Plectranthus* (Lamiaceae) illustrates that essential oil composition is a powerful tool to identify natural hybrids in this taxonomically challenging genus. A multidisciplinary study on indigenous *Vitex* (Verbenaceae) species which are widely used in African traditional healing has provided some scientific rationale for the use of these species in healing rites. Furthermore, the chemotaxonomic information extrapolated from the data matrix confirms the present infrageneric placement of taxa. A molecular phylogeny has been constructed for the genus *Eriocephalus* (Asteraceae) and the essential oil data has been superimposed on the gene-tree confirming some of the infrageneric relationships. A study of indigenous *Salvia* (Lamiaceae) species will be discussed to illustrate the valuable contribution the essential oil has made to the understanding of the medicinal properties and chemotaxonomy of some taxa with potential commercial applications.

On the Stress Relieving Properties of Chiral Essential Oil Constituents: A New Assessment Method

M. Höferl and G. Buchbauer

Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Abstract

In folk medicine stress relieving essential oils, e.g. lavender oil, sandalwood oil, are known since ancient times. On account of modern analytical techniques these relaxant activities can be attributed in many cases to the main constituents of their matrix oils such as linalool, α -santalol, etc. Since the end of the eighties and especially in the nineties of the last century an increasing number of reports can be found in scientific journals dealing with pharmacological/physiological and/or psychological activities of essential oils and single fragrance compounds. A great variety of assessment methods, such as EEG-measurements, determination of ANS-parameters, for instance heart rate and skin conductance, as well as behavioural studies are described. In the last few years attention was in particular focussed on the analysis of endocrinological parameters. However, these studies frequently afford a special equipment or the need of invasive investigations straining the subjects and frequently lack an appropriate stress model.

A sophisticated parameter to determine the extent of the physiological reaction to a standardized laboratory stressor is the quantification of cortisol levels in plasma or saliva, the latter having the advantage of being a non-invasive sampling procedure. To avoid the disadvantages of using radioisotopes, a commercially available time-resolved fluoroimmuno- assay (DELFI[®]) was adapted to yield a determination method warranting the same sensitivity as a standard RIA.

In this report the stress relieving properties of some chiral monoterpenoids as well as of their matrix oils are presented utilizing this new assessment method.

Phytochemical Investigation of *Arnica montana* (L.) and *A. chamissonis* (Lessing) Grown in Scotland

K. Svoboda¹, J. Brooker², G. Ruzickova¹, D. Taylor³ and R. Greenaway¹

¹ SAC Auchincruive, Dpt of Life Sciences KA6 5HW Scotland UK

² Avian Science Research Centre, Auchincruive campus, SAC, West Mains Rd, Edinburgh EH9 3JG Scotland UK

³ CPL Aromas Ltd, Barrington Hall, Hatfield Broad Oak, Bishop's Stortford CM22 7LE UK

Abstract

Arnica montana is a traditional medicinal plant, used widely for its anti-inflammatory properties (sprains and bruises, muscle and joint pains, rheumatoid complaints, antiseptic activities). Other uses include homeopathic preparations, food flavouring, alcoholic beverages and cosmetic preparations. The European market demand is about 20-30 tons of dry flowers per annum. The plant grows in specific habitats in the Northern Hemisphere (acidic soils in alpine meadows and light coniferous woodlands) and due to over-collection is now becoming an endangered species. It is comparatively difficult to cultivate and *A. chamissonis* is being considered as a substitute plant species for its medicinal properties. The main chemical components in both species are sesquiterpene lactones, flavonoid compounds, carotenoids, alkaloids and volatile oils. The volatile oil yield and composition, specifically from roots and vegetative matter, have not been previously described. Seeds from various sources have been donated by Botanical Gardens and Research Institutes and purchased from commercial nurseries. Plants were grown in the greenhouse, harvested at different ontological stages, distilled and analysed by GC and GCMS. Roots, stems, leaves, buds and flowers were distilled separately for selected harvests. Volatile oil yield was the lowest for inflorescence (0.02-0.1% v/dw), followed by aerial parts (0.1-0.3% v/dw) and roots and rhizomes (0.3-0.8 %v/dw). In addition, carotenoid content and composition of flowers during ontogenesis was established.

The glands on the surfaces of the leaves, sepals, ray and disc florets, were observed and described. *A. montana* had approx. 400 glands per cm² and 60 glands per cm² on upper and lower leaf surface respectively, *A. chamissonis* had about 890 glands per cm² (upper surface) and 380 glands per cm² (lower surface). Sections of stem, leaves and roots did not reveal any obvious ducts, however, after cutting the fresh root sections abundant globules of light, clear, colourless oil appeared all over the sections, which supports the recovery of high yield of oil from underground organs (both roots and stolons).

Acknowledgement

A. Syred (Microscopix, Wales) for the SEM investigation of volatile oil glands and ducts, B. Galambosi (Finland) and Orkney Agronomic Institute for plant material.

High-speed GC Essential Oil Analysis with 50 and 100 μm ID Micro-bore Columns

P. Q. Tranchida¹, A. Casilli¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

The major objective of any GC method is the separation of the most critical sample components in the minimum time. This, obviously, becomes of fundamental importance for laboratories with a high sample throughput and/or where there is a need for quick and correct results. As a consequence, there has been an ever-present interest within the chromatographic community for the introduction of faster techniques. The primary aim, relative to any fast GC technique, is to maintain (compared to traditional GC) sufficient resolving power for the separation between the compounds of interest. In respect to this aspect, the narrow-bore column approach is a very efficient way of increasing analysis speed. A decrease in column internal diameter reduces resistance to mass transfer in the gaseous phase. Although the use and effectiveness of these columns was demonstrated many years ago, their routine use in fast GC applications is only quite recent. The reason behind this delay is merely technical and was due to the lack of suitable GC equipment. Modern GC systems are now capable of supplying the extreme experimental conditions that narrow-bore columns necessitate: high inlet pressures, highly controlled split flows, rapid oven temperature heating/cooling and fast electronics for detection.

The present research is based on the evaluation of the use of 50 and 100 μm ID columns in the very fast GC analysis of the volatile fraction of essential oils. Furthermore, the correspondence between theoretical and experimentally derived data are measured and discussed. Practical aspects such as column sample capacity and detector requirements are also considered.

Biological effects of Corsican Everlasting Essential Oil (*Immortelle Helichrysum italicum* (Roth) G. Don ssp. *serotinum* (Boiss.) on human skin in –vitro and ex-vivo assays

L. Decomme¹, G. Gueneva², D. Davenne³

¹ Laboratoire de biogénotoxicologie Faculté de Pharmacie 27 Boulevard Jean Moulin 13385 Marseille France cedex 5

² Laboratoire de biochimie cellulaire Faculté de Pharmacie 27 Boulevard Jean Moulin 13385 Marseille France cedex 5

³ Laboratoire Rosier Davenne 50 Rue Henri Bessemer 13854 Aix en Provence cedex 3

Abstract

Essential oils are widely used in cosmetics, however very few scientific data are available about their activities. This presentation will illustrate through the investigation of *Helichrysum italicum* oil, a various range of properties interesting for cosmetic or medical purposes.

Helichrysum italicum is an about 30 cm high perennial plant widely distributed in Mediterranean areas. This species belongs to Asteraceae's family, Gnaphalieae tribe.

The volatile oil obtained by steam distillation is now rather well known. The Corsican type is rather homogenous : esters (Neryl acetate 25-40 % Neryl propionate 3-8 %) , ar & gamma Curcumene 10-30 % diketones (3,5-dimethyloctan-4,6-dione , 2,5,7- trimethyldec-2-en-6,8-dione, 2,5,7,9 tetramethyldec-2-en-6,8-dione, 2,5,7- tetramethylundec-2-en-6,8-dione) (P. Weyerstalh)

alcohols (Linalol, Nerol 5-15 %) , alpha pinene 2-5 %, seli-11-en-4alpha-ol 2-5 %, . The smell is long-lasting, strong and pleasant , curry type. This Corsican type is very close to Sardinian type but both are rather different of those from other areas.

Our investigations were leaded according the technics we have adapted for testing these natural complex mixtures with hydrophobic and low molecular weight compounds.

We managed lipolytic activity with *Helichrysum italicum* the oil shows a high lipolytic efficiency on human adipocytes collected after plastic surgery. Comparison with other E.O. is shown.

We managed anti-oxidant studies versus superoxyde radical and hydroxyl radical. These methods are described and results are discussed.

Helichrysum italicum E.O. promotes the synthesis of Type I collagen. This E.O. also has an interesting anti-inflammatory effect through 5-lipoxygenase test.

Neoangiogenetic studies shows that this oil increase specifically the number of arteriolo-veinous anastomosis without modification of the length of vessels.

All these data are in concordance with the traditional indications of Everlasting : haematomas, articular diseases, healing promoter and phlebitis protector. The new data make relevant the use of IMMORTELLE as a strong anti-aging and also slimming active ingredient.

A Novel Type of Membranes based on Cholesteryl Phosphate

S. Sedaghat¹, S. Streiff², N. Ribeiro², L. Désaubry², Y. Nakatani², G. Ourisson²

¹ Department of Chemistry, Islamic Azad University, North Tehran Branch, P.O.Box 19585/93

² Centre de Neurochimie, 5 rue Blaise Pascal - 67084 Strasbourg, France

Abstract

Mixtures of the rigid amphiphile disodium cholesteryl phosphate (DCP) with the non-phosphorylated diacyl amphiphile dimyristoylglycerol (DMG), give self-organised systems in a wide range of pH, as demonstrated by differential microcalorimetry. These systems can be closed bilayer vesicles, as shown by optical microscopy (Nomarski and confocal). Neither partner, DMG nor DCP, taken alone, gives vesicles in these conditions but 10 % DMG is enough to lead to the formation of vesicles from pH 5.8 to 9.3. These novel self-organised systems are akin to the classical eucaryotic ones, built on a phosphorylated diacylglycerol and free cholesterol (or analogues), the only difference being the site of the phosphate head-group. The results described here are compatible with what one could have expected on the basis of our general understanding of vesicle formation, so convincingly expressed by Israelachvili, and illustrate once again the importance of the properties of phosphates in Nature, emphasised by Westheimer. It is however remarkable that as little of 10 % DMG is enough to carry cholesteryl phosphate into a bilayer system.

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Comprehensive Two-dimensional NP (Adsorption)-RP Liquid Chromatography for Essential Oil Analysis

M. Lo Presti¹, R. Luppino², P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

Many complex mixtures of a natural origin require analytical methods of an extremely high resolving power in order to provide the complete separation of all sample components. Multidimensional (MD) chromatography separations are carried out on two independent columns with a differing selectivity. Frequently MD LC separations are performed in an off-line mode, presenting a series of disadvantages, such as losses of sample or formation of artefacts. On line MD LC is achieved through coupling of a second column by means of a high pressure switching valve which traps a defined volume of collected sample, usually in a loop, and directs it into the second column (heart-cutting).

Recently, different approaches have been used to develop comprehensive multidimensional HPLC systems, for the analysis of complex samples such as peptides and proteins or synthetic polymers. These systems have been based mainly on two approaches: the use of eight or ten port valve equipped with two sample loops, that allow transfers from a primary micro-bore LC column to a second fast column, or the use of a valve that allows transfer from a conventional column to two fast secondary columns in parallel, without the use of storage loops.

The 2D LC comprehensive system developed in this investigation was formed by a micro-bore silica column operated in normal phase mode (NP) in the first dimension, and a monolithic type column operated in reversed phase mode (RP) in the second dimension. The interface was a ten-port two position valve equipped with two storage loops. The use of a microbore column in the first dimension permits to inject a small volume in the secondary column, thus obtaining re-concentration. The use of a monolithic type column in the second dimension, due to a higher permeability and increased mass transfer properties in comparison to conventional particulate columns, permits to perform very fast analysis operating at higher flow rates without loss of resolution.

The use of normal and reversed phased mode in the two dimensions can be useful in the separation of uncharged molecules of comparable dimension, but varying in polarity and hydrophobicity.

The system has been tested analysing the oxygen heterocyclic fraction of cold-pressed lemon and grapefruit oils, made up of coumarins and psoralens and polymethoxylated flavones. The oxygen heterocyclic components are the main parts of the non volatile fraction of cold-pressed citrus oils. The qualitative and quantitative composition of these components have an important role in the characterization of each citrus oil. When the analysis of this fraction is carried out by HPLC, using both normal and reversed phase modes, some co-elutions may occur. With the comprehensive 2D LC analysis, higher resolution and larger peak capacities than single column chromatography can be obtained. The relative location of the components in the 2D plane varied in relation to their chemical structure, and allowed a positive peak identification. The UV spectra recorded with the photodiode array detector supplied an additional information used for the characterisation of the studied sample.

Aromatic production of Morocco

S. Zrira and B. Benjilali

Département des Sciences Alimentaires et Nutritionnelles, Institut Agronomique et Vétérinaire Hassan II ; BP 6202. Rabat Institut Rabat, Morocco

Abstract

By its situation between two seas and a desert, Morocco constitutes a geographical entity whose characteristics model a completely original natural framework; it offers a complete range of Mediterranean bioclimats allowing a rich flora and varied vegetation, with very marked endemism. The species with aromatic and/or medicinal interest are estimated at 500 to 600 species including great number of endemic species. Thus, one finds spontaneous and cultivated plants. The category of spontaneous plants includes a broad range of plants of which most important are thyme, rosemary, natural mint (*mentha pulegium*), oregano and bay leaves. The principal cultivated plants are: paprika, coriander, cumin, fennel, anise, verbena, mint, parsley and saffron. These plants (spontaneous or cultivated) are often exported in a rough state or in the forms of extracts (essential oils, concrete or résinoides).

Morocco exports a multitude of spices and aromatic and medicinal plants. Concerning spices, the value of exports represents (between 1995 and 2000) an average of 12, 8 million Euros per year (13 million \$ US approximately). The principal products are: paprika and its derivatives and Coriander. For aromatic herbs trade, the values of exports are about 4,7millions Euros (5 million \$ US). Among the principal exported medicinal plants one finds rosemary, thyme, verbena, sage and dried mint.

Morocco is traditionally one of the principal producer countries of Essential Oils (EO) and aromatic extracts. It has exported an average of 14.5 million Euros between 1995 and 2000. The exported products are: résinoides of paprika, Not Deterpened Essential Oils (NDEO), roses concrete, rosemary EO and Citrus fruits EO.

Determination of Antimicrobial Effects of Essential Oil Obtained from Some Natural Plants In Northern Cyprus

M. Akin and A. Uslu

Selcuk University, Science Faculty, Department of Biology, Konya, Turkey

Abstract

Antimicrobial effect of essential oil obtained from fifteen naturally growing plant species in Northern Cyprus were studied in the study.

There are antimicrobial effect of essential oil obtained from some plants e.g. *Pinaceae*, *Anacardiaceae*, *Labiatae*, *Umbelliferae*, *Myrtaceae*, *Compositae*, *Leguminosae*, *Cupressaceae* and *Liliaceae* on *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. There are also antimicrobial effect of essential oils obtained from *Pinus brutia*, *Pistacia lentiscus*, *Rosmarinus officinalis* and *Choridathymus capitatus* on *Bacillus subtilis* ATCC 6633, *M. luteus* ATCC 9341 and *S. aureus* ATCC 25923; *Cupressus sempervirens* on *Bacillus subtilis* ATCC 6633; *Chrysanthemum coronarium*, *Eucalyptus camaldulensis*, *Chrysanthemum coronarium*, *Helichrysum conglobatum*, *Salvia fruticosa* and *Acacia cyanophylla* on *S. aureus* ATCC 25923; *R. officinalis* and *Choridathymus capitatus* on *E. coli* ATCC 25922. There are no antimicrobial effect of essential oils obtained from all plants *Pseudomonas aeruginosa* ATCC 27853.

There is no antimicrobial effect of distillate obtained from some plants on all tested bacteria

Key Words

Antimicrobial effect, microorganism, disc method, essential oils.

Determination of pesticide and plasticizers residues in bergamot essential oils

D. Giuffrida¹, G. Di Bella², A. Bruzzese¹, L. Serrao¹, Giacomo Dugo¹

¹ Dipartimento di Chimica Organica e Biologica, University of Messina, 98168 Messina, Italy

² Dipartimento Farmaco-chimico, University of Messina, 98168 Messina, Italy

Abstract

Organophosphorus and organochlorine pesticides, phosphorated plasticizers, chloroparaffins and phthalate esters contamination in bergamot essential oils produced in Calabria in the crop years 1999-2000 was studied by HRGC in connection with detectors FPD, ECD, MS. Residues of dicofol and tetradifon were found in oils from both crop years. The mean dicofol concentration was 0.26 mg/L in samples from 1999 and 0.20 mg/L in those from 2000; the mean tetradifon content was 0.06 mg/L for both the crop years. Among plasticizers, residues of diisobutyl phthalate, di-*n*-butyl phthalate, and bis(2-ethylhexyl) phthalate were found in samples from crop years 1999 and 2000, the mean content were 1.22 and 1.23 mg/L, 1.51 and 1.65 mg/L, 1.38 and 1.42 mg/L respectively.

Effect of Mycorrhization on Amount and Composition of Essential Oils of Marjoram, Thyme, Sage and Caraway

J. Richter, D. Franz and I. Schellenberg

Department of Bioanalysis, Anhalt University of Applied Sciences, 06406 Bernburg, Germany

Abstract

Arbuscular mycorrhizal fungi form a symbiotic interaction with many plant species. Among a lot of positive effects of mycorrhization such as plant growth or plant resistance the influence of arbuscular mycorrhizal fungi on concentration and quality of essential oils in spice plants should be analysed.

The qualitative and quantitative composition of essential oils of mycorrhizal and non-mycorrhizal plants of marjoram, thyme, sage and caraway was determined by gas chromatography. The identification of the ingredients of the essential oils was carried out by mass spectrometry as well as with the help of reference substances.

Especially because of the high number of plant samples different extraction techniques have been compared for their suitability for the following determination of qualitative and quantitative differences of essential oil composition of inoculated and non-inoculated plants. Among Steam Distillation, Solvent Extraction, Accelerated Solvent Extraction (ASE), Supercritical Fluid Extraction (SFE) and Solid Phase Microextraction (SPME) the last technique turned out to be a very timesaving and practical method and was finally optimised for our research.

First results of the analyses of mycorrhizal and non-mycorrhizal plants show the influence of arbuscular mycorrhiza on amount and composition of essential oils.

Practicing Aromatherapy in El Salvador

Use of essential oils in basic health; Care in developing countries

J-V. Pineda Monge

AP 181 Sanlúcar La Mayor, 41800 Sevilla, España

Abstract

My lecture talks about the experience of the accumulation of the empirical knowledge of the common people at the rural area of my country, El Salvador, C.A.

The poor condition of the peasant at the rural areas, product of the economics system and the devastating effects of the structural adjust policy, dictates by the International policies of World Bank, have reduce the possibilities of develop the rural areas at my country. The challenge at this scenario is the use of local essential oils against the disease as follows:

Disease	Cause	E.O.	Main type constituent
Acute respiratory infections	Bacterial, Viral	<i>Lippia graveolens</i>	Phenols (carvacrol, thymol)
Diarrhoeal diseases	Bacterial, Viral, Protozoan	<i>Basil</i> <i>Chenopodium</i> <i>Cardamom</i> <i>Zingiber off.</i> <i>Artemisia</i> <i>absinthium</i> <i>Menta sp</i>	Phenylpropano Monoterpenes Ester, 1,8 cineol Sesquiterpene Coumarines Alcohols
Skin diseases	Fungus	<i>Myroxilum balsamum var. pereirae</i> <i>Cymbopogon martinii</i>	Cinnamic aldehyde Eugenol Terpene alcohols: Alcohol and esters
Pertussis	Bacterial	<i>Eucalyptus globulus</i>	Cineol
Anti-inflammatory		<i>Cymbopogon citratus</i> <i>Lantana camara</i>	Aldehydes Sesquiterpen hydrocarbons
Inmunodepressive states		<i>Vetiveria zizanooides</i>	Sesquiterpenol and Sesquiterpenones
Antiseptic		<i>Citrus latifolia vr persian</i>	Aldehydes
Sedative		Idem	Idem
Anti depressive		<i>Pinus sp</i>	Terpens

“The following considerations pertain to establishing the practice of aromatherapy in El Salvador as means to improve economic conditions and those of basic healthcare in rural and urban areas. As these considerations are based on the pharmacological effects found in essential oils all over the word, they may very well be attributed model character for developing self sufficient systems of basic healthcare in other developing nations as well”. (K. Schnaubelt, 1995; K.Duerbeck, 1997.) Understandingly that this will be the future of the next century for the rural and urban areas primary heath care and the most powerful holistic approached to the humankind treatments.

NEW COMPOUNDS FROM *PIMPINELLA* OILS

K.H.C. Baser¹, N. Tabanca^{1,3}, N. Kirimer¹, E. Bedir^{2,3}, I.A. Khan³

¹ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey

² Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, Izmir 35100, Turkey

³ National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, MS 38677, USA

Abstract

Essential oils from eighteen *Pimpinella* species (Umbelliferae) of Turkey were analysed by chromato-spectral techniques.

Of the twenty compounds isolated, 4-(2-propenyl)-phenyl angelate, 4-(3-methyloxiranyl)-phenyl 2-methylbutyrate, aureane [1-methyl-4-(5-methyl-1-methylene-hexa-4-enyl)-7-oxabicyclo[4.1.0]heptane] and traginone [4-(6-methyl-bicyclo[4.1.0]hept-2-en-7-yl)-butan-2-one] were new; 4-(1-propenyl)-phenyl 2-methylbutyrate, dictamnol, 4,6-guaiadiene, 4-(1-propenyl)-phenyl isobutyrate, alismol and 12-hydroxy- β -caryophyllene acetate were new for the family, and trans-isoosmorhizole was new for the genus.

Herbs and Essential Oils as Additives in Stock Farming and Pet Food - Actual Situation, Experimental Results and Outlook

R. Thomann¹, U. Bauermann¹, I. Halle², F. Bauer³

¹ IGV Institut für Getreideverarbeitung GmbH, D 14558 Bergholz-Rehbrücke, Germany, Arthur-Scheunert-Allee 40/41

² Institut für Tierernährung, Bundesforschungsanstalt für Landwirtschaft (FAL), Bundesallee 50, D 38116 Braunschweig, Germany

³ Veterinärwissenschaftliche Universität Wien, Veterinärplatz 1, A 1210 Wien, Austria

Abstract

Our research team is using cereals as carrier for active plant components to develop, produce and analyse functional food and feed. With our equipment we distil definite essential oils from herbs for scientific trials in stock farming and pet food.

The results of the feeding trials and expected questions will be lined out.

Caused by the prohibition of most of antibiotics as feed components in stock farming in the European Union the herbs and essential oils have got a chance to act as natural and well accepted growth enhancers.

Especially essential oils, containing carvacrol, are successful in application for chicken and pigs. The most useful source for carvacrol is oregano.

New savoury varieties with carvacrol concentrations up to 40% in the essential oil with about 4% essential oil in dry leaves are under investigation too.

In our experiments we compared oregano leaves and essential oil made from the same oregano batches and found that the active principle was completely transformed during the water steam distillation into the essential oil. The feed conversion (kg feed/kg body weight) in broiler feeding showed an optimum depending on essential oil concentration at about 0.2g/kg feed. The meat quality characterised by thiobarbituric acid (TBA) value of broilers was influenced by using feed additives containing carvacrol as component in feed.

Synergistic effects of carvacrol with other plant materials are found. In actual feeding trials we investigate the reasons for these effects caused by oregano and savoury together with university-partners. The interactions of essential oils, microbial gut parameters and blood composition of chicken and pigs are evaluated in the research project.

Problems which must be solved in future:

- The successful use of multi-component essential oils in commercial stock farming with active component concentrations of 50-500g per ton requires new reliable analytical methods to detect the presence of the active components in the “ready to feed” mixture, which is a very difficult and inconvenient complex matrix for analysts.
- Furthermore it is necessary to detect whether all the used components of the essential oil come from natural sources, if this is claimed, or synthetic components have been used too.
- The use of herbs, medical plants, extracts and essential oils for special feed for comrade animals is very popular because customers want naturally caused wellness for their best friends. Reliable product compositions and scientifically based knowledge on manufacturing are developed. Basic knowledge on essential oils is necessary.

We are interested in networking in scientific and commercial tasks in the fields of feed development and analysis.

Derivative stripping chronopotentiometry for heavy metals analysis in citrus essential oils

L. La Pera, D. Pollicino, M. Saitta, R. B. Lo Curto, Giacomo Dugo

Department of Organic and Biological Chemistry, University of Messina, Salita Sperone 31, 98166 Messina, Italy

Abstract

The wide use of citrus essential oils in food, cosmetic and pharmaceutical industry, implies a strict control of the presence contaminants. Literature reports few data about the presence of heavy metals in essential oils. Heavy metals as Cu (II), Zn (II), Mn (II) and Se (IV) naturally occur in citrus fruit since they are important micronutrient both for animals and plants; some others including Cd (II), Ni (II) and Pb (II) are potentially toxic and can be found in essential oils as a results of a contamination process. The purpose of this study was to use derivative stripping chronopotentiometry in the anodic (ASCP), cathodic (CSCP) and adsorptive (AdSCP) modes to determine the concentrations of some heavy metals in citrus essential oils. Derivative stripping chronopotentiometry (dSCP) was a rapid, sensitive and precise method to detect concentrations lower than 1 ppb both in oily and aqueous matrices without any laborious sample pre-treatment. Particularly ASCP was employed for the simultaneous analysis of Cd, Cu, Pb, Zn in bergamot essential oils from the crop years 1999 and 2000 and for Mn quantification in various types of citrus essential oils from 2003 and 2004. CSCP and AdSCP were respectively used to detect trace levels of Se and Ni in citrus essential oils from 2003 and 2004.

Forum Essenzia – Essential oil application and Aromatherapy in the European Context

K. Duerbeck

Forum Essenzia e.V., Meier-Helmbrecht-Str. 4, D-81377 Munich, Germany

Abstract

FORUM ESSENZIA with its approximately 1000 professional members from more than 10 countries worldwide (Europe, USA, Japan, and Korea) represents the leading sectorial interest group in the context of science based application of essential oils in aromatherapy.

The changes from medication to health care are eminent in the social and health care systems in Europe and around the world. Through its well established training and educational programme and the technical advisory board, FORUM ESSENZIA is spearheading the application of genuine and authentic essential oils in the primary health care and wellness.

The limitations for supply chain management are discussed together with the implications on availability and application are discussed in the context of the outlines for the updated training and education programme for professionals in the field of aromatherapy, aroma care and aroma culture.

GCxGC AND GCxGC/qMS for Complex Sample Analysis

A. Casilli¹, P. Q. Tranchida¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.FF.NN, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

Single column gas chromatography (GC) in combination with a flame ionization detector (FID) and/or a quadrupole mass spectrometer (qMS) is widely employed in the determination of complex matrix profiles. The latter, unfortunately, often can only be partially separated even on long capillaries. Inevitably, several monodimensional peaks are the result of two or more overlapping components hindering reliable identification and quantification. Consequently, a major objective in analytical chemistry concerns the continuous improvement and development of more powerful techniques.

Two-dimensional comprehensive gas chromatography (GCxGC) is a powerful multidimensional on-line technique for the analysis of very complex matrices providing a true comprehensive separation.

The present work, which can be considered the sum of distinct investigations, is based on the analysis of a variety of samples, ranging from medium to highly complex and with different chemical group compositions. The thorough separation/identification of all matrices was achieved through the exploitation of GC x GC peak capacity, the formation of group types on the 2D space plane and MS hyphenation. Different methods were developed in function of the specific sample-type: SPME sampling, orthogonal and non-orthogonal column sets, wide-bore 2D column separation under low pressure conditions, etc. The overall result was that a much deeper insight was acquired on the composition of a variety of real world samples.

ESSENTIAL OILS- VISION 2007 (India Emerging as Major producer of many bulk Essential Oils)

R. Harlalka

Nishant Aromas, Milan Ind. Estate, 424, Off T.J. Road Cotton Green, Mumbai 400033, India

Abstract

- 1) India is blessed with many positive factors, which enables it to stand at unique position in agriculture-based product.
- 2) After achieving self-dependence on food front now India is in position to put lot of focus on growing of Aromatic Plants.
- 3) Aromatic chemical ex Essential Oils can compete synthetic isolates with better production quality like Menthol ex Mentha oil. Similarly derivatives like Citronellol, Geraniol & Linalool are produced essential oils.
- 4) Apart from above, due to large scale production of many oil like Basil Oil, Citronella Oil, Eucalyptus Globulus Oil, Eucalyptus Citroedora Oil, Geranium Oil, Lemon Grass Oil Mentha Piperita Oil, Mentha Avensis Oil, Palmrosa /Jamrosa Oil, Spearmint Oil, Lavender/Lavandin/Sikimmia Lavender/ Clary Sage Oil, Patchouli Oil, Rose Oil, Vetiver (Khus Oil), many bioactive molecules can be derived from these essential oils in large qty for extended demand & need of world market for natural flavour & fragrance industry as well as for skin care products.

A Comparison of Microwave-Assisted Hydro Distillation, Hydro Distillation and Supercritical Fluid Techniques for the Preparation of Essential Oils

M. Lo Presti¹, A. Trozzi³, S. Ragusa³, P. Dugo², G. Colnaghi⁴, F. Visinoni⁴, Giovanni Dugo¹ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, Facoltà di Farmacia, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.NN.FF., University of Messina, 98166 Messina, Italy

³ Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università di Catanzaro, 88021 - Roccelletta di Borgia, Catanzaro, Italy

⁴ Milestones S.r.l., Via Fatebenefratelli, 1/5 – 24010 Sorisole (BG), Italy

Abstract

Traditional hydro distillation (HD), Supercritical Fluid Extraction (SFE) and water microwave extraction techniques were compared for their effectiveness in the extraction of essential oils. The microwave extraction technique was optimized in terms of delivered power and time duration. The oils extracted by the three different techniques were analyzed by gas chromatography and gas chromatography coupled to mass spectroscopy (GC-MS) in order to determine their chemical composition. Microwave extraction, which exploits the physical action of microwaves on plants, showed a series of advantages in respect to the HD technique: low costs, the use of water in sample pre-treatment, the greatly reduced extraction time (18-30 min against 4-6 hours) and the attainment of high quality essential oil as the risks linked to thermal degradation are avoided. Moreover, the absence of environmental impact of this innovative technique must be emphasized.

Essential Oils Composition of the Six Populations of *Foeniculum vulgare* Mill. ssp. *capillaceum* from Iran

M.M.Barazandeh¹, M.B.Rezaei¹ and K. Jaimand¹

¹ Research Institute of Forests and Rangelands, Tehran, Iran, P.O.Box:13185-116

Abstract

The seeds of *F.vulgare* Mill. ssp. *capillaceum* were collected from six different locations of Iran and their essential oils were produced by steam distillation. The oils were produced at the yields of 1.38% (Hamedan) to 1.74% (Marivan) and analyzed by Capillary Gas Chromatography. Because of similarity between all chromatograms, only one of them was injected to GC/MS. Eighteen compounds were identified among which (E)-anethole (62.1-77.5%), α -fenchone (8.8-14.4%) and methyl chavicol (3.5-5.5%) were the major constituents, respectively.

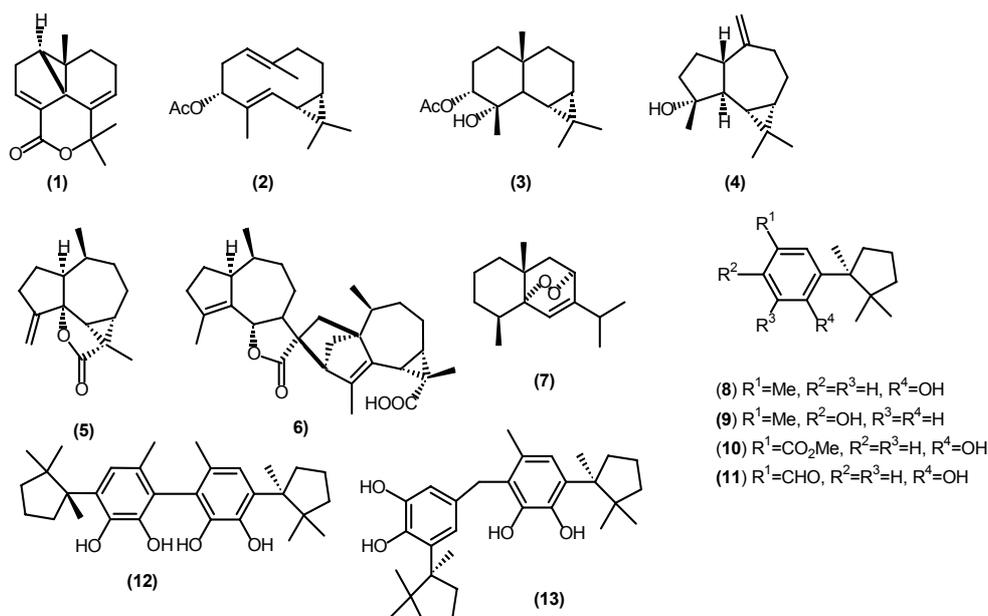
New Sesquiterpenoids from three southern hemispheric liverworts: *Lepidozia spinosissima*, *Chiloscyphus subporosus* and *Mastigophora diclados*

F. Nagashima, L. Harinantenaina and Y. Asakawa¹

¹ Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

Abstract

We are continuing to study the biologically active constituents of southern hemispheric liverworts (Asakawa 2001, 2004; Asakawa et al., 2003, Bardon et al., 1999, 2000; Nagashima et al., 1999, 2003; Toyota et al., 2004). The ether extracts of New Zealand *Lepidozia spinosissima* and *Chiloscyphus subporosus* and Malagasy *Mastigophora diclados* were chromatographed on silica gel and Sephadex LH-20 to give a new cyclogorgonane sesquiterpenoid (1) with 3 α -acetoxy-*ent*-bicyclogermacrene (2), (-)-3 α -acetoxy-*ent*-maalian-4 β -ol (3) and *ent*-spathulenol (4) from *L. spinosissima*, and a new aromadendrane sesquiterpenoid (5) and an aromadendrane-guaianolide dimer (6) with 5,6-epidioxy-*ent*-eudesmene (7) and *ent*-spathulenol from *C. subporosus*, and a new herbertane sesquiterpenoid (10), together with 13 known compounds including (8-13) from *Mastigophora diclados*. The structures of newly isolated compounds were elucidated by ¹H- and ¹³C-NMR spectral and X-ray crystallographic analyses.



The isolation and the structure determination of the new compounds and chemosystematics of these liverwort species will be discussed.

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New Potent Aldehydes and Related Alcohols in Fresh Lemon Peel

R. Naef and A. Jaquier

Firmenich SA, Corporate R&D Division, PO Box 239, 1211 Geneva 8, Switzerland

Abstract

A solvent extract of freshly grated flavedo of lemon (*Citrus limon* L.) was fractionated on silicagel into twelve fractions. Three unsaturated and two saturated branched aldehydes together with their corresponding alcohols were identified as trace components. Their structures could be confirmed by syntheses. Spectral data and organoleptic descriptors will be given.

In addition, interesting observations of citrus chemistry in general will be discussed.

P.01) Effect of sowing dates on the essential oil content and composition of *Foeniculum vulgare* cv. Soroksari

R. Omidbaigi¹, F.Sefidkon², G. De Mastro³ and K. Sadrai¹

¹Department of Horticulture, College of Agriculture, Tarbiat Modarres University, Teheran, Iran

²Research Institute of Forests and Rangelands, Teheran, Iran

³Department of Crop Production, College of Agriculture, Bari University, Bari-Italy.

P.02) Isolation of podophyllotoxin from suspension culture of *Linum album* L.

S. Sedaghat¹, E. Ezatzadeh¹, A. W. Alfermann²

¹Department of Chemistry, Islamic Azad University, North Tehran Branch, Iran

²Institute for development and molecular biology of the plants, H.H.U, Duesseldorf, Germany.

P.03) Changes in Lemon Oil Composition During Fruit Ripening

F. Badalamenti

Agrumaria Corleone S.p.A., Palermo, Italy.

P.04) Essential oil composition of *Croton cuneatus* and *Croton malambo* growing in Venezuela

A.I. Suárez, L. J. Vásquez¹, M. A. Manzano² and R. S. Compagnone²

¹Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela

²Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.

P.05) Composition of essential oils extracted from some selected Fijian Plants.

J-P. Dufour¹, K. Patel², S. Sotheeswaran², and S. Ali²

¹Department of Food Science, University of Otago, Dunedin, New Zealand;

²Department of Chemistry, The University of the South Pacific, Suva, Fiji Islands.

P.06) Essential Oils from Leaves, Stems and Flowers of *Salvia verticillata* L.

M. Jamzad¹, S. Masoudi² and A. Rustaiyan¹

¹Department of Chemistry, Science and Research Campus I. A. University, Tehran, Iran;

²Department of Chemistry, Central Tehran Branch I. A. University, Tehran, Iran.

P.07) The Volatile Constituents of the Leaves and Flowers of *Kigellia africana* Benth.

O. T. Asekun¹ and O. Adebola²

¹Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria;

²Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria.

P.08) Essential Oil Composition of leaves and Roots of *Heracleum persicum*

H. Vahidi, F. Mojab, B. Nickavar

Pharmaceutical Sciences Research Center and School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

P.09) Essential Oils of Three Iranian *Nepeta*

F. Mojab, B. Nickavar and H. Vahidi

Pharmaceutical Sciences Research Center, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

P.10) Phytochemical Study of *Thymus* Volatile Oils (*T. daenensis* subsp. *daenensis* and *T. kotschyanus*)

B. Nickavar, F. Mojab and H. Vahidi

Pharmaceutical Sciences Research Center and School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

P.11) Supercritical CO₂ extraction of essential oils from plant materials

B. Marongiu, A. Piras and S. Porcedda

Dipartimento di Scienze Chimiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, Cagliari, Italy.

P.12) Comparative analysis of the oil and supercritical CO₂ extract of *Artemisia arborescens* L. and *Helichrysum splendidum* (Thunb.) Less.

B. Marongiu, A. Piras and S. Porcedda

Dipartimento di Scienze Chimiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, Cagliari, Italy.

P.13) Volatile Constituents of *Lotus michauxianus* Ser

M. A. Khalilzadeh¹; J. Ghanaat²; M. Tajbakhsh²

¹Department of Chemistry; I.A. University; Ghaemshahr campus, Iran

²Department of Chemistry; Mazandaran University, Babolsar; Iran.

P.14) Composition of the Essential Oil of *Anchusa iranica*

J. Ghanaat^{1,2}; M. A. Khalilzadeh¹; J. Raouf²

¹Department of Chemistry; I.A. University; Ghaemshahr campus, Iran

²Department of Chemistry; Mazandaran University; Babolsar; Iran.

P.15) Comparative Study of the Essential Oil of Three *Salvia* Species Grown Wild in Iran

S. Masoudi¹ and A. Rustaiyan²

¹Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran, Iran;

²Department of Chemistry, Science & Research Campus, Islamic Azad University, Tehran, Iran.

P.16) Volatile components of selected Japanese medicinal plants

M. Nishiki¹, E. Achleitner², G. Buchbauer², F. Nagashima¹ and Y. Asakawa¹

¹Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan;

²Institute of Pharmacy, University of Vienna, Austria.

P.17) Comprehensive Two-Dimensional NP (Adsorption)-RP Liquid Chromatography for the Analysis of Oxygen Heterocyclic Compounds of Citrus Essential Oils

M. Lo Presti¹, R. Luppino², T. Kumm¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

²Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy.

³Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Position of *Citrus meyerii* Y. Tan, *Citrus medica* L. cv. Diamante and their hybrids

I. Zappalà³, F. Gazea⁴ and A. Cotroneo⁵

1 Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Messina, Italy;

2 Dipartimento di Scienze Farmaceutiche, Università di Catanzaro, Italy;

3 Dipartimento di Scienze e Tecnologie Alimentari, Università di Catania, Italy;

4 Dipartimento di Agrumicoltura, Reggio Calabria, Italy;

5 Dipartimento di Chimica, Università di Messina, Italy.

Chemical composition and composition of Lovage (*Levisticum officinale* Koch.) in reproductive stages

M. A. Ebrahimi^{1,2}, S. N. Ebrahimi²

1 Department of Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran;

2 Department of Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran.

Chemical composition and composition of commercial mandarin essential oil for aromatherapy

M. Thi Minh Tu^{1,2}, N. Thi Lan Phi¹ and Y. Onishi¹

1 Department of Resources Science, Faculty of Agriculture, Kochi University, Nankoku, Kochi, Japan;

2 Department of Food Technology, Hanoi University of Technology, Hai Ba Trung, Hanoi, Vietnam.

Chemical Constituent of Essential Oils in Fresh and Dried Strobiles of *Humulus lupulus* by GC-MS Analysis.

M. Hadi Soleimani^{1,2}, H. Farsam¹

1 Department of Medicinal Chemistry, Faculty of Pharmacy Tehran University of Medical Science, Tehran, Iran;

2 Medicinal Chemistry Research Center, Tehran University, Tehran, Iran.

Chemical composition of volatile oils of aerial parts and roots of *Dracocephalum kotschyi* Boiss. from Iran

M. Kamalinejad^{1,2}, M. Khoshneviszadeh¹

1 Department of Medicinal Chemistry, Faculty of Pharmacy, The Medical Sciences University of Shiraz, Shiraz, Iran;

2 Medicinal Chemistry Research Center, The Medical Sciences University of Shiraz, Shiraz, Iran, P. O. Box: 71345-1149

3 Medicinal Chemistry Research Center, Faculty of Pharmacy, The Medical Sciences University of Shaheed Beheshti, Tehran, Iran.

Chemical composition of essential oil of *Ferula persica* Wild. essential oil from Iran

M. Kamalinejad^{1,2} and N. Edraki¹

1 Department of Medicinal Chemistry, Faculty of Pharmacy, The Medical Sciences University of Shiraz, Shiraz, Iran;

2 Medicinal Chemistry Research Center, Faculty of Pharmacy, The Medical Sciences University of Shaheed Beheshti, Tehran, Iran; 3 Medicinal & Natural Products Chemistry Research Centre, The Medical Sciences University of Shiraz, Shiraz, Iran.

Chemical composition of Volatile Oils of *Satureja Atropatana* Bunge. and *Satureja Mutica* Fisch. et C. A. Mey. From Iran

M. Kamalinejad^{1,2} and A. Feizbakhsh

1 Department of Medicinal Chemistry and Medicinal Plants, Institute of Pure and Applied Science, Islamic Azad University, Central Tehran Branch, Tehran, Iran.

Chemical composition and differentiation among indigenous populations of wild spices in Israel: *Foeniculum vulgare* Mill. and *Micromeria fruticosa* (L.) Druce

M. Y. Cohen¹, O. Barazani¹, A. Fait¹, N. Dudai², U. Ravid² and L. Almog²

1 Department of Medicinal Chemistry, Tel Aviv University, Israel

2 Department of Medicinal Plants and Spices, Nevea Yaar Research Center, Ramat Ishai, Israel.

P.26) Composition of the essential oil of *Solidago virgaurea* ssp. *virgaurea* L. from Austria

G. Radoias¹, A. Bosilcov¹, J. Novak²

¹Erste Tiroler Latschenölbrennerei „Brüder Unterweger“ GmbH, Thal-Assling, Austria;

²Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Wien, Austria.

P.27) California Lomatiums. Analysis of the Essential Oils of *Lomatium nevadense* var. *parishii* (J. Coulter & Rose) Jepson

P. S. Beauchamp, B. C. Dev, V. Dev

Department of Chemistry, California State Polytechnic University, Pomona, CA, U.S.A.

P.28) Isoprenoid Biosynthesis in *Artemisia annua*: Functional Expression of α (+)-Germacrene A Synthase from a Glandular Trichome cDNA Library

C. Berteau¹, F. Verstappen², J. Beekwilder² and H. Bouwmeester²

¹Department of Plant Biology and Centre of Excellence CEBIOVEM, University of Turin, Turin, Italy;

²Plant Research International, AA Wageningen, The Netherlands.

P.29) Essential Oil Composition of *Nepeta involucrata* (Bunge) Bornm. from Iran

L. Allahyari¹, A. Sonboli², P. Salehi¹

¹Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, , Evin, Tehran, Iran;

²Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, , Evin, Tehran, Iran.

P.30) Essential Oil Variation of Sage (*Salvia officinalis* L.) Aerial Parts During its Phenological Cycle

M. M. Vala¹, P. Salehi^{1,2}, M.H. Mirjalili²

¹Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, Tehran, Iran;

²Department of Agriculture, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, Tehran, Iran.

P.31) Morphology and essential oil composition from *Plagiochila stricta* Lindenb. and *P. maderensis* Gottsche ex Steph. grown on Madeira

A. C. Figueiredo¹, M. M. Costa¹, M. Sim-Sim², J. G. Barroso¹, L. G. Pedro¹, M. G. Esquivel³, C. Lobo⁴, S. Fontinha⁴

¹Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCUL, C2, Lisbon, Portugal;

²Centro de Ecologia e Biologia Vegetal, Dep. de Biologia Vegetal, FCUL, C2, Lisbon, Portugal;

³Centro de Botânica Aplicada à Agricultura, Dep. Botânica e Engenharia Biológica, ISA, UTL, Lisboa, Codex, Portugal

⁴Serviço do Parque Natural da Madeira. Caminho do Meio, Quinta do Bom Sucesso, Funchal, Madeira, Portugal

P.32) Essential oil composition and glandular trichomes of *Marrubium vulgare* L. grown wild in Algeria

R. Belhattab¹, L. Larous¹, A. C. Figueiredo², P. A. G. Santos², M. M. Costa², J. G. Barroso² and L. G. Pedro²

¹Department of Biology, Faculty of Sciences, Ferhat Abbas University, Setif, Algeria;

²Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCL, C2, Lisbon, Portugal.

P.33) High-speed GC Analysis with a 50 μ m ID Micro-bore Column: Theory, Practical Aspects and Application on a Complex Essential Oil Sample

P. Q. Tranchida¹, A. Casilli¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy;

²Dipartimento di Chimica Organica e Biologica, University of Messina, Messina, Italy

³Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

P.34) Antimicrobial Activity of Essential Oils of the Selected *Stachys L. Species*

G. Stojanović¹, N. Ristić², R. Palić¹ and J. Lazarević¹

¹Department of Chemistry, Faculty of Natural Sciences and Mathematics, Niš, Serbia;

²Department of Chemistry, Faculty of Natural Sciences and Mathematics, Kosovska Mitrovica, Serbia.

P.35) *Stachys milanii* Essential Oil –Chemical Composition and Antimicrobial Activity

R. Palić¹, J. Lazarević¹, V. Ranđelović², G. Stojanović¹

¹Department of Chemistry, Faculty of Natural Sciences and Mathematics, Niš, Serbia;

²Department of Biology, Faculty of Natural Sciences and Mathematics, Niš, Serbia.

P.36) Root essential oils from *Artemisia absinthium* and *Artemisia vulgaris* growing wild in Serbia

N. Radulović^{1,2}, P. Rašić¹, Ž. Bošković¹ and G. Stojanović

¹University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Serbia and Montenegro;

²Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, Japan.

P.37) Antimicrobial activity and composition of the essential oil of *Equisetum arvense L.*

N. Radulović^{1,2}, G. Stojanović¹ and R. Palić¹

¹University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Serbia and Montenegro

²Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, Japan.

P.38) Sedative and Memory Enhancing Effects of the Essential Oil Fragrance from *Nardostachys jatamansi* After Inhalation and Oral Administration

B-S. Koo¹, S-I. Lee², and D-U. Lee³

¹Department of Oriental Neuropsychiatry, College of Oriental Medicine, Dongguk University, Seoul, Korea

²School of Life and Health, Sorabol College, Gyeongju, Korea

³Department of Biotechnology, College of Natural Science, Dongguk University, Gyeongju, Korea.

P.39) Inhibitory Effects of the Essential Oil from SuHeXiang Wan on the Central Nervous System after Inhalation

D-U. Lee¹, B-S. Koo² and S-I. Lee³

¹Department of Biotechnology, College of Natural Science, Dongguk University, Gyeongju, Korea;

²Department of Oriental Neuropsychiatry, College of Oriental Medicine, Dongguk University, Seoul, Korea

³School of Life and Health, Sorabol College, Gyeongju, Korea.

P.40) Antibacterial Activity and Essential Oil Composition of *Grammosciadium platycarpum* Boiss. from Iran

A. Sonboli, M. Yousefzadi and M. Reza Kanani

Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, Tehran, Iran.

P.41) The Effect of Collection Region on the Essential Oil Yield and Composition of *Eucalyptus camaldulensis* Dehn. from Iran

M.M. Barazandeh¹, M.B.Rezaei¹ and H.Shaker²

¹Research Institute of Forests and Rangelands, Tehran, Iran;

²University of Shahid Beheshti, Faculty of Biology, Tehran, Iran.

P.42) Essential Oils Composition of *Eucalyptus camaldulensis* Dehn. from Fars province of Iran during four seasons

M. M. Barazandeh, M.H.Asareh and M.B.Rezaei

Research Institute of Forests and Rangelands, Tehran, Iran.

[P.43\) Essential Oil Composition of *Geranium rotundifolium* L. from Iran](#)

M. M. Barazandeh

Research Institute of Forests and Rangelands, Tehran, Iran.

[P.44\) "Microwave Dry Distillation" of essential oil from aromatic plants: a rapid, clean and green approach](#)

F. Chemat¹, M. E. Lucchesi¹, J. Smadja¹, L. Favretto², G. Colnaghi² and F. Visinoni²

¹Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Faculté des Sciences et Technologies, Université de la Réunion, Saint Denis messag cedex 9, La Réunion, France D.O.M,

²MILESTONE srl, Sorisole, Bergamo, Italy.

[P.45\) Pesticide and plasticizers residues in biological citrus essential oils from 2003-2004 years](#)

G. Di Bella¹, V. Lo Turco², M. Alfa², F. Salvo² and Giacomo Dugo²

¹Dipartimento Farmaco-chimico, University of Messina, Messina, Italy;

²Dipartimento di Chimica Organica e Biologica, University of Messina, Messina, Italy

[P.46\) Variability of the Essential Oil of *Thymus zygis* from South Spain](#)

R. Pérez Sánchez¹; J. L. Uberta Jiménez¹ and C. G. Ramírez²

¹Department of Plant Biology, University of Córdoba, Campus of Rabanales, Córdoba, Spain

²emillas Silvestres, Córdoba, Spain.

[P.47\) Application of HPLC-MS with a C-30 Reversed Phase Column for the Separation and Identification of Carotenoid Esters in Citrus Essential Oils.](#)

D. Giuffrida, L. La Torre, M. Stelitano, T. M. Pellicanò and Giacomo Dugo.

Dipartimento di Chimica Organica e Biologica, University of Messina, Messina, Italy.

[P.48\) Research on Essential Oils of 32 *Citrus Limon* L. Burm. F. Clones](#)

G. Arcoleo¹, V. Marsolo¹, F. Badalamenti³, L. La Scala³ and M. A. Germanà²

¹Dipartimento di Ingegneria e Tecnologie Agro-Forestali, Facoltà di Agraria, Palermo, Italy.

²Dipartimento di Colture Arboree, Facoltà di Agraria, Palermo, Italy

³Agrumaria Corleone s.p.a., Palermo, Italy.

[P.49\) GC-MS Analysis Of Volatile Oil From *Achillea filipendulina* Lam. And The Preliminary Biological Activities](#)

A. Kowalczyk¹, J. Dąbrowska² and W. Cisowski¹

¹Department of Pharmacognosy, Wrocław Medical University, Wrocław, Poland;

²Institute of Plant Biology, Wrocław University, Wrocław, Poland.

[P.50\) Effect of location on the essential oil of Moroccan *Ormenis mixta*](#)

S. Zrira¹, J. M. Bessiere², C. Menut³ and B. Benjilali¹

¹Institut Agronomique et Vétérinaire Hassan II, Rabat Instituts, Rabat, Morocco;

²Laboratoire de Chimie appliquée Ecole Nationale Supérieure de Chimie USTL Montpellier 8, Montpellier, France;

³Laboratoire de Chimie organique Université Montpellier II Sciences et Techniques du Languedoc, Montpellier, France.

[P.51\) Ultrastructural Analysis of the Secretory Tissues During Ontogenesis of *Citrus bergamia* Risso & Poit. Fruit](#)

C. De Domenico¹, A. Trozzi², A. Cotroneo³ and A. Rapisarda¹

¹Dipartimento Farmaco-Biologico, Università di Messina, Italy;

²Dipartimento di Scienze Farmacobiologiche, Università di Catanzaro, Italy;

³Dipartimento Farmaco-Chimico, Università di Messina, Italy.

P.52) Composition of the essential oils from leaves and cones of *Pinus* species

K. Kurose, D. Okamura, K. Hori and M. Yatagai

Department of Global Agricultural Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.

P.53) Microbial Transformation of 2-Methyl-2-adamantanol and 1,3-Dimethyladamantane

Y. Noma¹, Y. Takahashi¹, T. Hashimoto² and Y. Asakawa²

¹Faculty of Human Life Sciences, Tokushima Bunri University, Japan;

²Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan.

P.54) Chemical Characteristics and Termiticidal Activity of Essential Oils from Root of Bogwood and Fresh Wood of *Cryptomeria japonica* D. Don

H. Narita¹, K. Hori¹, M. Yatagai¹ and T. Ohira²

¹Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan;

²Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan.

P.55) Chemical composition of the essential oil of *Juniperus phoenicea* from Algeria

T. Dob^{1,2}, D. Dahmane¹, T. Berramdane¹ and C. Chelguom²

¹Laboratoire de molécules bio-actives et valorisation de la biomasse, Ecole Normale Supérieure, Kouba Alger, Algeria;

²Laboratoire de chromatographie, Faculté de chimie, U.S.T.H.B.

P.56) Chemical composition of the essential oil of *Annona senegalensis* Leaves from Burkina Faso

A. Bélanger¹, R. H. C. Nébié², R. T. Yaméogo² and F. S. Sib³

¹Agriculture et Agroalimentaire Canada, Centre de R & D en horticulture, Saint-Jean-sur-Richelieu, Québec, Canada;

²Centre National de la Recherche Scientifique et Technologique, Département Substances Naturelles, Ouagadougou, Burkina Faso;

³Laboratoire de Chimie Organique: Structure et Réactivité; Département de Chimie, UFR-SEA, Université de Ouagadougou, Burkina Faso.

P.57) Chemical composition of essential oils of *Vitex diversifolia* from Burkina Faso

A. Bélanger¹, R. H. C. Nébié², R. T. Yaméogo² and F. S. Sib³

¹Agriculture et Agroalimentaire Canada, Centre de R & D en horticulture, Saint-Jean-sur-Richelieu, Québec, Canada;

²Centre National de la Recherche Scientifique et Technologique, Département Substances Naturelles, Ouagadougou, Burkina Faso;

³Laboratoire de Chimie Organique: Structure et Réactivité; Département de Chimie, UFR-SEA, Université de Ouagadougou, Burkina Faso.

P.58) Effects of some essential oils on the germination and the emergency of seeds

M. Macchia, L. Ceccarini and L. Andolfi

Dipartimento di Agronomia e Gestione dell'Agroecosistema, University of Pisa, Pisa, Italy.

P.59) Gas-chromatographic Fatty-acid Fingerprints and Partial Least Squares Modeling As a Basis for the Simultaneous Determination of Edible Oil Mixtures

M. Hajimahmoodi¹, Y. Vander Heyden², N. Sadeghi¹, B. Jannat¹, M. R. Oveisi¹ and S. Shahbazian¹

¹Department of Food Science and Nutrition, Faculty of Pharmacy, Tehran Medical Sciences University, Tehran, Iran;

²Vrije Universiteit Brussel- VUB, Farmaceutisch Instituut, Dept. Pharmaceutical and Biomedical Analysis, Brussels, Belgium.

P.60) Cloning the Diversity: a Multi-Functional Sesquiterpene Synthase as an Example of Product Maximization in *Citrus sinensis*.

A. Voster^{1,2}, C. M. Berteau^{1,2}, M. Maffei¹ and H. J. Bouwmeester²

¹Dipartimento di Biologia Vegetale Università degli Studi di Torino, Italy.

²Plant Research International, Wageningen, The Netherlands.

P.61) Composition and biological activity of essential oil from *Marrubium cuneatum* Soland. (LAMIACEAE)

D. Rigano¹, A. Grassia¹, F. Piozzi², A. Basile³ and F. Senatore¹

¹Dipartimento di Chimica delle Sostanze Naturali, University of Naples "Federico II", Napoli, Italy;

²Dipartimento di Chimica Organica, University of Palermo, Palermo, Italy;

³Dipartimento di Biologia Vegetale, University of Naples "Federico II", Napoli, Italy.

P.62) Molluscicidal activity of *Cymbopogon schoenanthus* ssp. *proximus*, *Geigeria alata* and *Nigella sativa* aromatic water extracts

H. H. EL-Kamali¹, R. O. EL-Nour¹ and H. EL-Subki Khalid²

¹Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan.

²Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

P.63) Volatile Fraction from *In Vitro* and *In Vivo* Biomass of Several *Caryopteris* Plants

M. Luczkiewicz¹, W. Dembińska-Migas¹, P. Migas¹, W. Cisowski^{1,2}, A. Parchowska¹, R. Zarate³ and M. Mardarowicz⁴.

¹Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland;

²Department of Pharmacognosy, Wrocław University of Medicine, Wrocław, Poland;

³Instituto Universitario de Bio-organica A. Gonzalez, Universidad de la Laguna, La Laguna, Tenerife, Spain;

⁴Department of Chemical Physics, Faculty of Chemistry, Maria Curie-Skłodowska University, Lublin, Poland.

P.64) Glandular trichomes and secreted essential oil in *Salvia wagneriana* Polak

A. Bisio¹, G. Romussi¹, C. Bicchi², P. Rubiolo² and L. Maleci³

¹Università di Genova, Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari, Genova, Italy;

²Università di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, Torino, Italy;

³Università di Firenze, Dipartimento di Biologia Vegetale, Firenze, Italy.

P.65) Rosewood Leaves: New Essential Oil from Amazon

L. E. S. Barata, B. Patelli and K. F. Discola

Natural Products Laboratory, Institute of Chemistry, State University of Campinas (Unicamp), Campinas-SP, Brazil.

P.66) Influence of Processing on the Chemical Composition of Valerian oil (*Valeriana officinalis* L.)

D. Lopes^{1,3}, H. Strobl² and P. P. Kolodziejczyk¹

¹Olds College Centre for Innovation, Olds, Alberta, Canada;

²Botanical Specialties, Cultus Lake, British Columbia, Canada;

³Embrapa Food Technology, Rio de Janeiro, Brazil.

P.67) Influence of Vacuum Distillation Parameters on the Chemical Composition of Lemon Concentrates

F. Torres¹, D. Lopes² and F. L. P. Pessoa³

¹Instituto de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia Bl. A, Ilha do Fundão, Rio de Janeiro, Brazil;

²Embrapa Food Technology, Rio de Janeiro, Brazil;

³Escola de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia Bl.E, Ilha do Fundão, Rio de Janeiro, Brazil.

[P.68\) Comprehensive GC \(GCxGC\) - for Citrus Volatile Fraction Analysis](#)

A. Casilli¹, P. Q. Tranchida¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy;

²Dipartimento di Chimica Organica e Biologica, University of Messina, Messina, Italy

³Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

[P.69\) Compositional analysis of essential oil of *Lallemantia roleana*\(Benth. In Wall.\) Benth. From Iran](#)

A. Ghannadi and B. Zolfaghari

Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

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M. C. Pietrogrande¹, L. Mondello² and Giovanni Dugo²

¹Department of Chemistry, University of Ferrara, 44100 Ferrara; e-mail: mpc@unife.it

²Dipartimento Farmaco-chimico, University of Messina, Messina, Italia

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L. Merza-Bahoo, U. Mitteregger, J. Novak and C. Franz

Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Wien, Austria.

[P.72\) The effect of mycorrhization on qualitative and quantitative parameters of *Origanum* sp.](#)

H. Vierheilig¹, J. Novak² and C. Franz²

¹Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Wien, Austria;

²Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Wien, Austria.

[P.73\) Molecular Expression of Flavonoids Pathway Enzymes in Italian Bean Ecotypes.](#)

A. Bonetti, I. Marotti, G. Trebbi, G. Dinelli and P. Catizone

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy.

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A. Bonetti, I. Marotti, G. Trebbi, G. Dinelli and P. Catizone

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy.

[P.75\) The AFLP analysis confirms the taxonomical relationships among *Thymus* species occurring in Portugal](#)

D. Vasques da Silva, M.G. Miguel and J. Leitão

Laboratório de Genética e Melhoramento – Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Faro, Portugal.

[P.76\) Further contributions in the field of antimicrobial testings and gas chromatographic analyses of aroma chemicals](#)

E. Schmidt¹, L. Jirovetz², G. Buchbauer², Z. Denkova³, A. Stoyanova³, I. Murgov³ and M. Geissler⁴

¹Kurt Kitzing GmbH, Wallenstein, Germany;

²Department of Pharmaceutical Chemistry, University of Vienna, Pharmacy-Center, Vienna, Austria;

³Department of Essential Oils, University of Food Technologies, Plovdiv, Bulgaria;

⁴Shimadzu Germany, Department of GC and GC/MS, Duisburg, Germany.

GC-MS of Essential Oil of *Rhododendron anthopogon* and Biological Properties

Innocenti¹, S. Dall'Acqua¹, M. Carrara², A. Tubaro³, M. Barbera² and K. Gurung⁴

Dipartimento di Scienze Farmaceutiche, University of Padova, Padova, Italy;

Dipartimento di Farmacologia ed Anestesiologia, University of Padova, Padova, Italy;

Dipartimento di Economia e Merceologia, University of Trieste, Trieste, Italy;

Partha Herbal Industry, Kathmandu, Nepal.

Essential Oil from *Echinacea pallida*: Characterization and Biological Activity

Dall'Acqua¹, M. Carrara², M. Barbera² and G. Innocenti¹

Dipartimento Scienze Farmaceutiche, University of Padova, Padova Italy;

Dipartimento di Farmacologia ed Anestesiologia, University of Padova, Padova, Italy.

Physical and Chemical Variations of *Pinus brutia* Essential Oils Based on Needles to Twigs Proportions

Ghosh¹, S. Talhouk² and N. A. Saliba¹

Chemistry Department, American University of Beirut, Beirut, Lebanon;

Culture Department, American University of Beirut, Beirut, Lebanon.

Breeding of *Thymus x citriodorus* (Pers.) Schreb. and the Analysis of Essential Oils

Buscus-Plescher, I. Zobel, and S. Thust

MAPLANT Medicinal - and Aromatic Plants, Research and Breeding, Ltd.; Arten, , Germany.

Changes in the qualitative and quantitative chemical composition during steam distillation in pilot plant scale of essential oils of *Achillea millefolium* L., *Salvia sclarea* L. and *Melissa officinalis* L.

Wagner, M. Mandl, H. Berghold and H. Böchzelt;

Forum Research – Institute of Sustainable Techniques and Systems, Graz, Austria.

Synthesis and Olfactory Properties of 1-Isopropylbicyclo[2.2.2]-octane Derivatives

Bella, M. Felici, I. Popolla, F. Leonelli, L. M. Migneco and R. Marini Bettolo

Istituto di Chimica Biomolecolare del CNR, Sezione di Roma and Dipartimento di Chimica Università degli Studi "La Sapienza", Roma, Italy.

New Preparation of 1,3,3-Trimethylbicyclo[2.2.2]octan-2,6-dione, a Never Isolated Key Intermediate in a Total Synthesis of (+)-Norpatchoulenol. A Formal Total Synthesis of (±)-Iso-Norpatchoulenol

Bella, F. Leonelli, I. De Salve, L. M. Migneco and R. Marini Bettolo

Istituto di Chimica Biomolecolare del CNR, Sezione di Roma and Dipartimento di Chimica, Università degli Studi "La Sapienza", Roma, Italy.

Chemical Profiling of *Ocimum Basilicum* L. Cultivated in Ligurian Areas (Italy)

Leonelli¹, A. Bertoli¹, G. Minuto², A. Bisio³ and Romussi³

Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Pisa, Italy;

Istituto Regionale di Sperimentazione e Assistenza Agricola, Camera di Commercio I.A.A. di Savona, Albenga, Italy;

IFA-Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari-Università degli Studi di Genova, Genova, Italy.

Composition of the leaf, stem and root bark oils from *Isolona cooperi*

De Boti,¹ G. Koukoua,² T. Yao N'Guessan,² A. Muselli,³ A-F. Bernardini³ and J. Casanova¹

Université de Corse, Equipe «Chimie et Biomasse», UMR-CNRS 6134, Ajaccio, France;

Université de Cocody-Abidjan, Laboratoire de chimie organique, UFR SSMT, Equipe des Produits Naturels, Abidjan 22, Côte d'Ivoire ;

Université de Corse, Equipe Chimie des Produits Naturels, UMR-CNRS 6134, Corte, France.

P.87) Leaf and root oils from *Peucedanum paniculatum*: composition and antimicrobial activity

M. Vellutini, N. Baldovini, D. de Rocca Serra, F. Tomi and J. Casanova

Université de Corse, Equipe Chimie et Biomasse, UMR-CNRS 6134, Ajaccio, France.

P.88) Composition and Antibacterial Activity of the Essential Oil from *Zanthoxylum hyemale*.

E. Simionatto, C. Porto, A. F. Morel, I. I. Dalcol

Departamento de Química, Núcleo de Pesquisa de Produtos Naturais, Universidade Federal de Santa Maria (UFSM), Santa Maria RS, Brazil.

P.89) Separation of chiral constituents of essential oils using a new selectively substituted cyclodextrin as stationary phase

I. I. Dalcol, E. M. Ethur, A. S. Mallmann, U. F. da Silva, E. Simionatto, and A. F. Morel

Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

P.90) Essential oil composition of *Poiretia latifolia*

C. Porto, C. Z. Stuker, L. V. Becker, I. I. Dalcol, A. F. Morel

Departamento de Química, Universidade Federal de Santa Maria, Santa Maria RS, Brazil.

P.91) Composition and antimicrobial activity of essential oils from *Poiretia bahiana* C. Muller (Papilionoideae-Leguminosae)

F. M. Araújo¹, M. G. V. M. Passos², E. de O. Lima³, N. F. Roque¹, D. Martins¹, M. L. S. Guedes⁴, F. Guaré Cruz¹

¹Grupo de Estudos de Substâncias Naturais Orgânicas (GESNAT), Instituto de Química, Universidade Federal da Bahia, Salvador, Bahia, Brasil;

²Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Bahia, Brasil;

³Laboratório de Micologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, 58.059-900, João Pessoa, Paraíba, Brasil;

⁴Instituto de Biologia, Universidade Federal da Bahia, Salvador, Bahia, Brasil.

P.92) Seasonal composition and antimicrobial activity of essential oils from *Myrcia myrtifolia* DC (Myrtaceae)

M. D. de Cerqueira¹, L. de Souza-Neta¹, M. G. V. M. Passos², E. de O. Lima³, N. F. Roque¹, D. Martins¹, M. L. S. Guedes⁴, F. Guaré Cruz¹

¹Grupo de Estudos de Substâncias Naturais Orgânicas (GESNAT), Instituto de Química, Universidade Federal da Bahia, Salvador, Bahia, Brasil;

²Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Bahia, Brasil;

³Laboratório de Micologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Paraíba, Brasil;

⁴Instituto de Biologia, Universidade Federal da Bahia, Salvador, Bahia, Brasil.

P.93) Monoterpene Patterns of industrially produced Needle Tree Oils

J. Rohloff¹, B. O. Langlete² and R. Hafstad²

¹The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology Trondheim, Norway;

²Norske Eteriske Oljer AS (*Norwegian Essential Oils*), Alvdal, Norway.

P.94) Effect of Fibre Mulch Cultivation on Biomass and Essential Oil Production of *Melissa officinalis*, *Salvia officinalis* and *Thymus vulgaris*

J. Rohloff¹, G. Rakvåg¹, R. Mordal² and S. Dragland²

¹The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology Trondheim, Norway;

²The Norwegian Crop Research Institute (Planteforsk), Apelsvoll Research Centre Division Kise, Hedmark, Norway.

P.95) Essential Oils from Seeds and Pods of *Aframomum corrorima* and Rhizomes of *Zingiber officinale* from Ethiopia

J. Rohloff, A. Hymete and T-H. Iversen

The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology, Norway.

P.96) Innovative Analytical Methods To Characterize Citrus By-Products For Their Possible Use As Ingredients Of Functional Foods

C. Corradini¹, R. Galanti², I. Nicoletti² and S. Padula¹

¹Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università di Parma, Italy;

²Istituto di Metodologie Chimiche, C.N.R. of Roma, Italy.

P.97) Essential oil composition of sea fennel (*Crithmum maritimum* L.) from Italy and Portugal

R. Piccaglia, M. Marotti, S. Grandi and M. T. Amaducci

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy

P.98) Marigold (*Tagetes* spp.) as source of thiophenes

M. Marotti, R. Piccaglia, I. Marotti and G. Venturi

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy.

P.99) Characterization and biological activity of essential oils of several aromatic plants

I. Stefanini, M. Marotti, R. Piccaglia and B. Biavati

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, Bologna, Italy.

P.100) Effect of Extraction Methods on the Chemical Composition and Antimicrobial Activity of *Nigella sativa* Seed Essential Oils

L. Kokoska¹, J. Havlik¹, I. Valterova², H. Sovova³ and V. Rada¹

¹Czech University of Agriculture Prague, Kamycka 129, Czech Republic;

²Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic;

³Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Rozvojova 2, Prague, Czech Republic.

P.101) Chemical Composition of the Essential Oils from Roots of Two *Leuzea* spp.

J. Havlik¹, D. Janovska¹, S. Vasickova², I. Valterova² and L. Kokoska¹

¹Czech University of Agriculture Prague, Prague, Czech Republic;

²Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

P.102) Separation and analysis of water-soluble components from selected essential oil

H. Krüger

Federal Centre for Breeding Research on Cultivated Plants, Institute of Plant Analysis, Quedlinburg, Germany.

P.103) Essential Oils of New Citrus Fruits

G. Ruberto¹, D. M. Biondi¹, A. Renda¹, G. Russo² and P. Rapisarda²

¹Istituto del C.N.R. di Chimica Biomolecolare, Sezione di Catania, Valverde CT, Italy;

²Istituto Sperimentale per l'Agrumicoltura, Acireale CT, Italy.

P.104) A Simple Method to Obtain Essential Oils from *Salvia triloba* L. and *Laurus nobilis* L. by Using Microwave-Assisted Hydrodistillation

Z. Tunalier, M. Kosar, T. Ozek, M. Kürkcüoğlu and K. H. C. Baser

Faculty Pharmacy, Department of Pharmacognosy, Anadolu University, Eskisehir, Turkey.

M. Lo Presti¹, M. L. Crupi¹, P. Dugo², A. Trozzi³, S. Ragusa³, G. Colnaghi⁴, F. Visinoni⁴, Giovanni Dugo¹ and L. Mondello¹
¹Dipartimento Farmaco-Chimico, Facoltà di Farmacia, University of Messina, Messina, Italy;
²Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.NN.FF., University of Messina, Messina, Italy;
³Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università di Catanzaro, Catanzaro, Italy;
⁴Milestones S.r.l., Sorisole (BG), Italy.

P.106) Composition of the Essential Oil of *Centaurea dichroa* Boiss. & Heldr.

A. Altintas¹, Y. B. Kose², E. Yücel³, B. Demirci¹, K. H. C. Baser¹

¹Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, Eskisehir, Turkey;

²Faculty of Pharmacy, Department of Pharmaceutical Botany, Anadolu University, Eskisehir, Turkey;

³Faculty of Science, Department of Biology, Anadolu University, Eskisehir, Turkey.

P.107) New Terpenoid Lactones: Synthesis and Odour Characteristics

R. Obara¹, A. Wzorek¹, J. Kula² and C. Wawrzęńczyk³

¹Institute of Chemistry, Świetokrzyska Academy, Kielce, Poland;

²Institute of General Food Chemistry, Technical University of Łódź, Łódź, Poland;

³Department of Chemistry, Agricultural University of Wrocław, Wrocław, Poland.

P.108) Effect of a spray-dried *Opuntia Ficus Indica* extract on prostate cancer cells (DU145) *in vitro*

A. Panico¹, V. Cardile², F. Garufi¹, C. Puglia¹, F. Bonina¹ and A. Sajja³

¹Dipartimento di Scienze Farmaceutiche, Università di Catania Catania, Italia;

²Dipartimento di Scienze Fisiologiche, Università di Catania, Catania, Italia;

³Dipartimento Farmaco-Biologico, Università di Messina, 98168 Messina, Italia.

P.109) Essential oil pattern of indigenous *Achillea* populations in Hungary

Y. Holm¹, E. Nemeth², I. Laakso¹ and R. Hiltunen¹

¹Faculty of Pharmacy, Division of Pharmacognosy, University of Helsinki, Finland;

²Corvinus University, Faculty of Horticultural Sciences, Dept. of Medicinal and Aromatic Plants, 8 Budapest, Hungary.

P.110) Evaluation of antimicrobial activity of Citrus essential oils

M. A. Cannatelli¹, F. Procopio¹, S. Ragusa², V. Tullio³, G. Di Giacomo⁴, N. Mandras³, A. Marino¹, A. Nostro¹

¹Dipartimento Farmaco-Biologico, University of Messina, Messina, Italy;

²Dipartimento di Scienze Farmacobiologiche, University of Catanzaro, Catanzaro, Italy;

³Dipartimento di Sanità Pubblica e Microbiologia, University of Torino, Torino, Italy;

⁴Citrus Vita S.p.A., Giammoro, Messina, Italy.

P.111) Comparative *in vitro* activity of essential oils against dermatophytes and other filamentous fungi

A. Nostro¹, V. Tullio², P. Dugo³, M. A. Cannatelli¹, N. Mandras², A. M. Cuffini², N. Carlone², V. Alonzo¹.

¹Dipartimento Farmaco-Biologico, University of Messina, Messina, Italy;

²Dipartimento di Sanità Pubblica e Microbiologia, University of Torino, Torino, Italy;

P.112) Bioactivity of selected essential oils for the intensive livestock industries

J. D. Brooker¹, K. P. Svoboda², G. Ruzickova¹ and M. Ritter¹

¹Avian Science Research Centre.

²Dpt of Life Sciences , Auchincruive, Edinburgh, Scotland.

P.113) Essential Oils from *Elaeoselinum fontanesii* Boiss (Umbelliferae)

M. Akssira¹, J. Anaya², F. El Hanbali¹, M. Grande², M. C. Grande², C. Raposo², P. Torres³.

¹Laboratoire de Chimie Bioorganique et Analytique, Université Hassan , Mohammedia, Morocco.

²Departamentos de Q. Orgánica y de Q. Farmacéutica, Universidad de Salamanca, Salamanca, Spain;

³UNED, Facultad de Ciencias, Elche, Spain.

P.114) *In vitro* inhibition of the growth of *Helicobacter pylori* by Bergamot essential oil.

F. C. Pizzimenti¹, A. Nostro¹, A. Marino¹, G. Crisafi¹, F. Procopio¹, L. Mondello², D. Monteleone³ and V. Alonzo¹

¹Dipartimento Farmaco-Biologico, University of Messina, Messina, Italy;

²Dipartimento Farmaco-Chimico, University of Messina, Messina, Italy;

³ Collaboratore Farmacista, Ministero della salute, Direzione generale della sanità veterinaria e degli alimenti.

P.115) “Peratoner” Accelerates Wound Healing In Rat

M. R. Mondello, M. Giampà, M.F. Taviano, G. Contartese, G. F. Ciancio and E. M. Galati.

Dipartimento Farmaco-Biologico, Università di Messina, Messina, Italy.

P.116) Composition of the Essential Oil of *Tanacetum paradoxum* Bornm., from Iran

T. Biniyaz, T. Ghodrati and Z. Habibi

Dept. of Chemistry, Shahid Beheshti University, Tehran, Iran.

P.117) A Comparative Investigation of the Antimicrobial Properties of Indigenous South African and Popular Commercially Available Essential Oils

S. van Vuuren and A. Viljoen

Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

P.118) The Essential Oil of *Ferula Szowitsiana* DC. From Iran

Z. Habibi and H. R. Aghaei

Dept. of Chemistry, Shahid Beheshti University, Tehran, Iran

P.119) Composition of the Essential Oil of *Lagochillus aucheri* Boiss., from Iran

A. Laleh, R. Ghahremanzadeh and Z. Habibi

Dept. of Chemistry, Shahid Beheshti University, Tehran, Iran.

P.120) Analysis of Enantiomeric Ratios of Aroma Components in Flowers Using an Efficient GLC System with a Mixed Chiral Stationary Phase

M. Ishikawa¹, S. Tamogami¹, K-i. Awano¹, M. Amaike¹, Y. Takagi¹ and T. Kitahara²

¹Technical Research Center, T.Hasegawa Co.Ltd., Kawasaki, Japan;

²Department of Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo Japan.

P.121) Antimicrobial Activity of Natural Preserving System (Planning of a self-preserving product)

G. M. Ricchiuto¹, F. Nalin¹, M. Giampà² and F. Pizzimenti²

¹Specchiasol s.r.l., Verona, Italy

²Dipartimento Farmaco-Biologico Università degli Studi di Messina, Italy.

P.122 On the mechanisms of the antimicrobial activity of four monoterpenes of oregano essential oils
D. Trombetta, V. Venuti, M. Cristiani, M. D'Arrigo, G. Bisignano and A. Saija
Department Farmaco-Biologico, University of Messina, Messina, Italy.

P.123) Frequent sources of error when determining specific gravities and refractive indices
P. Wyss
Mettler-Toledo GmbH, Business Unit Analytical, Schwerzenbach, Switzerland.

P.124) Investigation of the essential oils from three *Cephalotaxus* species

W. Cisowski¹, I. Mażo² and M. Gleńsk²

¹Department of Pharmacognosy, Medical University Gen. J. Hallera, Gdańsk, Poland;

²Department of Pharmacognosy, Medical University of Medicine, Nankiera 1, Poland.

P.125) High speed & High resolution GC-FID analysis of Geranium and Limette essential oils

H-U. Baier

Shimadzu Deutschland GmbH, Duisburg, Germany.

P.126) The Determination of Allergens in Fragrance Products Fast GCMS with narrow bore columns

H-U. Baier

Shimadzu Deutschland GmbH, Duisburg, Germany.

P.127) A New Chemotype of *Foeniculum vulgare* Mill. (Umbelliferae)

J. Anaya¹, M. Grande¹, A. Pujadas-Salvà², C. Raposo¹, P. Torres³ and E. Triano-Muñoz⁴;

¹Departamento de Química Orgánica, Universidad de Salamanca, Salamanca, Spain;

²Dpto. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Córdoba, Spain;

³UNED, Facultad de Ciencias, Elche, Spain;

⁴Majadilla, Córdoba, Spain.

P.128) Essential Oils from *Eryngium dilatatum*, *E. bourgatii* and *E. aquifolium* s.l. (Umbelliferae)

J. Anaya¹, M. Grande¹, A. Pujadas-Salvà², C. Raposo¹, P. Torres³ and E. Triano-Muñoz⁴;

¹Departamento de Química Orgánica, Universidad de Salamanca, Salamanca, Spain;

²Dpto. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Spain;

³ UNED, Facultad de Ciencias, Elche, Spain;

⁴ Majadilla, Córdoba, Spain.

P.129) Synthesis and Odour Characteristics of some Compounds with Trimethylcyclohexane System.

K. Winska¹, C. Wawrzenczyk¹ and S. Lochynski²

¹Department of Chemistry, Agricultural University, Wrocław, Poland;

²Institute of Organic Chemistry, Biochemistry and Biotechnology, Wrocław University of Technology, Wrocław, Poland.

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G. Iscan¹, T. Ozek¹, G. Ozek¹, A. Duran² and K. H. C. Baser¹

¹Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskisehir, Turkey;

²Kirikkale University, Faculty of Sciences and Letters, Department of Biology, Kirikkale, Turkey.

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T. Özek¹, G. Özek¹, K. H. C. Baser¹ and A. Duran²

¹Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University Eskisehir, Turkey;

²Faculty of Letters and Sciences, Department of Biology, Kirikkale University, Kirikkale, Turkey.

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N. Kirimer, K. H. C. Baser and M. Kürkcüoğlu

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey.

P.133) Composition of the Essential Oils of *Phlomis rigida* Labill. and *P. samia* L.

B. Demirci¹, K. H. C. Başer¹ and M. Y. Dadandı²

¹Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey;

²Department of Biology, Faculty of Science and Letters, Erciyes University, Kayseri, Turkey.

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G. Özek¹, T. Özek¹, K. H. C. Baser¹, A. Duran² and M. Sagirolu²

¹Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University Eskisehir, Turkey;

²Faculty of Letters and Sciences, Department of Biology, Kirikkale University, Kirikkale, Turkey.

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M. Kosar, T. Ozek, F. Goger, M. Kürkcüoğlu, K. H. C. Baser

Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, Eskisehir, Turkey.

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I. Göhler¹, C. Franz¹ and A. Orellana²

¹Institute for Applied Botany, University of Veterinary Medicine, Vienna, Austria;

²Institute of Agricultural Research and Technology, ICTA/Chimatenango.

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M. T. Momol¹, P. Ji¹, S. M. Olson¹, P. M. Pradhanang¹ and J.B. Jones².

¹North Florida Research and Education Center, University of Florida, Quincy, FL, USA;

²Plant Pathology Department, University of Florida, Gainesville, FL, USA

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M. Miyazawa

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Osaka, Japan.

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Fakhari Zavareh^{1,2}, A. Sonboli², F. Sefidkon³ and N. Masoodian¹

¹Department of Chemistry, Science Faculty, Shahid Beheshti University, Tehran, Iran;

²Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, Tehran, Iran;

³Research Institute of Forests and Rangeland, Tehran, Iran.

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Bizzo¹, F. C. M. Chaves², P. C. S. Angelo² and J. J. B. N. Xavier²

¹Brapa Food Technology, Av. das Américas, Rio de Janeiro, Brazil;

²Brapa Amazonia Ocidental, Rod., Brazil.

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Bizzo¹ and R. F. Vieira²

¹Brapa Food Technology, Av. das Américas, Rio de Janeiro, Brazil;

²Brapa Genetic Resources and Biotechnology, Brasilia, Brazil.

5) Composition and antifungal activity of the essential oil from cultivated and wild Ecotypes of *Cymbopogon parkeri* (stapf), from Iran

Adadian¹, S. M. F. Tabatabaei¹, P. Salehi² and A. Khalighi³

¹Department of Horticultural Science, Faculty of Agriculture, Tehran University, Iran;

²Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, Tehran Iran;

³Department of Horticultural, Tehran Research Institute, Islamic Azad University.

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Kurkcuoglu¹, K. H. C. Başer¹, G. Iscan¹, H. Malyer² and G. Kaynak²;

¹Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey;

²Department of Biology, Faculty of Science & Letters, Uludag University, Bursa, Turkey.

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Salgueiro¹, E. Pinto², M. J. Gonçalves¹, S. Costa-de-Oliveira³, C. Pina-Vaz³, A. G. Rodrigues³, A. Palmeira², C. Cavaleiro¹, C. Tavares³ and J. Martinez-de-Oliveira⁴

¹Pharmacognosy, Faculty of Pharmacy, Coimbra, Portugal;

²Inst. Microbiology, School of Pharmacy, Porto, Portugal;

³Inst. Microbiology School of Medicine, Porto, Portugal;

⁴Inst. Obstetrics/Gynaecology, School of Medicine, Porto, Portugal.

8) GC-MS characterization of "*Ocimum basilicum* var. *Gigante Napoletano*". A preliminary study of the volatile fraction in function of different controlled atmosphere storage conditions

de Luca¹, M. Quinto¹, G. Colelli², D. Centonze¹ and M. L. Amodio²

¹Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Università di Foggia, Foggia, Italy;

²Dipartimento di Scienze delle Produzioni, dell'Ingegneria, della Meccanica e dell'Economia applicate ai Sistemi Agro-zootecnici, Università di Foggia, Foggia, Italy.

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F. Noudjou¹, H. Kouninki², T. Hance², E. Haubruge¹, L. Ngamo³, P. M. Maponmestsem³, M. Ngassoum³, F. Malaisse¹ and G. Lognay¹

¹Faculté Universitaire des sciences agronomiques, Passage des Dépoetés, Gembloux, Belgique;

²Unité d'écologie et de biogéographie, Louvain-la-neuve, Belgique;

³Université de Ngaoundéré, Ngaoundéré, Cameroun.

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A. Akrou¹, I. Chri², M. Hammami² and M. Neffati¹

¹Institut des Régions Arides, 4119 Medenine, Tunisia;

²Faculté de Médecine, Monastir, Tunisia.

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H. R. Monsef-Esfahani¹, M. Ali Faramarzi², M. Moghimi¹ and A. R. Shahverdi²

¹Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran;

²Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

P.149) Iranian Rose Oil

A. Massoudi

Department of R&D, KAF Co., Tehran, Iran.

P.150) Biological activities of the essential oils of *Anethum graveolens* and *Levisticum officinale* hairy roots

P.A. Santos¹, M. G. Miguel², S. Gomes², M. L. Faleiro², A. C. Figueiredo¹, J. G. Barroso¹, L. G. Pedro¹, S. G. Deans³ and J.J.C. Scheffer⁴

¹Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, Lisbon, Portugal;

²Universidade do Algarve, FERN, Campus de Gambelas, Faro, Portugal;

³Dept. of Pharmaceutical Sciences, University of Strathclyde, Glasgow, Scotland, UK;

⁴LACDR, Leiden University, Gorlaeus Laboratories, Leiden, The Netherlands.

P.151) Essential oil of *Dittrichia viscosa* subsp. *revoluta* (Hoffmans & Link) a possible new tool for combating *Helicobacter pylori* infection

M.L. Faleiro¹, M. G. Miguel¹, C. Cavaleiro², L. R. Salgueiro² and J. Casanova³

¹Universidade do Algarve-FERN Campus de Gambelas Faro, Portugal;

²Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal;

³Université de Corse, Équipe Chimie et Biomasse, Route des Sanguinaires, Ajaccio, France.

P.152) The Essential Oil of *Meristotropis xanthioides*

M. Tajbakhsh², M. A. Khalilzadeh¹ and J. Ghanaat²

¹Department of Chemistry; I.A. University; Ghaemshahr Campus, Iran;

²Department of Chemistry; I.A. University; Ayatollah Amoli Campus, Amol, Iran.

P.153) Chemical Composition of the Essential Oil of *Allium jestianum* Boiss. From Iran

H. Amiri¹, R. A. Khavari-Nejad² and A. Rustaiyan³

¹Biology Department, University of Lorestan, Khoramabad, Iran;

²Biology Department, University to Teachers Education, Tehran, Iran;

³Department of Chemistry, Science and Research Campus, I. A. University, Tehran, Iran.

P.162) Chemical Constituents and Biological Study of the Essential Oil of *Polylophium Involvucratum* (pall.) Boiss

M. Pirali-Hamedani¹, A. Hadjiakhoondi² and E. Esmaelii²

¹Department of Pharmaceutical Chemistry, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran

²Department of Pharmacognosy, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran

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Z. Alhani, H. R. Monsef-Esfahani, Y. Amanzade and M. A. Faramarzi

Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

P.164) Composition and antimicrobial activity of *Bupleurum plantagineum* Desf. essential oil

H. Laouer¹, A. N. Chaker¹, G. Ferretti², C. Reynaud³, G. de Billerbeck³, L. Haddioui³ and H. Belkhelfa³

¹Natural Biological Resources Valorization Laboratory (NBRVL), University Ferhat Abbas of Setif, Algeria.

²Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, Italy.

³ Université Paul SABATIER, Toulouse, France.

P.165) Chemotypical characterization of the Essential oil of *Rhodiola rosea* L from Mongolia

S. Shatar, S. Altantsetseg, O. Sarangerel

Institute of Chemistry and Chemical Technology of the Mongolian Academy of Science, Ulaanbaatar, Mongolia

P.166) Trigeminal Chemosensation and its Relation to Physiological Odor Effects

E. Heuberger, C. Springsics, R. Meddeb and G. Buchbauer

Department of Pharmaceutical Chemistry, University of Vienna, Austria.

P.167) Composition and antimicrobial activity of the essential oil of *Saccocalyx satureioides* Coss. et Dur.

H. Laouer¹, N. Chaker¹, G. Ferretti², C. Reynaud³, G. de Billerbeck³, L. Haddioui³, H. Belkhelfa³

¹Natural Biological Resources Valorization Laboratory (NBRVL), University Ferhat Abbas of Setif, Algeria.

²Dipartimento di Scienze Chimiche, Università degli Studi di Camerino, Italy.

³ Université Paul SABATIER, Toulouse, France.

Effect of sowing dates on the essential oil content and composition of *Foeniculum vulgare* cv. Soroksari

R. Omidbaigi¹, F. Sefidkon², G. De Mastro³ and K. Sadrai¹

¹ Department of Horticulture. College of Agriculture, Tarbiat Modarres University, P.O. Box 16415-381, Iran

² Research Institute of Forests and Rangelands, P.O. Box 13185-116, Iran

³ Department of Crop Production, College of Agriculture, Bari University, Bari, Italy

Abstract

Eight sowing dates of 5 March, 20 March, 5 April, 20 April, 5 May, 20 May, 5 June and 20 June were studied on the essential oil content and compositions of *Foeniculum vulgare* cv. Soroksari provided from Hungary. The seeds were sown on 5 and 20 June resulted in plant producing no flowers and fruit. The results of hydro distillation of the seeds and GC and GC/MS of the oil of seeds of *Foeniculum vulgare* cv. Soroksari showed that oil content and compositions were affected by sowing dates. The highest oil content (5.6%) was extracted from the seed of plants were sown on 20 April. Thirteen constituents of the oil of plants were sown on 5 March, 20 March, 5 April, 5 May and 20 May were identified, but ten components from the plants sown on 20 April were analysed. The main component of the oils was anethol, fenchone, estragol, α -pinene, p-cymene, limonene, δ -terpinen and myrcene by GC and GC/MS analysis. The highest amount of anethol (65.59%) was extracted from the plants were sown on 5 of March, then decreased in the oil of plants were sown till 20 May. The largest amount of other compounds were fenchone (28.38%), estragol (6.61%), α -pinene (3.78%) and limonene (3.67%) obtained from the plants were sown on 20 April, 5 May, 20 April and 5 April respectively. It could be concluded that the more suitable time for sowing of *Foeniculum vulgare* cv. Soroksari as a potential source of anethol in Tehran region, Iran was the first of spring, from 5 March to 20 April.

Key word: *Foeniculum vulgare* cv. Soroksari, sowing date, anethol, fenchon, estragol, α -pinene

Essential Oils of Three Iranian *Nepeta*

F. Mojab, B. Nickavar and H. Vahidi

Pharmaceutical Sciences Research Center, Shaheed Beheshti University of Medical Sciences, P.O. Box: 14155-6153, Tehran, Iran; Email: sfmojab@yahoo.com

Abstract

The genus *Nepeta* (Lamiaceae) has 67 species, mostly endemic in Iran. Some species are used in Iranian traditional medicine, *N. cataria* (catnip), for instance, is used as a fortifier, a disinfectant and cure against colds. *N. menthoides* has been used, at the name "Stoechas", as a relief agent for stomach pains, febrifuge and sedative. In recent years, the essential oil of several species of the genus *Nepeta* of Iran, have been examined. These oils are characterized by the presence of 1,8-cineole or several of nepetalactone isomers, as a major component. The composition of the essential oils of *Nepeta asterothrica* Rech. f., *N. crispa* Willd. and *N. menthoides* Boiss. & Buhse (Lamiaceae) - endemics of Iran - were investigated by means of GC and GC-MS. Plant materials were hydrodistilled in a Clevenger-type apparatus for 3 h. Yield of the distillation was 0.6, 0.9 and 0.5%, respectively. 1,8-Cineole was the most abundant component in three oils: *Nepeta asterothrica* (56%), *N. crispa* (71%) and *N. menthoides* (41.1%). The major components of the oil of *N. asterothrica* were thymol (10.6%) and neryl acetate (7.4%). In the oil of *N. crispa*, β -pinene (5%) and δ -terpineol (4.1%) were found to be the major constituents. Dihydromyrcen-1-ol (9.2%), 4-terpineol (7.1%), and geranyl acetate (6.1%) were the predominant compounds in the oil of *N. menthoides*.

Isolation of podophyllotoxin from suspension culture of *Linum album* L.

S. Sedaghat¹, E. Ezatzadeh¹, A. W. Alfermann²

¹ Department of Chemistry, Islamic Azad University, North Tehran Branch, Iran, P.O. Box 19585/93

² Institute for development and molecular biology of the plants, H.H.U, Duesseldorf, Germany

Abstract

Lignans are 8,8' linked dimmers of coniferyl alcohol which are widely distributed in the plant kingdom. Podophyllotoxin isolated from the roots of *Podophyllum peltatum* serves as a precursor for the semi-synthesis derivatives and used in cancer chemotherapy. We try to optimize the production of the callus from *Linum album* by selection of the best hormones compounds and the general cultivation conditions. Other interest is focused on the elucidation of the structure of podophyllotoxin as a Lignans compound and synthesis one of its derivative.

The best callus induction from *Linum album* L. as an Iranian species was obtained on MS medium with NAA (0.4 mg l⁻¹) and BA (0.2 mg l⁻¹) solidified with agar (1%) in dark. After cultivation period, we were used suspension cultures for isolate and characterize podophyllotoxin as glycosides compounds. We were separated fresh cells from the medium by filtration under suction. The cells were freezed, and lyophilized cells were ground in a mortar. The fine powder were suspended in methanol and incubated in an ultrasonic bath. After adding β -glycosidase to each sample, the supernatant was directly used for HPLC analysis by a gradient system with acetonitrile and H₃PO₄ in water. The structure of the Lignan was settled by extensive studies of their 1D and 2D NMR spectra and chemical correlations with the known compounds.

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Chemical Profiling of *Ocimum Basilicum* I. Cultivated in Ligurian Areas (Italy)

L. Pistelli¹, A. Bertoli¹, G. Minuto², A. Bisio³, G. Romussi³

¹ Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italia.

² Centro Regionale di Sperimentazione e Assistenza Agricola, Camera di Commercio I.A.A. di Savona, Regione Rollo, 98, 17031 Albenga, Italia

³ DICTFA-Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari-Università degli Studi di Genova, Genova, Italia

Abstract

Regarding to the commercial use of culinary and medicinal herbs, quality control is becoming an increasingly important issue. Species belonging to the genus *Ocimum* L., sweet basil (Lamiaceae), are examples of plants that are difficult to distinguish on the basis of their leaf morphology, because of the wide range of leaf shapes within most species. Causes of this variation include hybridization among species and selection by humans often associated with the different uses of essential oils. At least six essential oil chemotypes are known to be present in *O. basilicum*. Since the environment affects the characteristic aroma for the same species cultivated in different places, we studied several samples of the most common Italian selection named “Genovese” grown in Ligurian basil cultivation areas. The analyses were carried out on fresh samples both by SPME and GC-MS. The gas chromatographic fingerprints of these samples were compared with those of the “Genovese” plants grown in different Italian regions (Piemonte, Umbria, Campania, Lazio) and in different countries (France, Israel), where the sweet basil “Genovese” is cultivated for industry and consumer’s needs. Trials were designed varying environment (open field, greenhouse) and soil (native soil, Ligurian soil).

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The Volatile Constituents of the Leaves and Flowers of *Kigellia africana* Benth.

O. T. Asekun¹ and O. Adebola²

¹ Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria; taiwoasekun@yahoo.com

² Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria

Essential Oils from Leaves, Stems and Flowers of *Salvia verticillata* L.

M. Jamzad¹, S. Masoudi² and A. Rustaiyan¹

¹ Department of Chemistry, Science and Research Campus I. A. University, P. O. BOX 14515-775
Tehran, Iran; minajamzad@yahoo.com.

² Department of Chemistry, Central Tehran Branch I. A. University, Tehran, Iran

Abstract

The genus *Salvia* (Lamiaceae) comprises about 700 herbs and shrubs, growing in the temperate and warmer zones of the world. Some of these species are used as medicinal, aromatic, and ornamental plants. *Salvia officinalis* L. is one of the most widespread species and since ancient times, has been used in the treatment of various disorders, such as tuberculosis, psoriasis, and seborrheic eczema.

Previous chemical investigations on different species of *Salvia* essential oils have shown the presence of flavonoids, diterpenoids, and even the rare sesterterpenes¹⁻².

The essential oils obtained by hydrodistillation of the leaves, stems and flowers of *S. verticillata* L. were analyzed by a combination of GC and GC/MS.

The major components of the leaves oil were β -phellandrene (17.2%) and β -caryophyllene (14.0%). The oil obtained from the stems were rich in β -caryophyllene (20.4%) and tricyclene (17.4%). β -Caryophyllene (19.7%) and β -phellandrene (13.4%) were the predominant compounds in the flowers oil.

The composition of the oils are different mostly quantitatively than qualitatively.

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Microbial Transformation of 2-Methyl-2-adamantanol and 1,3-Dimethyladamantane

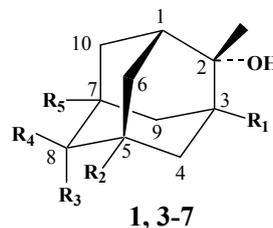
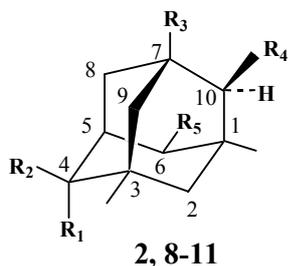
Y. Noma¹, Y. Takahashi¹, T. Hashimoto² and Y. Asakawa²

¹ Faculty of Human Life Sciences; Tokushima Bunri University, Japan

² Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan; e-mail address: ynoma@tokushima.bunri-u.ac.jp

Abstract

In connection with both the metabolism and the biological activities of adamantane and its derivatives the bioransformation of 2-methyl-2-adamantanol(**1**) and 1,3-dimethyladamantane (**2**) was carried out by using *Aspergillus niger*, strain NOMA, *A.niger* IFO4049, *A.cellulosae* IFO4040, *A.sojae* IFO4389 and *A.usami* IFO4338. Compound **1** was easily metabolized to afford 2 kinds of 2-methyladamantane-2,5-diol(**3** & **4**), 2-methyladamantane-2,6-diol(**5**), 2-methyladamantane-1,2-diol(**6**) and 2-methyl-2-hydroxy-6-adamantanone(**7**). Compound **3** was the major metabolite in case of *A.niger*, strain NOMA, *A.niger* IFO4049 and *A.usami*. Compound **4** was the major metabolite in case of, *A.sojae*, Compound **6** was the major metabolite in case of *A.cellulosae* and. showed the strong inhibition against the germination of lettuce seeds. On the other hand, **2** was also hydroxylated to afford 7-hydroxy-1,3-dimethyladamantane(**8**) and three kinds of 1,3-dimethyladamantanediols, namely 4,6-diol(**9**), 4,8-diol(**10**) and 4,7-diol(**11**) by *A. niger*, strain NOMA.



	R ₁	R ₂	R ₃	R ₄	R ₅		R ₁	R ₂	R ₃	R ₄	R ₅
2	H	H	H	H	H	1	H	H	H	H	H
8	H	H	OH	H	H	3	H	OH	H	H	H
9	H	OH	H	OH	H	4	H	H	H	H	OH
10	OH	H	OH	H	H	5	H	H	H	OH	H
11	H	OH	H	H	OH	6	OH	H	H	H	H
						7	H	H	O	O	H

Essential oil content and composition of Lovage (*Levisticum officinale* Koch.) in reproductive stages

M. H. Mirjalili¹, P. Salehi², S. N. Ebrahimi²

¹ Department of Agriculture, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran; m-mirjalili@sbu.ir

² Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran.

Abstract

Lovage (*Levisticum officinale* Koch.; Apiaceae) is well known as a spice plant in the food and fragrance industries (1). Here, the essential oil yield and composition of *Levisticum officinale* in four reproductive stages (anthesis, immature seed, green mature seed and ripen seed) is reported. The oils were obtained by hydrodistillation of air-dried samples. The yield of essential oil (w/w%) in different stages was in order of: immature seed (1.5%)> green mature seed (1%)> ripen seed (0.6%)> anthesis (0.1%). The essential oils were analyzed by GC and GC-MS. In total, 28, 31, 28 and 26 constituents were identified and quantified in the mentioned stages, respectively. Monoterpene hydrocarbons were the main group of compounds in green mature seed (79.2%), immature seed (78.4%), ripens seed (75.2%) and anthesis stage (44%). Cis-ocimene as one of the major constituents of all samples was lower in the first stage and increased gradually in subsequent stages. In contrast, β -pinene content was higher in the first stage and decreased gradually in subsequent stages.

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Mapping of Essential Oil Plants by NIR FT Raman Microspectroscopy

M. Baranska^{1,2}, H. Schulz¹, P. Rösch³, M. A. Strehle³, J. Popp³

¹ Federal Centre for Breeding Research on Cultivated Plants (BAZ), Institute for Plant Analysis, Neuer Weg 22-23, D-06484 Quedlinburg, Germany. E-mail: H.Schulz@bafz.de.

² Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

³ Institut für Physikalische Chemie, Friedrich-Schiller-Universität Jena, Helmholtzweg 4, D-07743 Jena, Germany

Abstract

Raman spectroscopy principally allows to identify non-destructively various components in fresh plant material if characteristic key bands of the individual analyt molecules can be found in the spectrum [1-3]. Especially NIR FT Raman spectroscopy has been described as a valuable tool for in vivo investigations because at these measurement conditions fluorescence and thermal decomposition of the plant tissue can be reduced to a minimum. This poster demonstrates the special potential of vibrational NIR FT Raman microspectroscopy for the study of selected essential oil tissues such as fennel fruits or chamomile inflorescence to obtain detailed information about their microstructure and chemical composition. The Raman area mapping was done by using an xy stage directly connected with the Raman spectrometer. This technique allows to obtain 2-dimensional spectroscopic images of the investigated samples which can be directly compared to the corresponding visual image. Microscopic Raman mappings of fennel fruits prove that anethole, which is the main essential oil component, is present in the whole mericarp with highest concentration at the top of the fruit. *In situ* measurements obtained from of the essential oil cells are dominated by two bands observed at 1657 cm^{-1} and 1609 cm^{-1} which are characteristic for anethole. Raman mappings of chamomile inflorescence show that spiroethers, identified by significant bands between 2150 and 2250 cm^{-1} , are accumulated in the middle part of the flower head. Generally, not only qualitative but also semi-quantitative interpretation of the spectroscopic measurements presented in this study is possible.

Acknowledgment

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Essential oil composition of *Croton cuneatus* and *Croton malambo* growing in Venezuela

A. I. Suárez, L. J. Vásquez¹, M. A. Manzano² and R. S. Compagnone²

¹ Facultad de Farmacia, Universidad Central de Venezuela, Apartado 40109 Caracas, Venezuela

² Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47102, Caracas, Venezuela

Abstract

Croton malambo, (euphorbiaceae) is a small tree that grows in Venezuelan west region, it is known as **palomatías**, The aerial parts of the tree have an aromatic smell and a pungent bitter taste. Infusions of *C. malambo* bark are widely used in traditional medicine for treatment of diverse diseases such as: diabetes, diarrheas, rheumatism, gastric ulcers, and as anti-inflammatory and analgesic agent. *Croton. cuneatus* is a medicinal plant used by the Amazonian natives to treat inflammations, gastrointestinal disturbances and as analgesic. It is a medium size tree which grows widely in the Amazons of Venezuela, and it is known with the name of **arapurina**. In this work we describe the essential oil composition of the bark of *Croton malambo* and the aerial parts of *C. cuneatus*. The composition of these samples were analysed by GC-MS and by NMR. The analysis of the *C. malambo* oil showed 36 constituents in which methyl eugenol, methyl isoeugenol, elemicine, isoelemicin, veratral and δ -cadinol were the major components. *C. cuneatus* oil was found rich in phenylpropanoids and sesquiterpene alcohols showing 43 compounds, where α -11-eudesmene, methyleugenol, 4- α -seleniol, cedryl propyl ether, τ -cadinol, cubenol, methyl isoeugenol, veratral, agarospirol were the most abundant compounds.

Seasonal composition and antimicrobial activity of essential oils from *Myrcia myrtifolia* DC (Myrtaceae)

M. D. de Cerqueira¹, L. de Souza-Neta¹, M. G. V. M. Passos², E. de O. Lima³, N. F. Roque¹, D. Martins¹, M. L. S. Guedes⁴, F. Guaré Cruz¹

¹ Grupo de Estudos de Substâncias Naturais Orgânicas (GESNAT), Instituto de Química, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

² Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

³ Laboratório de Micologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, 58.059-900, João Pessoa, Paraíba, Brasil

⁴ Instituto de Biologia, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

Abstract

The genus *Myrcia* (Myrtaceae) comprises more than 300 species which grows in all Brazilian territory. Indigenous tribes and traditional communities have used several species of the genus as astringent, against diabetes and diarrhea, as diuretic, to stanch hemorrhages, against the hypertension and ulcers of the mouth. This work reports the seasonal variation of composition of the volatile oils from leaves and the composition of volatile oils of flowers and fruits of *M. myrtifolia* a medium-sized shrub that was harvested in the sand dunes of Salvador, Bahia, northeastern region of Brazil between 2001 and 2003. One sample of the leaves oil had its antimicrobial properties tested against six bacteria, two yeasts and five filamentous fungi and toxicity tested with brine shrimp (*Artemia salina*) bioassay.

The volatile oils were obtained from fresh leaves, flowers and fruits by hydrodistillation (3 h) in a Clevenger modified apparatus. The identification of compounds was performed by comparison of their retention indices and mass spectra with those reported in the literature and stored in the NIST libraries (Mass Spectral Library, 1998). The retention indices were calculated by co-injection with a standard saturated *n*-alkanes homologous series.

The oils were analyzed by GC/FID and GC/MS being identified 28 components, α -pinene was predominant in a range from 61.5 to 90.9 % in all samples analyzed. The oil from leaves did not present inhibition against *Escherichia coli*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Cladosporium herbarum* and *Penicillium notatum*. The oil was active against *Staphylococcus aureus*, *Staphylococcus aureus methicilin resistant*, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* and was very active against *Microsporum canis* and *Trichophyton rubrum*. The oil toxicity against *Artemia salina* presented an LC₅₀ of 479.16 $\mu\text{g mL}^{-1}$.

Changes in Lemon Oil Composition During Fruit Ripening

F. Badalamenti

Agrumaria Corleone S.p.A. , via Salvatore Corleone , 90124 Palermo , Italy

Abstract

GC analysis of lemon essential oil extracted through industrial processing sometimes produces results that would be considered atypical with respect to the composition of the volatile fraction.

Often these phenomena cannot be explained solely through analysis of the production process as the results may be influenced by a variety of factors : the variety of the fruit, the stage of maturity, the growing area, the blossom and variables during production.

Analysis results obtained through GC analysis of lemon oil extracted by hand from winter lemons throughout the entire ripening period of the fruit on the other hand enable us to explain these oscillations in average values and at the same time to confirm what are the characteristics peculiar to a pure lemon essential oil.

Gas-chromatographic Fatty-acid Fingerprints and Partial Least Squares Modeling As a Basis for the Simultaneous Determination of Edible Oil Mixtures

M. Hajimahmoodi¹, Y.Vander Heyden², N. Sadeghi¹, B. Jannat¹, M.R. Oveisi¹ and S. Shahbazian¹

¹ Department of Food Science and Nutrition, Faculty of Pharmacy, Tehran Medical Sciences University, Tehran, Iran , hajimah@sina.tums.ac.ir

² Vrije Universiteit Brussel- VUB, Farmaceutisch Instituut, Dept. Pharmaceutical and Biomedical Analysis, Laarbeeklaan 103- 1090 Brussels, Belgium

Abstract

Partial least squares modeling and gas-chromatographic fatty-acid fingerprints are reported as a method for the simultaneous determination of cottonseed-, olive-, soybean- and sunflower-edible oil mixtures. In this work two sets of three- and four- components combinations of oils were prepared, hydrolyzed and the obtained free fatty acids analyzed by gas chromatography (GC) without any further derivatization. The normalized percentages of the myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic acids (18:3) were chromatographically measured in samples and used for constructing calibration matrix. The cross-validation method was used to select the number of factors and the proposed methods were validated by using two sets of synthetic oil mixture samples. The relative standard error for each oil in mixture samples was less than 10 %. This approach allows determining possible adulteration in each of the four edible oils.

Composition of essential oils extracted from some selected Fijian Plants.

J-P. Dufour¹, K. Patel², S. Sotheeswaran², and S. Ali²

¹ Department of Food Science, University of Otago, Dunedin, New Zealand;

² Department of Chemistry, The University of the South Pacific, Suva, Fiji Islands.

Abstract

The essential oils of *Spondias dulcis*, *Cinnamomum verum*, *Eryngium foetidum*, *Coriandrum sativum* and *Alpinia zerumbert* of Fijian origin were analyzed by GC-MS. *S.dulcis* (Sapindaceae) has edible fruits similar to that of mangoes and the leaves are sometimes used to flavour meat. The aerial parts have shown antispasmodic, antibacterial and antifungal activity. The local name for this species is *wi* (fijian) and *amra* (hindi). *C.verum* (Lauraceae) leaves are used for flavouring food and as a spice and the local name is *macovu*. *E.foetidum* and *C.sativum* both belong to the family Umbelliferae. *E.foetidum* is locally known as false *dhania* while *C.sativum* is known as *dhania* or coriander. *E.foetidum* is often mistaken for *C.sativum* as they both have similar taste and smell. *A.zerumbert* (Zingiberaceae) is commonly known as shell-flower ginger due to the shell shaped flowers. There is no traditional use of this species in Fiji.

This paper (Table 1) updates the essential oil data file of five species in Fiji and compares the results with the same species from different countries and reports components identified for the first time from the Fijian species.

Name of plant (a)	Number of components (b)	Major components (c)	Major components published (d)	New components reported (e)
<i>E.foetidum</i> (leaves)	27 (46-Cuba, 19-Vietnam)	E-2-dodecenal (71%) E-2-tetradecenal (11%) Dodecanal (8%)	2,4,5-trimethylbenzaldehyde (21%) hexadecanoic acid (12%) E-2-dodecenal (46%)	Tetradecenal (1.24%), caryophyllene oxide (0.78%), linalool (0.16%), E,E-2,4-dodecadienal (0.13%), E-beta-farnesene (0.09%)
<i>C.sativum</i> (leaves)	51 (41 and 62- USA)	2-decen-1-ol (24%) decanol (18%) E-2-tetradecenal (11%)	E-2-decenal (40%) E-2-dodecenal (10%) 2-decen-1-ol (9%)	E-2-dodecen-1-ol (3.7%), E or Z hexenol (1.32%), eucalyptol (0.50%), E-2-hexenol (0.48%), p-cymene (0.26%)
<i>C.verum</i> (leaves)	31 (24-Sri Lanka)	Eugenol (86%) E-caryophyllene (6%) Linalool (2%)	Cinnamic aldehyde (63%) Linalool (7%) β-caryophyllene (6%)	E-caryophyllene (5.7%), caryophyllene oxide (0.48%), Z-3-hexenal (0.31%), hexanal (0.3%), eucalyptol (0.22%)
<i>S.dulcis</i> (leaves)	22 (no published work)	α-Pinene (48%) Limonene (14%) β-pinene (13%)	No work reported	
<i>A.zerumbert</i> (leaves)	75 (10-Fiji)	4-terpineol (25%) 1,8-cineole (15%) γ-terpinene (13%)	4-terpineol (41%) 1,8-cineole (13%) β-pinene (10%)	Sabinene (5.66%), α-terpinene (2.44%), L-α-terpineol (1.80%), terpinolene (1.62%), limonene (1.57%)
<i>A.zerumbert</i> (rhizomes)	88 (9-Fiji)	4-terpineol (19%) 1,8-cineole (16%) β-cymene (14%)	4-terpineol (41%) 1,8-cineole (28%) γ-terpinene (8%)	limonene (2.88%), camphene (2.56%), L-α-terpineol (1.62%), terpinolene (1.46%), α-terpinene (1.37%)
<i>A.zerumbert</i> (flowers)	57 (no published work)	4-terpineol (20%) 1,8-cineole (19%) sabinene (16%)	No work found	

Table 1: (a) part of plant used for essential oil extraction; (b) number of components found in other countries; (c) only three major components identified in this study are listed; (d) three major components previously identified; (e) five major components identified in this study for the first time from the species.

Leaf and root oils from *Peucedanum paniculatum*: composition and antimicrobial activity

M. Vellutini, N. Baldovini, D. de Rocca Serra, F. Tomi and J. Casanova

Université de Corse, Equipe Chimie et Biomasse, UMR-CNRS 6134, Route des Sanguinaires, 20 000 Ajaccio, France.

Abstract

Peucedanum paniculatum Loisel, syn. *Peucedanum officinale* subsp. *paniculatum* (Loisel.) Frey. (*Apiaceae*) [1] is an endemic species to Corsica, growing wild in the centre and northwest of the island. This plant, 70 - 120 cm high, with sheets finely cut out in long narrow thin straps, looks like fennel [2] and is known for its important phototoxicity. It is also a determinant factor in growth and distribution of *Papilio hospiton*, an endemic corsican butterfly [3].

Continuing our research on essential oils bearing plants growing wild in Corsica, we report here on the chemical composition of the essential oils from leaves and roots of *P. paniculatum*. Identification of the individual components was carried out by combination of column chromatography, GC/RI and ¹³C-NMR spectroscopy according to an experimental procedure and a computerised method developed in our laboratories.

Both samples were characterised by the occurrence of irregular monoterpenes belonging to the cyclolavandulyl framework. The main components of leaf oil and root oil are β -cyclolavandulyl isobutyrate (respectively 17,8% and 6,2%), β -cyclolavandulyl acetate (16,1% and 13,9%), β -isocyclolavandulyl isobutyrate (5,3% and 7,3%), β -isocyclolavandulyl acetate (6,1% and 15,9%), β -cyclolavandulol (4,3% and 5,0%), β -isocyclolavandulol (2,2% and 5,7%) and lavandulyl acetate (3,0% and 9,8%). β -Isocyclolavandulol was fully characterised by a full set of NMR experiments (¹H, ¹³C, DEPT, NOESY, HMQC, HMBC) and the corresponding esters, not yet reported in the nature, were prepared from the alcohol.

Leaf and root oils were also tested against eleven bacterial strains. Both oils exhibited an antimicrobial effect particularly on *Staphylococcus aureus*.

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Morphology and essential oil composition from *Plagiochila stricta* Lindenb. and *P. maderensis* Gottsche ex Steph. grown on Madeira

A. C. Figueiredo¹, M. M. Costa¹, M. Sim-Sim², J. G. Barroso¹, L. G. Pedro¹, M. G. Esquível³, C. Lobo⁴, S. Fontinha⁴

¹ Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCUL, C2, Campo Grande, 1749-016 Lisbon, Portugal, acsf@fc.ul.pt

² Centro de Ecologia e Biologia Vegetal, Dep. de Biologia Vegetal, FCUL, C2, Campo Grande, 1749-016 Lisbon, Portugal

³ Centro de Botânica Aplicada à Agricultura, Dep. Botânica e Engenharia Biológica, ISA, UTL, 1399 Lisboa, Codex, Portugal

⁴ Serviço do Parque Natural da Madeira. Caminho do Meio, Quinta do Bom Sucesso, 9050-251 Funchal, Madeira, Portugal

Abstract

Madeiran *Plagiochila* species constitute 64% of the total species referred to Europe and Macaronesia. *P. stricta*^{1,2} and *P. maderensis* are frequent on the Madeiran Laurissilva in shaded slopes located along water rivulets. Previous phytochemical studies have examined only the solvent extracts of both species.^{3,4}

Morphological studies were carried out by scanning electron microscopy (SEM) and the essential oils of both species were isolated from deep-frozen plant material by distillation-extraction and analysed by GC and GC-MS.

allo-Ocimene (13-19%), *neo-allo*-ocimene (7-11%), peculiaroxide (16-17%) and bicyclogermacrene (9-17%) were the main components of the oils of the *P. stricta* specimens studied, whereas terpinolene (34-60%) was the main component from the oil isolated from *P. maderensis* specimens.

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Essential oil pattern of indigenous *Achillea* populations in Hungary

Y. Holm¹, E. Nemeth², I. Laakso¹ and R. Hiltunen¹

¹ Faculty of Pharmacy, Division of Pharmacognosy, P.O.Box 56, 00014 University of Helsinki, Finland

² Corvinus University, Faculty of Horticultural Sciences, Dept. of Medicinal and Aromatic Plants, 1118 Budapest, Villanyi str. 29-35, Hungary; drog@omega.kee.hu

Abstract

Achillea collina Becker has been a well-known and widely used species in Europe for many centuries and the majority of the drug originates from wild growing populations even today. During collection, the assurance of standard quality seems to cause significant difficulties, because of the big intraspecific variability of this species. Aim of the recent investigations was to clear up the chemical characteristics inside a tighter area, North to the lake Balaton, in Hungary. In addition, we examined the compositional differences between flowers and leaves and their possible correlation with the type of natural habitat.

Seed material at 8 habitats (meadows, oak forest, roadside, pastures, *etc.*) were collected in 2001 and experimental plots were established in the next year at the Research Station of the BKA University in Budapest in order to assure similar ecological circumstances during comparison. Representative samples were taken in the second year old stands in 2003 at full flowering. The flowering shoots had been divided to flower and leaf samples for each population. The essential oil was distilled according to the PhHgVII.

The essential oils from 7 leaf and flower samples were analysed by GC-MS. Altogether 79 compounds were detected of which 35 were identified for this study. Chamazulene was the main compound in all studied oils (range 24–45%). Relatively high spathulenol (range 1–6%; mean 4%) and low germacrene-D (range 1–3%) levels were characteristic for leaf oils, while low spathulenol (range 1–2%) and high germacrene-D (5–13%; mean 8%) contents were typical for flower oils. β -Caryophyllene levels in individual plants formed two distinct groups. Low (1–4%) and high (7–10%) β -caryophyllene chemotypes in the leaves could be classified as well as low (5–8%) and high (10–15%) groups also in the flower oils.

The present results provide further data on the marked variability of sesquiterpenoids in essential oils of *Achillea* species.

Essential Oil Composition of leaves and Roots of *Heracleum persicum*

H. Vahidi¹, F. Mojab¹, B. Nickavar¹

¹ Pharmaceutical Sciences Research Center and School of Pharmacy, Shaheed Beheshti University of Medical Sciences, P.O. Box: 14155-6153, Tehran, Iran; hovahidi@yahoo.com

Abstract

The leaves and roots of *Heracleum persicum* Desf. ex Fischer (Syn. *H. glabrescens* Boiss. & Hohen, *H. pubescens* Rech.) (Fam. Apiaceae) collected in July-August 2003 in Kandavan Area, North of Tehran (Iran). The oils, which were obtained by hydrodistillation, were analysed by GC, GC/MS and ¹H-NMR.

Major components of leaves and roots were *trans*-anethole (82.8 %) and viridiflorol (23.05 %), respectively. Other components of leaves were β -pinene, *p*-cymene and terpinolene (monoterpenes), α -caryophyllene, α -bergamotene, α -farnesene, zingiberene and spathulenol (sesquiterpenes), *cis*-anethole, stragole and 2,5-dimethyl styrene (aromatic compounds) and β -springene (an aliphatic and hydrocarbonic diterpene). In conclusion, *trans*-anethole was identified as a major component of this oil. Other components of root oil constituents were elemol (3.63 %), β -maliene (3.07 %), spathulenol (3.34 %) and 2-tetradecanol (3.38 %).

Antimicrobial Activity of Natural Preserving System (Planning of a self-preserving product)

G. M. Ricchiuto¹, F. Nalin¹, M. Giampà², F. Pizzimenti²

¹ Specchiasol s.r.l., Verona, Italy

² Dipartimento Farmaco-Biologico Università degli Studi di Messina, Italy

Abstract

During the period in which they are used, cosmetic products go through serious aggressions caused by oxidation and microbial action: in this extract we are going to consider some preserving system made up of natural substances, whose intent is to create an “uncomfortable” environment for bacteria and mould.

In fact, to make sure that cosmetics will live for more than 30 months, we need to weight the risk of microbial pollution and protect cosmetics from contamination during the daily use, with appropriated mixture of antimicrobial substances.

Using two cosmetic products (an O/W emulsion and a gel), we have noticed how, a pH tampon, an antioxidant, a natural antibacterial molecule and an essential oil, can create a system with a large range of action which develops an antibacterial activity towards the products considered before.

In particular we have exploited the properties of the lactic tampon to create a pH of 5,5; the use of the capryloyl glycine mixture with an extract of *Cinnamomum zeylanicum* as an antibacterial and finally a natural, innovative extract which derives from the working process of *Citrus Bergamia* and which is called “PERATONER”. The valuation of the preserving activity has been executed through the determination of the total microbial power and the Challenge Test, referring as regards methods and microbiological limits, to the values suggested by the European Pharmacopoeia test.

Ultrastructural Analysis of the Secretory Tissues During Ontogenesis of *Citrus bergamia* Risso & Poit. Fruit

C. De Domenico¹, A. Trozzi², A. Cotroneo³, A. Rapisarda¹

¹ Dipartimento Farmaco-Biologico, Università di Messina, Italia;

² Dipartimento di Scienze Farmacobiologiche, Università di Catanzaro, Italia;

³ Dipartimento Farmaco-Chimico, Università di Messina, Italia.

Abstract

There are few and contradictory pharmaco-botanical investigations about the morphofunctional aspects of the glandular system of *Citrus* fruits, from which essential oils are mainly extracted. Therefore the fruit of *Citrus bergamia* Risso & Poit., a typically Mediterranean species, was examined in order to obtain information about tissues where the biogenesis and/or storage of the active principles takes place, and also to identify phytochemical markers able to differentiate the cultivars that represent the vegetable source for the bergamot essential oil production.

The ultrastructural study of the fruit peel showed the schizolysigenous nature of the secretory tissues, where the essential oil is produced and stored. In unripe fruit the glandular structures are initially formed by separation of walls of neighbouring cells; this phenomenon begins in the mesocarp, then during the following stages of gland development, due to the disintegration of cells surrounding the central cavity, esocarp is affected too. The micromorphometric analysis of cross-sectioned fruit peels of *C. bergamia* provided a key factor for differentiation of the cultivars castagnaro, fantastico and femminello. The glands number/cm² in unripe and ripe fruits is constant, even if the sizes of oil cavities change. The mean value of the volume of the glandular cavities/glandular cavities number per cm² ratio evidences that the essential oil productivity for the three cultivars of *C. bergamia* fruits is: fantastico > castagnaro (-13%) > femminello (-21%).

Pesticide and plasticizers residues in biological citrus essential oils from 2003-2004 years

G. Di Bella¹, V. Lo Turco², M. Alfa², F. Salvo², Giacomo Dugo²

¹ Dipartimento Farmaco-chimico, University of Messina, 98168 Messina, Italy; gdibella@pharma.unime.it

² Dipartimento di Chimica Organica e Biologica, University of Messina, 98168 Messina, Italy

Abstract

Organophosphorus and organochlorine pesticides, phosphorated plasticizers, chloroparaffins and phthalate, adipate and sebacate esters contamination in citrus essential oils produced in Sicily in the crop years 2003-2004 was studied by HRGC in connection with detectors FPD, ECD, MS.

All the samples, 40 from 2003 and 10 from 2004, were grown using biological cultivation and were industrially produced.

Biological farming is based on the abolition of chemicals and produces food free from contaminants. Unfortunately residues of organophosphorus and organochlorine pesticides were found in some of the analysed citrus essential oils from both the crop years.

Also plasticizers residues were found in some of the studied oils, probably as a result of the use of different types of plastic components during the extraction process.

Phytochemical Study of *Thymus* Volatile Oils (*T. daenensis* subsp. *daenensis* and *T. kotschyanus*)

B. Nickavar, F. Mojab and H. Vahidi

Pharmaceutical Sciences Research Center and School of Pharmacy, Shaheed Beheshti University of Medical Sciences, P. O. Box: 14155-6153, Tehran, Iran

Abstract

The genus *Thymus* (Lamiaceae) consists of about 215 species widespread throughout the world and is represented by 14 species in Iran. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants in the food and pharmaceutical industries. Among the species grown in Iran, *T. daenensis* Celak. and *T. kotschyanus* Boiss. & Hohen., are widely used as herbal tea, flavoring agents (condiment and spice), carminative, digestive, antispasmodic, antitussive, expectorant, anti-inflammatory. Therefore, due to the importance of *Thymus* species, it was decided to investigate the chemical composition of the essential oils of two Iranian *Thymus* species, *T. daenensis* subsp. *daenensis* and *T. kotschyanus*.

The flowering aerial parts of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* were collected from the west of Iran, Hamadan province in June 2002. The essential oils were isolated by hydrodistillation in a Clevenger-type apparatus for 3 h and analyzed by using GC/FID and GC/MS. Identification of compounds of the oils were based on retention indices relative to *n*-alkanes and computer matching with the WILEY275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the library. The relative percentages of the oils constituents were calculated from the GC peak areas.

The hydrodistillation of the aerial parts of *T. daenensis* subsp. *daenensis* and *T. kotschyanus* gave oils with a yield of $2.4 \pm 0.1\%$ (v/w) and $1.2 \pm 0.1\%$ (v/w), on dry weight basis, respectively. The main components in the oil of *T. daenensis* subsp. *daenensis* were thymol (74.7%), *p*-cymene (6.5%), β -caryophyllene (3.8%) and methyl carvacrol (3.6%). *T. kotschyanus* oil contained thymol (38.6%), carvacrol (33.9%), γ -terpinene (8.2%) and *p*-cymene (7.3%) as major constituents.

The results of this study indicate that both oils are rich in monoterpene phenols, especially thymol and carvacrol. In addition, there are many qualitative similarities between the oils although the amounts of some corresponding compounds are different.

Supercritical CO₂ extraction of essential oils from plant materials

B. Marongiu, A. Piras and S. Porcedda

Dipartimento di Scienze Chimiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, SS 554, km 4,500 09042, Cagliari, Italy

Abstract

The characteristic smell of plant materials is usually the result of the complex interactions occurring among hundreds of compounds. Correct reproduction of natural fragrance in a concentrated extract is therefore a complex task. The presence of thermolabile compounds and the possibility of hydrolysis are serious obstacles in the reproduction of natural fragrance. Moreover severe legislative restrictions are currently being proposed to eliminate solvent residues in these products when used in the food, pharmaceutical and cosmetic industries. Extraction by means of carbon dioxide, CO₂, in the supercritical state (SFE) is a good technique for the production of flavors and fragrances from vegetable matter. Indeed, there is considerable interest in replacing the steam distillation, hydrodistillation and solvent extraction process traditionally used to obtain these products.

The following plant materials were selected for extraction: oleo-gum resin of incense (*Boswellia carterii*), oleo-gum resin of myrrh (*Commiphora myrrha*), rhizome of calamus (*Acarus calamus*) and wood of sandal (*Santalum album*). The effect of extraction conditions (pressure, temperature, flow rate and particle size) on the yields and compositions of the resultant extracts is examined. GC/MS analysis of the extracts allowed the identification of oils composition. The main constituents in the extracts were: (Z)- α -santalol (45%) and (E)- β -santalol (18%) in sandalwood, furanoeudesma-1,3-diene (34.9%) and lindestrene (12.9%) in myrrh, (Z)-sesquilavandulol (22%) and acorenone (11%) in calamus, incensole acetate (35%) and incensole (10%) in incense.

The AFLP analysis confirms the taxonomical relationships among *Thymus* species occurring in Portugal

D. Vasques da Silva, M. G. Miguel, and J. Leitão

Laboratório de Genética e Melhoramento – Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; dvsilva@ualg.pt

Abstract

Thymus is a widely spread genus in the Mediterranean region with several species endemic to the Iberian Peninsula. Some species show an even more restricted dispersal and are endemic to Portugal. AFLP (Amplified Fragment Length Polymorphisms) analyses were performed to assess the genetic relationships among 12 *Thymus* species occurring in Portugal: *T. albicans* Hoffmanns. & Link, *T. caespititius* Brot., *T. camphoratus* Hoffmanns. & Link., *T. capitellatus* Hoffmanns. & Link, *T. carnosus* Boiss, *T. lotocephalus* G. López & R. Morales, *T. mastichina* L. ssp. *mastichina*, *T. pulegioides* L., *T. villosus* L. ssp. *lusitanicus* (Boiss) Coutinho, *T. villosus* L. ssp. *villosus*, *T. zygis* Loefl. ex L. ssp. *silvestris*, *T. zygis* Loefl. ex L. ssp. *zygis*. The genetic relationship between these *Thymus* species and *Thymbra capitata* was also determined. An exceptional concordance was found between the genetic relatedness estimated on the basis of AFLP analysis and the accepted taxonomic classification of these species established according to morphological traits. The need of a new subsection to include the Portuguese endemism *Thymus carnosus* Boiss, is also discussed.

Molluscicidal activity of *Cymbopogon schoenanthus* ssp. *proximus*, *Geigeria alata* and *Nigella sativa* aromatic water extracts

H. H. EL-Kamali¹, R. O. EL-Nour¹ and H. EL-Subki Khalid²

¹Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, P.O. Box # 382, Omdurman, Sudan.

²Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, P.O. Box # 11496, Khartoum, Sudan.

Abstract

Molluscicidal activity of the aromatic water extracts of the *C. schoenanthus* ssp. *proximus* leaves, *G. alata* aerial parts and *N. sativa* seeds were investigated against *Biomphalaria pfeifferi*, intermediate host of schistosomiasis. Molluscicidal activity was evaluated according to the established procedures (WHO, 1965) [1]. The lethal concentration (LC50) value was calculated after 24 hrs and was found that *N. sativa* extract showed the highest toxicity with LC50 value of 308 ppm followed by that of *C. schoenanthus* ssp. *proximus* and *G. alata* with 35.7 ppm and 435.2 ppm respectively. Further studies of these plants as possible agents for molluscs control are warranted.

[1] WHO, 1965. Molluscicide screening and evaluation. Bulletin WHO 33, 567-581.

Essential oil composition of *Citrus meyerii* Y. Tan., *Citrus medica* L. cv. Diamante and their hybrids.

A. Verzera¹, A. Trozzi², M. Zappalà³, F. Gazea⁴, A. Cotroneo⁵.

¹ Dipartimento di Chimica Organica e Biologica, Università di Messina, Italy;

² Dipartimento di Scienze Farmaco-biologiche, Università di Catanzaro, Italy;

³ DOFATA, Sez. Scienze e Tecnologie Alimentari, Università di Catania, Italy;

⁴ Istituto Sperimentale per l'Agricoltura, Reggio Calabria, Italy;

⁵ Dipartimento Farmaco-chimico, Università di Messina, Italy.

Abstract

The hybridization project represents one of the most important research lines in the Citrus genetic improvement since genus Citrus is open to countless possibilities for crossbreeding. As regards the lemon, the early crosses, which eventually led to the genesis of new species, were performed to obtain cultivars resistant to the disease “mal secco”.

Recently, new hybrids, by crossbreeding the diploids *Citrus meyerii* Y. Tan. and the *Citrus medica* cv. Diamante with the tetraploid *Citrus limon* Burm. cv. Cavone, have been obtained. These hybrids have potential commercial value because they have shown interesting agro-biological characteristics, particularly a good resistance to diseases, a good and steady fructification, and seedless fruits with easy removable peel.

Although the programs of genetic improvement of lemon in Sicily are mainly aimed at the fresh fruit market, the economic relevance of the essential oil should not be disregarded. In the current study, the volatile fraction of the essential oils of *Citrus meyerii* Y. Tan. and the *Citrus medica* cv. Diamante, for what the information reported in literature are limited, has been characterized. Moreover, the new hybrids oils have been analysed and compared with those of the parents.

The oils were laboratory-extracted from the peel fruits and the volatile fraction was analyzed by HRGC-MS and HRGG-FID. In all, 63 components were fully characterized and grouped into classes (hydrocarbons, monoterpenes, sesquiterpenes, oxygenated compound, carbonyl compounds, alcohols, esters) for an easier comparison with the oils of the parents. Since limonene is by far the main component of all the essential oil examined, representing from 50 to 80% of the total, significant hybrid and parent differences can be observed. The level of most components in the hybrid peel oils was intermediate between their levels in the parent oils. Multivariate analysis using principal component afforded more information on the similarities and differences of each hybrid to its parents.

Comparative analysis of the oil and supercritical CO₂ extract of *Artemisia arborescens* L. and *Helichrysum splendidum* (Thunb.) Less.

B. Marongiu, A. Piras and S. Porcedda

Dipartimento di Scienze Chimiche, Università degli Studi di Cagliari, Cittadella Universitaria di Monserrato, SS 554, km 4,500 09042, Cagliari, Italy

Abstract

Isolation of volatile concentrates from the dried leaves of *Artemisia arborescens* and of *Helichrysum splendidum* have been obtained by supercritical extraction with carbon dioxide. To obtain a pure volatile extract devoid of cuticular waxes, the extracts were fractionated in two separators operating in series. A good extraction process was obtained operating at 90 bar and 50 °C in the extraction vessel, at 90 bar and at -5 °C in the first separator and at a pressure between 20 and 15 bar and temperatures in the range (10 - 20) °C in the second one.

The composition of the volatile concentrate has been analyzed by GC/MS.

The volatile concentrate of *Artemisia arborescens* was found to contain: trans-thujone (13.96%), camphor (6.15%) and chamazulene (5.95%).

The main constituents in the extract of *Helichrysum splendidum* were: germacrene D-4-ol (17.08%), germacrene D (9.04%), bicyclogermacrene (8.79%) and δ -cadinene (8.43%). A comparison with the oils obtained by hydrodistillation is also given.

The differences observed between the composition of the SFE volatile concentrates and of the hydrodistilled oils one were relevant. Indeed, in the both cases, the HD oils had a blue color whereas the volatile concentrates were pale yellow. The HD oil of *Helichrysum splendidum* had a blue color due to presence of guaiazulene (0.53% versus 0%, in the HD and SFE respectively), whereas the coloration of the HD oil of *Artemisia arborecens* is due to high concentration of chamazulene (26.64% versus 3.37%) in aqueous medium.

Volatile Fraction from *In Vitro* and *In Vivo* Biomass of Several *Caryopteris* Plants

M. Luczkiewicz¹, W. Dembińska-Migas¹, P. Migas¹, W. Cisowski^{1,2}, A. Parchowska¹, R. Zarate³, M. Mardarowicz⁴.

¹ Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Gdańsk, al. Gen. J.Hallera 107, 80-416 Gdańsk, Poland

² Department of Pharmacognosy, Wrocław University of Medicine, pl. Nankiera 1, 50-140 Wrocław, Poland.

³ Instituto Universitario de Bio-organica A. Gonzalez, Univesidad de la Laguna, Avda. Fco. Sanchez 2, 38206 La Laguna, Tenerife, Spain

⁴ Department of Chemical Physics, Faculty of Chemistry, Maria Curie-Skłodowska University, sq. M.Curie-Skłodowskiej 3, 20-031 Lublin, Poland

Abstract

The subject of the presented research was biotechnological research into plant cell cultures. Three selected species of the *Caryopteris* genus were used to establish *in vitro* cultures with the aim of developing plant material rich in volatile oils. The researched species contain volatile oil rich in cineol and due to this property they are used in traditional Chinese medicine to make anti-inflammatory, anti-rheumatic and antiseptic preparations. According to recent reports volatile oils containing cineol act as acetylcholinesterase inhibitors.

Presently, with increasing incidence of microbic resistance to antibiotics and antiseptics, there is a growing interest in volatile oils with proven, safe antimicrobial properties. Also it must be noted that in the temperate climatic zone there are no natural sources of cineolic oils.

The research was based on the herb, callus, suspension and organ cultures of *C. incana*, *C. mongolica* and *C. cladonensis*. Considering the reported lack of volatile oil production in unorganised callus cultures, this research focused on organ cultures. In order to check the possibility of volatile oil production in *in vitro* conditions, callus, suspension and shoot cultures were established on Knudson's medium modified with various growth regulators. Moreover, hairy root cultures of three *Caryopteris* species were grown too (transformation with *Agrobacterium rhizogenes*- strain ATCC 158344).

Chromatographic analysis of all investigated materials was carried out using gas chromatograph coupled with mass spectrometer. Resulting spectra were compared with the data from the library. The differences in the chemical composition of the volatile oil in the biomass from *in vitro* and *in vivo* cultures were studied and revealed that *in vitro* material is a very valuable source of volatile fraction rich in cineol, many terpenoids and sesquiterpenoids.

Essential Oil Variation of Sage (*Salvia officinalis* L.) Aerial Parts During its Phenological Cycle

M. Mohammadi Vala¹, P. Salehi^{1,2}, M. H. Mirjalili²

¹ Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran; e-mail:p-salehi@sbu.ac.ir

² Department of Agriculture, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran.

Abstract

Sage (*Salvia officinalis* L.; Lamiaceae) is well known as a common medicinal and aromatic plant widely used in food and herbal products (1). Here, variation in the quantity and quality of the essential oil from aerial parts of *Salvia officinalis* during its life cycle (vegetative, floral budding, flowering and fruiting set) is reported. The oils were obtained by hydrodistillation of air-dried samples. The yield of essential oil (w/w%) in different stages was in order of: floral budding (0.9%)> vegetative (0.7%)> flowering (0.5%)> fruiting set (0.3%). The essential oils were analyzed by GC and GC-MS. In total, 36, 41, 40 and 38 constituents were identified and quantified in the mentioned stages, respectively. Oxygenated monoterpenes were the main group of compounds in fruiting set stage (49.3%), vegetative stage (48.5%), flowering stage (47.7%) and floral budding stage (45.3%). 1,8-cineole as one of the major constituents of all samples was lower in the first stage and increased gradually in subsequent stages. In contrast, borneol and β -caryophyllene content were higher in the first stage and decreased gradually in subsequent stages.

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Volatile Constituents of *Lotus michauxianus* Ser

M. A. Khalilzadeh¹; J. Ghanaat²; M. Tajbakhsh²

¹ Department of Chemistry; I.A.University; Ghaemshahr campus, Ghaemshahr, Iran

² Department of Chemistry; Mazandaran University; Babolsar; Iran

Abstract

The genus *Lotus* (Fam. Papilionaceae) presents to species that are found wild all over Iran, one of which are endemic.[1,2] The purpose of the investigation was to analyse the chemical composition of the aerial parts of *Lotus michauxianus* which was collected from Haranj Area, Taleghan, north of Tehran, Iran, in June 2002. The literature survey shown that, there was no report about determination of the components in the essential oil of this plant. Traditional medical application of this plant is very important because of several effective ingredients, which has antibacterial properties. Another application of the essential oil of this plant is in perfumery industry. The essential oil of the plant was taken by hydro distillation and was investigated by means of GC and GC/MS instruments. Some of the important components have been identified by using of Kovats indices, fragmentation of the components from mass spectra and GC Chromatogram. About 97.9% (10 Components) of the oil of *Lotus michauxianus* was identified. The oil of *Lotus michauxianus* consisted of five monoterpene hydrocarbons (65.2%), four sesquiterpenes (29.6%) and one C₁₃ – compound (3.1%). The major component of this oil was limonene (43.4%), Z-β-Ocimene (18.9%) and α-eudesmol (15.9%) followed by germacrene D (8.9%).

References:

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2. V. Mozaffarian, *A dictionary of Iranian plant names*, Farhange Moaser publishers, Tehran, Iran (1996).

Volatile components of selected Japanese medicinal plants

M. Nishiki¹, E. Achleitner², G. Buchbauer², F. Nagashima¹ and Y. Asakawa¹

¹ Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514; Japan

² Institute of Pharmacy, University of Vienna, Austria: asakawa@ph.bunri-u.ac.jp

Abstract

In order to obtain the essential oils and crude extracts from medicinal plants and herbs the whole plants have been usually steam-distilled or extracted with organic solvents. Each part of the plants elaborate different type of components and the content of the focused constituents are often quite different. In this paper five Japanese medicinal and aromatic plants, *Alpinia japonica* (Zingiberaceae), *Solidago altissima* (Asteraceae), *Polygonum hydropiper*, (Polygonaceae), *Zanthoxylum piperitum* (Rutaceae) and *Cryptotaenia japonica* (Umbelliferae) were selected and the volatile components of each part of the above mentioned plants analyzed by GC/MS.

1) *Alpinia japonica* (Zingiberaceae).

A. japonica which possesses a strong sweet camphoraceous-like odor and a pungent and bitter taste, is used as an aromatic bitter to increase the appetite and to benefit the digestion. The predominant volatile components of both crude extracts of the leaves and rhizoma are fenchone (**1**), fenchyl alcohol and its acetate. These fenchane-type monoterpenoids play an important role in the characteristic strong aroma of both leaves and rhizome of this plant.

2) *Solidago altissima* (Asteraceae).

Because of an effective diuretic-antiseptic activity some *Solidago* species are beneficial in prevention and treatment of various types of bladder and kidney diseases. The plant material was collected at three different sites around Tokushima city at the end of the flowering periods. They are separated in flowers, leaves, stems and roots. Monoterpenes were in all parts of the plant. The highest amount (75.6%) was detected in the oil of roots. Limonene amounted even to 48%. Comparing sesquiterpenoids of the roots striking differences of the composition were found.

The sesquiterpenoids of the oil obtained from flowers, leaves and stems were dominated by germacrene D (**2**) accompanied by isogermacrene D (**3**), δ -elemene and β -elemene. By using a β -cyclodextrin containing capillary GC column the enantiomers could be separated. (-)- α -pinene, (+)- β -pinene, (+)- β -sabinene and (+)-limonene were predominant monoterpenes. On the other hand, germacrene-D was present as almost racemate.

3) *Polygonum hydropiper* (Polygonaceae).

Polygodial (**4**), responsible for the pungency of this plant, possesses strong antimicrobial, antifungal, insecticidal and mosquito repellent activities. Very high relative concentration of **4** in the leaves (62%) and seeds (21%) is remarkable. Further constituents found in both parts were *cis*- and *trans*- β -caryophyllene and its oxide nerolidol and drimenol. While *trans*- α -bergamotene, (*E*)- β -farnesene and (*Z*)- β -farnesene were characteristic components in *P. hydropiper* from Poland. Their isomers *trans*- β -bergamotene and (*E,E*)- α -farnesene were significant constituents of the Japanese species.

4) *Zanthoxylum piperitum* (Rutaceae).

The leaves and fruits emit a very strong pleasant aroma and are therefore used as a spice for grilled eel and soup. The mature seeds are employed in the treatment of stomach pain and aromatic stomachic and bitter tincture. α -Pinene was the main component in both leaf and stem, followed by limonene, *trans*- β -caryophyllene and 2-tridecanone. By comparing this data with those of *Z. piperitum* harvested in spring, some significant difference could be observed. Relative amount of α -pinene in *Z. piperitum*, in autumn was four times bigger than that of spring, whereas for limonene a slight degradation was noticed. Moreover, linalyl acetate, 4-terpinyl acetate and a high content of α -tocopherol were detected in the material of spring and missing in leaves.

5) *Cryptotaenia japonica* (Umbelliferae).

The whole plant has been used as antiphlogistic, antidote and for pneumonia and gonorrhoea. The young leaves and stems elaborate characteristic scent and has been used as spice for Japanese consomme soup. The major volatile constituents of the leaves, stems, flowers and feces of the butterfly's larva *Papilio zuthus*, which eat the leaves, are α -selinene, β -selinene and (*E*)- β -farnesene. Interestingly, this larva exhausted a large amount of fatty acid with feces.

Composition of the Essential Oil of *Anchusa iranica*

J. Ghanaat^{1,2}; M. A. Khalilzadeh¹; J. Raouf²

¹ Department of Chemistry; I.A.University; Ghaemshahr campus, Ghaemshahr, Iran

² Department of Chemistry; Mazandaran University; Babolsar; Iran

Abstract

One of the most famous species from Boraginaceae family is *Anchusa iranica* is known locally by the name 'Golgavzaban'. The genus *Anchusa* is represented in the flora of Iran by five species, of which *A.iranica* Rech.f. and *Esfand* is endemic plant [1,2]. In this research, the aerial parts of *A.iranica* was collected from Gadok (Province of Mazandaran), Iran, in June 2003, at full flowering stage. Hydro-distilled volatile oil from the aerial parts of *A.iranica* was analyzed by a combination of GC and GC/MS. Some of the important components have been identified by using of Kovats indices and fragmentation of the components from mass spectra and GC chromatogram. Twenty components were identified that approximately constitute more than 96% of the oil. The main constituents of the essential oil were α -eudesmol (24.1%), germacrene D (18.4%), spathulenol (12.2%) and α -Pinene (8.8%).

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Comparative Study of the Essential Oil of Three *Salvia* Species Grown Wild in Iran

S. Masoudi¹ and A. Rustaiyan²

¹ Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran, Iran;

² Department of Chemistry, Science & Research Campus, Islamic Azad University, Tehran, Iran

Abstract

Fifty-eight species of the genus *Salvia* (Lamiaceae) are found in Iran, seventeen of which are endemic. Several species of *Salvia* are used as medicinal, aromatic, and ornamental plants.

S. officinalis L. is one of the most widespread species and, since ancient times, has been used in the treatment of various disorders, such as tuberculosis, psoriasis and seborrheic eczema. It shows strong antibacterial and antifungal activities.

The rhizomes of *S. miltiorrhiza* Bunge. have been used widely to treat coronary heart diseases, particularly angina pectoris and myocardial infarction.

Water distilled essential oils from aerial parts of *S. rhytidea* Benth., *S. xanthocheila* Boiss.ex Benth. and *S. palaestina* Benth. were analyzed by GC and GC/MS.

Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices with those given in the literature and those authentic samples.

Thirty-three constituents, representing 93.8% of the total components in the oil of *S. rhytidea*, was characterized by β -phellandrene (22.7%) and sabinene (13.5 %) as the main compounds.

S. xanthocheila oil contained germacrene D (17.6%), caryophyllene oxide (15.5%), β -caryophyllene (14.8%) and α -copaene (14.0%) among the twenty constituents characterized, comprising 91.8% of the total components detected.

β -caryophyllene (38.4%) followed by germacrene D (11.2%) and spathulenol (10.5%) were the main constituents among the sixteen characterized, comprising 87.6% of the total components detected in the oil of *S. palaestina*.

As can be seen from the above information, in *S. rhytidea* oil monoterpenes (72.0%) predominated over sesquiterpenes 21.8%, while the two oils of *S. xanthocheila* and *S. palaestina*, consisted mainly of sesquiterpenes (91.4% and 74.7%, respectively).

Rosewood Leaves: New Essential Oil from Amazon

L. E. S. Barata, B. Patelli and K. F. Discola

Natural Products Laboratory, Institute of Chemistry, State University of Campinas (Unicamp), CP 6154, (13083-970), Campinas-SP, Brazil, lbarata@iqm.unicamp.br

Abstract

In the year 2002 Amazon lost ca. 25,000km² of forests, consumed by fire, pastures and wood cutting. About 625,000 km² had been destroyed in the previous four decades, corresponding to an area twice bigger than Italy. The essential oil of Rosewood (*Aniba rosaeodora* Aublet.) used in fine perfumes, comes from the annual falling of 4,000 trees of the Amazonian rainforest. Their trunk is cut down to small pieces (wood splinters) and steam-distilled (1% yield), leading up to 40 tons of essential oil/year¹, sold at US\$ 33/kg. Due to the predatory exploration, this species is in danger of extinction. Four days on a boat, or 10 hours walking into deep forest are necessary to arrive to the Rosewood lasting sources. Our studies² have shown that trimming leaves of Rosewood from young plantations (3-5 years), and then steam distillation (1% yield also), generates an essential oil equivalent to the one from their wood. These data have resulted in the plantation of 10,000 Rosewood trees in 30ha, alongside other species in devastated areas of the Amazon, resulting in vegetal recovers. The eventual production of 1,000 kg of essential oil from the leaves of these cultivations is due to generate an income of US\$ 50,000.00. Physical-chemical analyses of the oil from Rosewood leaves have shown properties slightly different to the oil from the wood, as shown by color, density, refractive index and optical rotation. Its chemical composition has shown a similar percentage of Linalool in the leaves (79%) as in the wood (81%). The chromatographic analysis (GC-MS) shows similarities between the two oils, with small, but sensible differences in the number of peaks and substances present in the oil from the leaves, showing a higher percentage of oxygenated sesquiterpenes, and a lower ratio of Linalool oxides. However, the sensorial analysis³ of the oil from the leaves shows only 2/3 of the olfactory fragrance when compared to the oil from the wood. Experiences to ameliorate aroma of oil from the leaves dealt with purification using silica, active charcoal and NaOH washings, improves significantly the fragrance of the oil when using alkaline solution. A fractional distillation with and without vacuum, has produced concentrated Linalool (up to 99%), sensorial and chemically similar to that distilled from the wood. The floral/rose/fruity aroma of this new essential oil from leaves could be directed to the fine perfumery industries, liberating the pressure of the destruction upon the remaining native Rosewood.

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GC-MS Analysis Of Volatile Oil From *Achillea filipendulina* Lam. And The Preliminary Biological Activities

A. Kowalczyk¹, J. Dąbrowska² and W. Cisowski¹

¹ Department of Pharmacognosy, Wrocław Medical University, Nankiera 1, 50-140 Wrocław, Poland: akow2@poczta.onet.pl

² Institute of Plant Biology, Wrocław University, Maxa Borna 9, 50-206 Wrocław, Poland

Abstract

The genus *Achillea* L. numbers over 100 species and is a polyploid complex of di-, tetra-, hexa- and octaploids. *Achillea filipendulina* belongs to the *Filipendulina* section and it is a diploid (2n=18) distributed in central Asia, Caucasus, Iran, Afghanistan and Turkey.

The examined material was collected during blooming by J.Dąbrowska in Wrocław (Poland) collection.. The herb of *A.filipendulina*, previously air-dried, was distilled with water in Deryng apparatus, according to the method of obtaining and measuring volatile oils in Polish Pharmacopoeia.

Chromatographic analysis was carried out using GS-MS chromatograph. The main identified compound of the analysed sample was 1,8-cineole (10.7%). Others substances were: boreol (8.5%), iso-asceridol (4.8%), camphor (2.2%), p-cymene (1.3%), terpinene-4-ol (1.5%), 2,3-dehydro-1,4-cineol (1.7%), bornyl acetate (1.8%).

The preliminary biological activities of water extracts from the herb of *A.filipendulina* were made by the observation of the ability of the germination of different plant's seeds on Petri dishes. In some cases the germination was completely inhibited or the growth of the roots was slower in comparison with the control test. Because of the short time and simple equipment, this method of the observation of the biological activities is very useful.

Changes in the qualitative and quantitative chemical composition during steam distillation in pilot plant scale of essential oils of *Achillea millefolium* L., *Salvia sclarea* L. and *Melissa officinalis* L.

S. Wagner, M. Mandl, H. Berghold and H. Böchzelt

Joanneum Research – Institute of Sustainable Techniques and Systems, Elisabethstraße 16, A – 8010 Graz, Austria

Abstract

Several data published in literature show that the quality of essential oils and the oil yield depend on genetic, environmental, climatic and soil conditions. Relative amounts of the compounds and the chemical composition of essential oils may also vary due to the applied distillation process itself and the duration of the distillation. Essential oils from the herbs *Achillea millefolium* L., *Salvia sclarea* L. and *Melissa officinalis* L. (two various sorts), all grown by organic farming in three habitats in the northeast of Styria (Austria), were analysed. The essential oils were obtained using a hundred litres distillation plant of the type Herba-tec.

During the distillation process samples were taken in order to investigate the changes of essential oil composition within a steam distillation process. The samples were subjected to gas chromatographic investigation (GC/MS and GC/FID). Progression and development of the qualitative constitution and the relative amounts of the most important components were examined. These are important factors for the pharmaceutical application of essential oils. The results provide the possibility to determine the optimised distillation time with the highest relative amount of the main compounds. In addition trends and correlations among these compounds will be shown.

Comprehensive Two-Dimensional Liquid Chromatography in the Study of Essential Oils

M. Lo Presti¹, R. Luppino¹, T. Kumm¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

Food products are very complex mixtures that contain many nutrients of organic and inorganic nature. In addition to natural constituents, food products may contain xenobiotic substances that can come from technological processes, agrochemical treatments or packaging materials.

Complex samples require analytical methods of an extremely high resolving power in order to provide reliable analysis of the sample components. Multidimensional chromatography is certainly valid for food analysis; but the transferring step can only involve a certain number of target fractions and so, the 2D advantage cannot be extended to the entire matrix. In recent years, this disadvantage has been fully overcome with the introduction of comprehensive multidimensional chromatography.

The comprehensive two dimensional HPLC system developed in this study is based on the use of a microbore silica column for the first dimension and an RP monolithic column for the second dimension. The interface was a ten-port two positions switching valve equipped with two loops. The use of a micro-bore column in the first dimension permits the injection of a small volume in the secondary column, so the problems of solvent incompatibility (NP-RP) can be avoided and the maximum injection volume in the second dimension to have re-concentration of the sample is not exceeded.

The use of a monolithic type column in the second dimension permits the performance of very fast analysis operating at higher flow rates without loss of resolution, due to a higher permeability and increased mass transfer properties in comparison to conventional particulate columns. Due to the brief reconditioning time necessary for monolithic columns, repetitive gradients can be carried out.

The system has been tested analysing the oxygen heterocyclic fraction of cold-pressed lemon and grapefruit oils, made up of coumarins and psoralens and polymethoxylated flavones. The oxygen heterocyclic components are the main parts of the non volatile fraction of cold-pressed citrus oils. The qualitative and quantitative composition of these components have an important role in the characterization of each citrus oil. The 2D space chromatogram derived from LCXLC analysis has a great potential for identification, because the contour plot position give characteristic patterns for specific classes of compounds. The UV spectra and the MS spectra supplied additional information for the characterization of the studied samples.

Comparison of the Volatile Oils of *Satureja Atropatana* Bunge. and *Satureja Mutica* Fisch. et C. A. Mey. From Iran

N. Ameri, A. Rustaiyan and A. Feizbakhsh

Department of Organic Chemistry and Medicinal Plants, Institute of Pure and Applied Science, Islamic Azad University, Central Tehran Branch, Tehran, Iran (e-mail: nazak_ameri@hotmail.com).

Abstract

To the genus *Satureja* (Lamiaceae, tribe Mentheae) belong about 200 species of herbs and shrubs often aromatic, widely diffused in Mediterranean area, Asia and boreal America. The flora of Iran comprises 12 species of *Satureja*, of which 8 are endemic. Some *Satureja* species are used as flavouring agents and for medical purposes, e.g. an infusion of the aerial parts of *S. boliviana* is used as a digestive, an anti-spasmodic and in the treatment of colds. An infusion of the aerial parts of *S. breviculix* is used as a digestive, a gastralgic, an anti-spasmodic and to help in parturition. *S. kitaibelii* is used to treat bronchitis in adults and children and skin, respiratory, digestive and Urinary inflammation. An infusion of *S. brownei* is used as a remedy for respiratory diseases and cough. The oil obtained by hydrodistillation of the aerial parts of *Satureja atropatana* Bunge., endemic in Iran, and *S. mutica* Fisch. et C. A. Mey. Was analyzed by GC/MS. Thirty seven compounds representing 99.3% of the oil of *S. atropatana* were identified, among them carvone (21.5%), menthol (18.1%), 1,8-cineol (13.1%), methyl chavicol (11.1%) and menthone (10.5%) being the major ones. The oil of *S. mutica* was characterized by higher amount of also menthol (37.4%), menthone (17.2%) and 1,8-cineol (9.3%) among the thirty nine components comprising 95.1% of the total oil detected. Both oils were richer in oxygenated monoterpenes than sesquiterpenes.

Compositional changes in commercial mandarin essential oil for aromatherapy

M. Sawamura¹, U-S. Son¹, N. Thi Minh Tu², N. Thi Lan Phi¹ and Y. Onishi¹

¹ Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan; sawamura@cc.kochi-u.ac.jp

² Institute of Biological and Food Technology, Hanoi University of Technology, 1 Dai Co Viet Street, Hai Ba Trung, Hanoi, Vietnam

Abstract

Compositional changes in commercial mandarin essential oil for aromatherapy were investigated under four different conditions for practical use over a one-year period. Sample S-1 was stored at 25°C with the cap open for 3 min everyday; sample S-2 was stored at 25°C with the cap open once a month for analysis; sample S-3 was stored at 5°C with the cap open for 3 min everyday; sample S-4 was stored at 5°C and was opened once a month for analysis. The oil samples were analyzed quantitatively over a 12-month period using methyl myristate as an internal standard by GC and GC-MS. Compositional changes occurred predominantly in the S-1 condition followed by the S-3 condition during the storage period, where monoterpene hydrocarbons decreased from 97.7% to 27.3% and from 97.7% to 63.1%, respectively. The main compound, limonene decreased remarkably in the S-1 and S-3 conditions, from 93.2% to 26.6% and from 93.2% to 61.1%, respectively. On the other hand, the changes of S-2 and S-4 were not significant. During a 12-month period, many compounds belonging to the alcohol, oxide and ketone groups increased in concentration due to oxidation of terpene hydrocarbons. No antioxidative compounds such as tocopherols and BHA were detected in the commercial oil.

Essential oil composition of *Poiretia latifolia*

C. Porto, C. Z. Stuker, L. V. Becker, I. I. Dalcol, A. F. Morel

Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria RS, Brazil

Abstract

Poiretia latifolia, locally called “erva de touro”, belongs to the Leguminosae, and it is a plant that grows in Southern Brazil. It has been used in folk medicine for the treatment of hemorrhoids and renal diseases. Leaves and flowers of *P. latifolia* were submitted to hydrodistillation, to yield 1.0% and 0.6%, respectively. The essential oils were analyzed by GC using a Varian 3800 Gas Chromatograph equipped with a capillary fused silica column (25 m x 0.25 mm) coated with SE-54. The GC conditions used were: carrier gas He (1 ml min⁻¹); on column injector 200°C; FID 280°C; column temperature 50°C to 250°C at 4°C/min. GC-MS analyses were performed on a HP 5973-6890 GC-MSD system operating in the EI mode at 70 eV, equipped with a HP-5 cross-linked capillary column (30 m x 0.25 mm). Column and the injector temperatures were as above. The identification of the components was based on comparison of their mass spectra with those of a NBS Libraries (Massada, 1995) and/or published by Adams (Adams, 1995). The leaves was bioactive against some Gram-positive and Gram-negative bacteria and two yeast.

Comparison of Chemical Constituent of Essential Oils in Fresh and Dried Strobiles of *Humulus lupulus* by GC-MS Analysis.

S. Ataie¹, M. Amanlou^{1,2}, M. Hadi Soleimani, H. Farsam¹

¹ Department of Medicinal chemistry, Faculty of Pharmacy Tehran University of Medical Science, Tehran, Iran, sevin_ataie@yahoo.com

² Pharmaceutical science Research Center, Tehran University, Tehran, Iran

Abstract

Hop (*Humulus Lupulus* L.) have been used world wide as a flavoring agent in beer industry and for medicinal purposes. The aroma and bitter taste of this plant can be change in different cultivated climate or methods of drying and processing. The main objective of this study was to compare the essential oils obtained from fresh and heat dried hop strobiles cultivated in Gorgan province, north of Iran.

Essential oils (EO) of female flowers of hop (*Humulus lupulus* L.) cultivated in Gorgan, Iran obtained by hydro-distillation and the main components was identified and assessed by GC-MS analysis, with subsequent statistical treatment. Percentage of the main constituents of EOs obtained from fresh and dried flowers were: β -caryophyllene (13.08, 2.67), β -myrcene (12.82, 0.03), alloaromadendrene (3.59, 1.53), β -farnesene (1.94, 0.74), α -limonene (1.77, 0.04), linalool (1.12, 0.03), β -pinene (0.78, 0.01), α -humulen (0.38, 0.18) and α -pinene (0.11, 0.01), respectively. The data shows that regular drying dramatically changes and decreases most of major components of EO. Due to high ratio of β -caryophyllene and alloaromadendrene in Essential oil of fresh flower compared with dried one, and the role of these two components in bitter taste in hop, fresh hop of Iran can be used for obtaining EO with predominant bitter characteristic.

Variability of the Essential Oil of *Thymus zygis* from South Spain

R. Pérez Sánchez¹, J.L. Ubera Jiménez¹ and C. G. Ramírez²

¹ Department of Plant Biology. University of Córdoba, Campus of Rabanales, 14071 – Córdoba, Spain

² Semillas Silvestres, S.L. c/ Aulaga, 24, 14012 – Córdoba, Spain

Abstract

Composition and variability of the essential oils of 21 populations of *Thymus zygis* from South Spain were investigated by GC-MS. Chemometric investigation of the infraspecific variability of the essential oils of these populations led to the differentiation of seven main chemotypes: thymol, carvacrol, linalool, terpinyl acetate, thymol/p-cymene/ γ -terpinene, eucalyptol/ β -myrcene/espátulenol and eucalyptol/terpineol. The phenolic compound timol was the most common constituent in the majority of the samples studied. The concentration of its precursors, p-cymene and γ -terpinene is significant. There is no chemotaxonomical differentiation between the two subspecies (*gracilis* and *sylvestris*) of *Thymus zygis* studied. All data were statistically processed by a Cluster analysis to establish the different groups/chemotypes within the individual essential oils. Principal Components Analysis (PCA) to check for partition among the identified compounds was used too. This multivariate analysis brings a new set of coordinates that make up the highest variability in the lowest number of these coordinates, as much to the individuals as to the compounds. All data have been graphically represented to interpret and deduce the relationship between individuals and compounds. Statistical analysis have supported us in the characterization of chemotypes.

Constituents of the volatile oils of aerial parts and roots of *Dracocephalum kotschyi* Boiss. from Iran

K. Javidnia^{1,3}, R. Miri^{1,3}, M. Kamalinejad², M. Khoshneviszadeh¹

¹ Dept. of Medicinal Chemistry, Faculty of Pharmacy, The Medical Sciences University of Shiraz, Shiraz, Iran, javidniak@sums.ac.ir

² Medicinal & Natural Products Chemistry Research Center, The Medical Sciences University of Shiraz, Shiraz, Iran, P. O. Box: 71345-1149

³ Department of Pharmacognosy, Faculty of Pharmacy, The Medical Sciences University of Shaheed Beheshti, Tehran, Iran

Abstract

The genus *Dracocephalum* belonging to the Lamiaceae family is represented by six species growing in Iran. *Dracocephalum Kotschyi* is distributed in many parts of Iran. The chemical composition of the essential oils obtained from aerial parts and roots of *Dracocephalum kotschyi* Boiss was investigated by GC and GC/MS. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra. The yield of the oils of the aerial parts and roots of the plant were 0.016% and 0.013% (w/w) respectively. GC and GC/MS analysis resulted in the identification of a total of 69 and 59 compounds from the aerial parts and roots, respectively. The main component of the oils of the aerial parts and roots of the plant was longibornyl acetate (11.2% and 57.6%), respectively.

Glandular trichomes and secreted essential oil in *Salvia wagneriana* Polak

A. Bisio¹, G. Romussi¹, C. Bicchi², P. Rubiolo², L. Maleci³

¹ Università di Genova, Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari

² Università di Torino, Dipartimento di Scienza e Tecnologia del Farmaco

³ Università di Firenze, Dipartimento di Biologia Vegetale

Abstract

Salvia wagneriana is a shrub of the subgenus Calosphace, growing in Central America, particularly in Mexico and Costa Rica, frequently cultivated in the gardens of the warm-temperate regions for his beautiful, red inflorescences.

The subject of this work is a study of the glandular trichomes present on the plant and of their type of secretion. The leaves of *Salvia wagneriana* bear numerous glandular trichomes both on abaxial and adaxial sides. They occur as peltate and capitate. The peltate trichomes consist of one basal epidermal cell, a stalk cell and a broad head of 4 secretory cells. Three types of capitate trichomes are present: capitate type I, consisting of one basal epidermal cell, one stalk cell and a round head of one single broad cell; capitate type II, consisting of one basal epidermal cell, one stalk cell and an elongated head of one cell; capitate type III, consisting of a basal epidermal cell, 1-6 stalk cells and a single rounded head cell. All these types of trichomes are present on the leaf, although type III is rare; however it is frequent on the flowers, particularly on the calix. The histochemical and ultrastructural observations evidence in peltate hairs a lipophylic secretion constituted mostly of essential oil and in capitate type I hairs a mainly hydrophilic secretion. The other two types of capitate hairs show a lipophylic secretion constituted mainly of essential oil. Flavonoids have been evidenced in all the hair types.

In the essential oil, obtained by hydrodistillation of the leaves, twenty nine components, have been identified by GC-MS: monoterpene hydrocarbons account for 0.3%, oxigenated monoterpenes 6.3%, sesquiterpene hydrocarbons 47.1%, oxigenated sesquiterpenes 2.9%, non-terpenoidic compounds 13.2%.

Chemical composition of *Ferula persica* Willd. essential oil from Iran

R. Miri^{1,3}, K. Javidnia^{1,3}, M. Kamalinejad² and N. Edraki¹

¹ Department of Medicinal Chemistry, Faculty of Pharmacy, The Medical Sciences University of Shiraz, Shiraz, Iran, P. O. Box: 71345-1149, mirir@sums.ac.ir

² Department of Pharmacognosy, Faculty of Pharmacy, The Medical Sciences University of Shaheed Beheshti, Tehran, Iran

³ Medicinal & Natural Products Chemistry Research Centre, The Medical Sciences University of Shiraz, Shiraz, Iran

Abstract

The genus *Ferula* belongs to the Umbelliferae family and comprises of fifty-three species grown wildly in Iran. *F. assa-foetida* L. is one of the most important natural oils, which is used as flavoring in many food products. The sesquiterpenes isolated from the rhizomes of *F. communis*, showed antibacterial activity. Some extracts of *F. gummosa* were useful for the alleviation of morphine withdrawal syndrome in mice and also the seed acetone extract of the plant showed anticonvulsant effect.

The essential oil of *F. gummosa* exhibit spasmolytic activity on ileum contraction.

Composition of the volatile oil of *Ferula persica* Willd. (Umbelliferae) was investigated by GC and GC-MS. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literatures and by comparison of their mass spectra with the Wiley library or with the published mass spectra. Sixty-one components representing 93.7% of the total oil were characterized. The major components of the oil were dill-apiole (57.3%) and elemicine (5.6%).

Antimicrobial activity and composition of the essential oil of *Equisetum arvense* L.

N. Radulović^{1,2}, G. Stojanović¹ and R. Palić¹

¹ University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia and Montenegro

² Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Yamashiro-cho, Tokushima 770-8514, Japan

Abstract

Equisetum arvense L. (Equisetaceae, subgenus *Equisetum*) - horsetail is well known and widespread pteridophyte distributed in the northern hemisphere. Its sterile stems are used as medicines in various countries, constituting «Equiseti herba» of European Pharmacopoeias.

The volatile constituents obtained by hydrodistillation of the sterile stems of *Equisetum arvense* L. (Equisetaceae) were investigated for the first time using GC/MS, and ¹³C-NMR. Twenty-five compounds were identified making up 99.64 % of the oil obtained in a yield of 0.01 %. Hexahydrofarnesyl acetone (18.34 %), *cis*-geranyl acetone (13.74 %), thymol (12.09 %) and *trans*-phytol (10.06 %) were the major constituents.

A disk diffusion method was used for the evaluation of the antimicrobial activity of this oil against a panel of microorganisms (bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enteritidis* fungi: *Aspergillus niger* and *Candida albicans*). The essential oil of *Equisetum arvense* L. was shown to possess a broad spectrum of a very strong antimicrobial activity against all tested strains. The diameters of growth inhibition zones ranged from 23 to 37 mm (including the diameter of the disk, 12.7 mm) with the highest inhibition zone values observed against Gram-negative *S. enteritidis* (35 mm) and *K. pneumoniae* (37 mm). The essential oil of *E. arvense* inherent to the composition showed strong antimicrobial activity *in vitro* and may despite the small yield but cosmopolitan spreading and availability of *E. arvense* ultimately be used therapeutically in this respect although further studies on a much larger number of strains of microorganisms are necessary.

Isoprenoid Biosynthesis in *Artemisia annua*: Functional Expression of α (+)-Germacrene A Synthase from a Glandular Trichome cDNA Library

C. Berte¹, F. Verstappen², J. Beekwilder² and H. Bouwmeester²

¹ Department of Plant Biology and Centre of Excellence CE BIOVEM, University of Turin, Viale P.A. Mattioli 25, 10125 Turin, Italy; cinzia.bertea@unito.it

² Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Abstract

Artemisia annua L. (Compositae) is the source of artemisinin, an endoperoxide sesquiterpene lactone that it is effective against multidrug-resistant strains of *Plasmodium*, the parasite responsible for malaria.

The aim of this project was to isolate and characterise genes involved in terpenoid biosynthesis in *A. annua*, by using glandular trichomes, structures highly specialised in the production of isoprenoids, as an enriched source for biochemical and molecular biological studies.

Intact gland secretory cells were incubated in the presence of radio-labelled farnesyl diphosphate (FPP) and products of conversion were analysed by radio-GC and GC-MS. A number of sesquiterpenes, including germacrene A were detected.

The random sequencing of ~ 900 clones of a plasmid cDNA library from glands, revealed that up to 6% of these expressed sequence tags (ESTs) encodes for enzymes related to isoprenoid biosynthesis. A full-length clone, encoding for a putative (+)-germacrene A synthase, was heterologously expressed in *Escherichia coli*. Bacterial cell lysates incubated according to the standard terpene synthase enzyme assay, were analysed by GC-MS and germacrene A was detected as the main reaction product.

To our knowledge, this work represents the first report about the use of *A. annua* glandular trichomes as a starting material for studying isoprenoid biosynthesis in this plant species.

Chemotypic differentiation among indigenous populations of wild spices in Israel: *Foeniculum vulgare* Mill. and *Micromeria fruticosa* (L.) Druce

J. Friedman¹, M. Gross¹, Y. Cohen¹, O. Barazani¹, A. Fait¹, N. Dudai², U. Ravid² and L. Almog²

¹Dept. of Plant Sciences, Tel Aviv University, Israel jfried@post.tau.ac.il

²Department of Medicinal Plants and Spices, Newe Yaar Research Center, Ramat Ishai, Israel.

Abstract

Foeniculum vulgare (Apiaceae), or bitter fennel, is an aromatic perennial widely spread in Israel. Indigenous populations occur in areas with annual rainfall from 900mm/year in the North, down to 100mm in the South. Due to the anise sweet taste of *t*-anethole and the bitterness of fenchone, leaves and particularly fruits are implied as a spice, mainly for seasoning fish. When crushed fruits were hexane extracted and analyzed by GC-MS chemical variability was observed, in some natural populations *t*-anethole was most dominant and was associated with low levels of estragole; Whereas, in other populations, where estragole was most dominant, small amounts of *t*-anethole were associated. When representatives from 11 natural populations were grown in the Newe Yaar Research Center under summer irrigation, representatives of three populations exhibited a phytochemical composition similar to that found in nature, suggesting occurrence of 2 different chemotypes: a *t*-anethole and an estragole chemotype. The chemical composition of the other 7 populations exhibited chemical plasticity. In a similar way we studied the chemical variations among indigenous populations of *Micromeria fruticosa* (Lamiaceae). In Israel this species is restricted to the Mediterranean phyto-geographical territory (400-800 mm/annual rainfall), despite pulegone is most common in its shoots, it is often used as a tea plant. In a phyto-chemical survey, hexane extracted shoots of *M. fruticosa*, from six populations showed that in 4 populations, pulegone was dominant (70-80 %), nevertheless, in 2 populations the amount of pulegone was considerably lower. Instead, amounts of piperitenone-oxide and piperitenone had increased; When plants from natural populations were propagated by cuttings and grown under irrigation in the Newe Yaar Research Center, the chemical composition observed in nature had also been manifested under cultivation. By that way, new pulegone-free (p-f) cultivars of *M. fruticosa* were selected.

Composition of the essential oil of *Solidago virgaurea* ssp. *virgaurea* L. from Austria

G. Radoias¹, A. Bosilcov¹, J. Novak²

¹ Erste Tiroler Latschenölbrennerei "Brüder Unterweger" GmbH, A-9911 Thal-Assling, Austria, labor.bu-oils@tirol.com

² Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Veterinärplatz 1; A-1210 Wien, Austria

Abstract

The genus *Solidago* (fam. *Asteraceae*), commonly known as "goldenrod", comprises about 120 species, most of them growing wild in North America. One of the most widespread species is *Solidago virgaurea* L., which is native to Europe and is used for medical purposes, especially in the treatment of urological diseases, due to its diuretic, spasmolytic and antiphlogistic properties.

The European *S. virgaurea* is subdivided into ssp. *virgaurea* and ssp. *minuta* (L.) Arcang., both subspecies being widely distributed. *S. virgaurea* ssp. *virgaurea* is a perennial mountain plant in Central and Southern Europe, growing wild at altitudes up to 1600 m or even more in the Southern Alps. In Austria, it can be found on the edge of the woods, in the valleys and on the slopes of the mountains, growing up to 1 m tall. The name "goldenrod" derives from the abundant yellow flowers that are used also for decorative purposes.

In this study, the essential oil from the aerial parts of *S. virgaurea* ssp. *virgaurea* growing wild was investigated. The plant material was collected during the flowering period ashore of the river Drau near Lienz (Eastern Tyrol) in August/September 2002 and August 2003. The essential oils were obtained by hydrodistillation of the fresh herb using a Clevenger type apparatus. The average oil yield was 0.5%.

The oils were analyzed by GC and coupled GC-MS, by dual channel analysis using two 60m fused silica capillary columns with stationary phases of different polarity (SPB-1 and Supelcowax-10). Identification of the compounds was performed using MS library search in combination with retention indices.

24 Compounds were identified, representing up to 96% of the total oil content. The main compounds were: α -pinene (22,0 ÷ 31,9%), α -phellandrene (1,4 ÷ 4,7%), limonene (12,5 ÷ 24,2%), germacrene-D (0,8 ÷ 20,0%) and β -turmerone (3,4 ÷ 31,9%).

The enantiomeric distribution of the main chiral monoterpene hydrocarbons was determined by GC separation using a 30 m fused silica capillary column coated with modified cyclodextrins as the stationary phase (β -Dex 325). The ratios were found to be as follows:

- (1R,5R)-(+)- α -pinene (3,7 %) : (1S,5S)-(-)- α -pinene (96,3%)
- (5S)-(+)- α -phellandrene(100%): (5R)-(-)- α -phellandrene(0%)
- (4R)-(+)-limonene(97%): (4S)-(-)-limonene(3%)

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California Lomatiums. Analysis of the Essential Oils of *Lomatium nevadense* var. *parishii* (J. Coulter & Rose) Jepson

P. S. Beauchamp, B. C. Dev, V. Dev

Department of Chemistry, California State Polytechnic University, Pomona, CA 91768, U.S.A.

Abstract

Many species of the genus *Lomatium*, commonly known as "desert parsley" or "desert biscuit" have been used by the natives of California for their needs for food, medicinals, hunting aid, as well as tribal rituals.

Following a summary of the composition of the essential oils of several species of this genus, the current results on the composition of *Lomatium nevadense* var. *parishii* will be presented.

Essential Oil Composition of *Nepeta involucrata* (Bunge) Bornm. from Iran

L. Allahyari,¹ A. Sonboli,² P. Salehi¹

¹ Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, P. O. Box 19835-389, Evin, Tehran, Iran, e-mail: p-salehi@sbu.ac.ir

² Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box-19835-389, Evin, Tehran, Iran, e-mail: a-sonboli@sbu.ir

Abstract

The genus *Nepeta* L. includes more than 250 species distributed in Asia, Europe and some areas of Africa. In Flora of Iran this genus represented by 67 species.¹ The chemical composition of the essential oil obtained by hydrodistillation from the aerial flowering parts of *N. involucrata* (Bunge) Bornm. was analyzed by GC and GC-MS.² Fifty constituents accounting for 98.2% of the total oil were identified. 1,8-Cineol(23.1%), germacrene-D (15.1%) and β -pinene (12.2%) were the predominant compounds followed by sabinene (6.7%) and α -pinene (4.9%).

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Chemical composition of the essential oil of *Juniperus phoenicea* from Algeria

T. Dob^{1,2}, D. Dahmane¹, T. Berramdane¹ and C. Chelguom²

¹ Laboratoire de molécules bio-actives et valorisation de la biomasse, Ecole Normale Supérieure, Kouba Alger, Tel 21321297511, Fax 21321282067, E-Mail t_dob2010@yahoo.co.uk

² Laboratoire de chromatographie, Faculté de chimie, U.S.T.H.B

Abstract

The volatile oil of *Juniperus phoenicea* from Algeria obtained by hydrodistillation, yielded 0.82% of essential oil, were analysed by GC and GC-MS. A total of 119 compounds identified from arial part representing 95,87% of the total oil.

The oil consisted mainly of monoterpene hydrocarbons (62,08%), whereas oxygen-containing monoterpenes were hardly present (10,50%). α -thujene (40,17%) was the main component of the oil analysed and α -phellanfrene (14,66%) the second most important monoterpene. The sesquiterpene fraction of the oil (12,10%) was characterized by oxygen-containing sesquiterpene of wich elmol (3,90%).

Key words : *Juniperus phoenicea*. L. Cupressaceae; essential oil, GC and GC-MS, α -thujene, α -phellanfrene.

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Essential oils composition of hops (*Humulus lupulus* L.) growing wild in north-east Lithuania

G. Bernotiene, O. Nivinskiene, R. Butkiene, D. Mockute

Institute of Chemistry, A.Goštauto 9, LT-2600 Vilnius, Lithuania, Fax: 370-2 617018, e-mail: gbernotiene@yahoo.com

Abstract

Wild hop (*Humulus lupulus* L.) cones were collected in five localities of north-east Lithuania. Essential oils were produced by hydrodistillation and analysed using GC and GC / MS. Different mixtures of compounds were found in volatile oils. Only two samples contained the same major constituents α -humulene (17.1-33.4 %), β -bisabolol (13.1-15.0 %), β -caryophyllene (6.2-9.8 %) and myrcene (6.1-6.7 %). The main compounds of the other three essential oils were different. One sample of the oil included α -humulene (33.2 %), β -caryophyllene (14.5 %), α -zingiberene (5.6 %) and myrcene (5.1 %), the second sample contained α -humulene (14.2 %), myrcene (10.5 %), β -farnesene (9.5 %) and β -bisabolol (9.6 %) and the third one – γ -elemene (14.0 %), myrcene (8.5 %), β -farnesene (7.8 %) and α -humulene (6.6 %) as major constituents. The largest part (47.8-73.5 %) of the essential oils comprised sesquiterpene hydrocarbons. Three hop essential oils included 47.2-59.8 % of monocyclic sesquiterpenoids and other two samples contained only 28.6-35.4 % of these compounds. The constituents with five carbon skeletons humulane, bisabolane, caryophyllane, farnesane and elemene made up 57.6-70.8 % in the above three oils and 42.7-53.6 % in the other two samples. Ninety eight identified constituents made up 86.1-98.9 % of the essential oils.

Essential oil composition and glandular trichomes of *Marrubium vulgare* L. grown wild in Algeria

R. Belhatab¹, L. Larous¹, A. C. Figueiredo², P. A. G. Santos², M. M. Costa², J. G. Barroso² and L.G. Pedro²

¹ Department of Biology, Faculty of Sciences, Ferhat Abbas University, 19000 Setif, Algeria, rbelhat@yahoo.fr

² Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCL, C2, Campo Grande, 1749-016 Lisbon, Portugal, acsf@fc.ul.pt

Abstract

Marrubium vulgare L., commonly known as horehound or hoarhound, is native of Europe, western Asia and North Africa. In Algeria it is used in folk medicine to cure several diseases of the digestive tract such as diarrhoea, as well as diabetes, rheumatism, cold and respiratory pains.

The SEM study of the aerial parts of *M. vulgare* shows a dense woolly indumentum of stellate, nonglandular, point-shaped trichomes that completely hide both the adaxial and abaxial epidermal leaf, as well as the stem and flower surfaces bearing the glandular trichomes. Peltate- and two types of capitate trichomes constitute the morphological distinct glandular trichomes which are spread over the vegetative and reproductive organs. *Marrubium* indumentum seems not only to have a protective function by increasing the reflectiveness of the leaves and lowering the leaf surface temperature, but also to contribute to the dispersal strategies.

The essential oils of *M. vulgare* were obtained by hydrodistillation and distillation-extraction from the aerial parts collected during the flowering (F) and vegetative phases (V), and analysed by GC and GC-MS. The essential oils were obtained in a yield $\leq 0.05\%$ (v/w). Eugenol was the main component in the F oil (50%) but attained only 16% in V oil. β -Bisabolene was the dominant component of the V oil (29%) and second main component in F oil (11%). Major differences were found between *M. vulgare* essential oil composition here studied and those formerly reported since in no previous study was the phenylpropanoid fraction dominant.

High-speed GC Analysis with a 50 μm ID Micro-bore Column: Theory, Practical Aspects and Application on a Complex Essential Oil Sample

P. Q. Tranchida¹, A. Casilli¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

It is well known that conventional GC methods produce effective results but have one substantial limitation: the cost in time. In fact, satisfactory separations concerning moderately to highly complex matrices such as essential oils, can take an hour or more. This factor becomes particularly important for those laboratories where a great number of analyses are carried out and/or where there is a need for quick results. The growing necessity, over the years, for fast GC techniques has seen the application of several different theories with the general aim of decreasing analysis time while contemporaneously maintaining acceptable efficiency for a given separation. The most successful instrumental tool in this direction has been the micro-bore capillary column. In comparison to traditional columns, they are characterized by a higher resolving power and thus are capable of maintaining the same efficiency as these, while working at a higher speed. The increase in solute velocity is obtained through the application of high inlet pressures and fast temperature program rates. These and other instrumental requirements have seriously delayed the use of fast techniques because of the lack of adequate equipment.

Almost all applications described in the literature have been achieved with 10 m x 0.1 mm ID x 0.1 μm columns. The latter are characterized, approximately, by the same resolving power as a 25 m x 0.25 mm ID x 0.25 μm column (100000 theoretical plates). The amount of research dedicated to 5 m x 0.05 mm ID x 0.05 μm capillaries (approx. 100000 theoretical plates) is very limited. This is due to the fact that these columns have become available only recently, as the coating of such a thin stationary phase presents a series of technical difficulties. Furthermore, the operational conditions required are obviously more extreme in respect to 100 μm ID columns.

This present research is based on the minimization of GC analysis times through the use of a 5 m x 0.05 mm ID x 0.05 μm (film thickness) column. Experimental minimum plate height (H_{min}) and optimum linear velocity values were derived from standard compound applications, under various analytical conditions, and then related to classical chromatographic theory. Deviations from the latter are measured and discussed. Practical aspects linked to the use of such capillaries, such as column sample capacity and detector acquisition rates, are also considered. Furthermore, what can be considered a very fast method was applied to the separation of a complex essential oil sample.

Research on Essential Oils of 32 *Citrus Limon* L. Burm. F. Clones

G.Arcoleo¹, V. Marsolo¹, F. Badalamenti³, L. La Scala³, M.A. Germanà²

¹ Dipartimento di Ingegneria e Tecnologie Agro-Forestali. Facoltà di Agraria. Viale delle Scienze, 11. 90128. Palermo, Italy.

² Dipartimento di Colture Arboree. Facoltà di Agraria. Viale delle Scienze, 11. 90128. Palermo, Italy agermana@unipa.it

³ Agrumaria Corleone s.p.a. v.s. Corleone, 12. Palermo, Italy.

Abstract

An important aspect in the essential oil industry is the enhancement of the quality of the oil. The oil quality level depends not only on the processing system employed, but also on the raw material, and on the genotype.

In Italy, although the main lemon cultivars are Femminello, Monachello, Interdonato and Lunario, it is possible to find many clones selected in the different areas of cultivation. This paper refers to a part of lemon germplasm collected in Italy and grown in an experimental orchard in Lascari (Palermo, Italy, 38°N).

This research has been carried out to characterize many Italian lemon clones in order to indicate the best genotypes from the industrial point of view. Particularly, the contents of the peel essential oil obtained by manual extraction as well as the oil quality in fruit of different genotypes have been determined.

Among the best clones, the differences in the quality and quantity of the essential oils due to the different stages of ripening have been investigated.

The morphological parameters of the fruits were also determined to better identify the specific features of the observed material.

Antimicrobial Activity of Essential Oils of the Selected *Stachys* L. Species

G. Stojanović¹, N. Ristić², R. Palić¹, J. Lazarević¹

¹ Department of Chemistry, Faculty of Natural Sciences and Mathematics, Višegradaska 33, 18 000 Niš, Serbia

² Department of Chemistry, Faculty of Natural Sciences and Mathematics, Kosovska Mitrovica, Serbia

Abstract

The antimicrobial activity of essential oils of three Balkan endemic species: *Stachys iva*, *Stachys plumosa* and *Stachys scardica* was determined, as for the *Stachys germanica*, European widely distributed specie.

Essential oils were obtained from the aerial parts of plants by hydrodistillation using Clevenger type apparatus. The antimicrobial investigations were performed using the disc diffusion technique placing 50 µL of the diluted oil (1: 10) on the disc (12.7 mm in diameter). Disc moistened with absolute ethanol was used as the control.

The **results** are given in Table.

Table. Antimicrobial activity of essential oils of selected *Stachys* L species (the width of the growth inhibition zones in mm)

Microorganisms	Selected <i>Stachys</i> L. species			
	<i>St. germanica</i>	<i>St. iva</i>	<i>St. plumosa</i>	<i>St. scardica</i>
<i>Pseudomonas aeruginosa</i>	5.3	8.3	7.3	12.3
<i>Escherichia coli</i>	7.3	5.3	4.3	17.3
<i>Staphylococcus aureus</i>	/	5.3	6.3	18.3
<i>Salmonella enteritidis</i>	3.3	7.3	5.3	19.3
<i>Klebsiella pneumoniae</i>	1.3	3.3	0.3	1.3
<i>Candida albicans</i>	9.3	6.3	3.3	4.3
<i>Aspergillus niger</i>	/	5.3	8.3	5.3

It is worthwhile noting that *St. scardica* oil showed very strong activity towards *S. enteritidis*, *S. aureus* and *E. coli*.

Effects of some essential oils on the germination and the emergency of seeds

M. Macchia, L. Ceccarini and L. Andolfi

Dipartimento di Agronomia e Gestione dell'Agroecosistema, University of Pisa, via S. Michele degli Scalzi, 2, 56142 Pisa, Italy; macchia@agr.unipi.it

Abstract

The use in agriculture of biological products is increasing and many studies have been completed in the last years to verify the possibility to use essential oils as insecticides, fungicides and herbicides. The aim of the present research was to evaluate the effects of three essential oils (Cinnamon, Oregano and Sandal oil) on the germination and the emergency of seeds of some weeds (*Rumex crispus* L., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Datura stramonium* L) and to assess their eventual phytotoxicity on of three crops (maize, soybean and durum wheat), operating in growth chamber. The results evidence the activity of essential oils, on the emergence of weed species, while regarding the cultivated species only in maize light phenomena of phytotoxicity (increase of the mean time of emergency) have been observed. In particular the cinnamon oil show the best activity against all weed species, excluding the seeds of rumex whose germination does not seem compromised by using essential oils. The problems linked to the use of essential oils are however various. It will be necessary to ascertain if these oils can influence negatively on several aspects of the soil fertility and which are their times and products of degradation.

Chemical Composition of the Essential Oils from Roots of Two *Leuzea* spp.

J. Havlik¹, D. Janovska¹, S. Vasickova², I. Valterova² and L. Kokoska¹

¹ Czech University of Agriculture Prague, Kamycka 129, 165 21, Prague 6-Suchdol, Czech Republic; E-mail address: havlik@itsz.czu.cz

² Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10, Prague 6, Czech Republic

Abstract

Leuzea carthamoides Willd DC. and *L. centauroides* (L.) Holub (Asteraceae) are perennial herbaceous plants occurring in southern Siberia and Pyrenees, respectively.

Roots and rhizomes of *L. carthamoides* were traditionally used in folk medicine in cases of overstrain and common weakness. The drug has been shown to possess a stimulatory and tonic effect [1]. Main groups of previously isolated components are ecdysteroids, flavonoids, sesquiterpene lactones and polyacetylenes [2]. In *L. centauroides* long chain aldehydes, fatty acids and esters were identified in root and aerial part extracts [3].

The steam distillation of fresh roots of *L. carthamoides* and *L. centauroides* using a Clevenger-type apparatus yielded 0.43 % and 0.24 % (w/w) of essential oils, respectively. Analyses of both oils by GC-MS and GC-FTIR showed a predominant content of unsaturated aliphatic hydrocarbons and sesquiterpene hydrocarbons.

Acknowledgement

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***Stachys milanii* Essential Oil - Chemical Composition and Antimicrobial Activity**

R. Palić¹, J. Lazarević¹, V. Randelović², G. Stojanović¹

¹ Department of Chemistry, Faculty of Natural Sciences and Mathematics, Višegradska 33, 18 000 Niš, Serbia; stgocaus@yahoo.com

² Department of Biology, Faculty of Natural Sciences and Mathematics, Višegradska 33, 18 000 Niš, Serbia

Abstract

The chemical composition and antimicrobial activity of the aerial parts hydrodistilled essential oil of *Stachys milanii* Petrović, the Balkan endemic, were examined for the first time.

The ionic chromatogram of the *St. milanii* oil indicated 29 peaks, 14 of which (92.2 % of the total oil) were identified. The major component of the oil was borneol (49.5 %) followed by terpinen-4-ol (13.2 %), camphor (7.1 %), myrtenol (5.0 %), α -terpineol (4.6 %) linalool (3.2 %) and 1-octen-3-ol (2.4%).

The antimicrobial assay was performed in three replicates using the agar diffusion technique against the *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, and the fungi *Aspergillus niger*. The oil dilutions (1:10 and 1:30) were tested at the dose of 50 μ L on 12.7 mm in diameter disks. Ethanol (99.8 %) moistened disk was used as a control.

The *St. milanii* oil in dilution 1:10 had no bactericidal activity against the *E. coli* and the fungi *A. niger*. The isolated oil showed minor bactericidal activity against the following microorganisms: *K. pneumoniae*, *P. aeruginosa*, *S. enteritidis* and *S. aureus*. In dilution 1:30 there was no bactericidal activity against the tested microorganisms what so ever. Zones that represented partial inhibition (bacteriostatic activity) of microbial growth were wider in dilution 1:30 (for the following microorganisms: *K. pneumoniae*, *E. coli*, *S. aureus*) compared to 1:10 dilution. The isolated oil had the strongest effect against *S. enteritidis*.

Iranian Rose Oil

A. Massoudi

Department of R&D, KAF Co. PO Box 15815-3478, Tehran, Iran, nasser_massoudi@yahoo.com

Abstract

Rosa damascena Mill. is known for best aroma and fragrance in the perfumery world.

This plant is native to the orient, the birth of the cultivated rose is believed to be Persia and is now mainly cultivated in Bulgaria, Turkey and France.

In Iran the *R.damascena* is cultivated in different regions especially around Kashan city in Isfahan province. Annually a lot of Rose oil and Rose water is produced with traditional and modern methods.

Iranian rose oil was obtained by hydro distillation method and the main components were identified by GC. The main components were: Citronellol 58.1%, Nerol 5%, Geraniol 9.34%, Phenylethylalcohol 2%, Heptadecane 0.87%, Nonadecane 5.8%, Heneicosane 1.8%.

The main components of Iranian Rose oil is compared with Bulgarian and Turkish Rose oils.

Root essential oils from *Artemisia absinthium* and *Artemisia vulgaris* growing wild in Serbia

N. Radulović^{1,2}, P. Rašić¹, Ž. Bošković¹ and G. Stojanović¹

¹ University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, 18000 Niš, Serbia and Montenegro

² Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Yamashiro-cho, Tokushima 770-8514, Japan

Abstract

The genus *Artemisia* is one of the largest of the Asteraceae family, consisting more than 800 species that are widespread all over the world. In this genus, many species, and among them especially *A. absinthium* and *A. vulgaris*, have been used since ancient times as folk remedies and credited with a long list of medical uses, including antimalarial, antiviral, antitumor, spasmolytic, and others.

The volatile oil distilled from the dried aerial parts of *A. absinthium* and *A. vulgaris* has been studied extensively, but little work has yet been done on the volatile secondary metabolites in the root system of these species. To the best of our knowledge this is the first report of the composition of the essential oils extracted by hydrodistillation from the dried roots of *A. absinthium* and *A. vulgaris* collected in Serbia.

Both oils, obtained in the following yields: 0.22 % *A. absinthium* and 0.04 % *A. vulgaris* (w/w of dried roots), were yellow in color and highly fragrant in smell. Sixteen (*A. vulgaris*) and thirty-five (*A. absinthium*) components were identified by GC and GC/MS, representing 97.66 % and 87.28 % of the oils.

The root essential oil of *A. absinthium* was dominated by the geranyl (17.94 %), neryl (16.24 %) and linalyl (22.39 %) esters of acetic, 2-methylpropanoic, 2-methylbutanoic, 3-methylbutanoic and pentanoic acid. However, the major component α -fenchene (19.18 %) belonged to the monoterpene fraction of the oil (37.35 %) that also contained large amounts of *p*-cymene (9.86 %) and α -phellandrene (4.04 %).

The chemical composition of the root oil of *A. vulgaris* was characterized by the sesquiterpenoids (43.17 % of which about 2/3 were hydrocarbons), with β -eudesmol (15.18 %) as the greatest contributor. However, similar to the *A. absinthium* root oil, the major component of the oil was an ester linalyl 3-methylbutanoate (20.09 %). Also the oil contained significant amounts of esters of borneol with acetic (7.57 %), propanoic (1.19 %) and 3-methylbutanoic (12.68 %) acid.

Sedative and Memory Enhancing Effects of the Essential Oil Fragrance from *Nardostachys jatamansi* After Inhalation and Oral Administration

B-S. Koo¹, S-I. Lee², and D-U. Lee³

¹ Department of Oriental Neuropsychiatry, College of Oriental Medicine, Dongguk University, 135-010 Seoul, Korea

² School of Life and Health, Sorabol College, 780-711 Gyeongju, Korea

³ Department of Biotechnology, College of Natural Science, Dongguk University, 780-714 Gyeongju, Korea; dulee@dongguk.ac.kr

Abstract

The essential oil fragrance of *Nardostachys jatamansi* was investigated after inhalation and/or oral administration with different concentration for its analgesic effects, anticonvulsant action, hypnotic effect, inhibitory activity of monoamine oxidase, and memory enhancement. The components of the essential oil were determined by GC/MS analysis. The results showed that the fragrance oil increased the analgesic effect after 12 hour inhalation by 25.8% against the control in the phenylquinone-induced Writhing test and increased the effect dose-dependently after oral administration, showing better activity than inhalation. Its anticonvulsant action was increased by 12 hour inhalation to reveal 28.9% delay of onset time and 39.5% reduction of duration, of which effect was weaker than that of oral administration. The fragrance oil inhalation for 6 or 12 hour lengthened the pentobarbital-induced sleeping time by ca. 26%, this effect was further increased by oral administration. The learning and memory enhancement of the fragrance oil inhalation was greater than that of oral administration, exhibiting 65.2% increase against control group. The fragrance oil inhibited monoamine oxidase activity in a dose-dependent manner by 50.6% at a dose of 5.0mg/ml. The GC/MS measurement showed that the fragrance contains 13 kinds of the essential oil components, among them, calarene was a main component with 36.4% content

Application of HPLC-MS with a C-30 Reversed Phase Column for the Separation and Identification of Carotenoid Esters in Citrus Essential Oils.

D. Giuffrida, L. La Torre, M. Stelitano, T. M. Pellicanò, and Giacomo Dugo.

Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy.

Abstract

Liquid chromatography-atmospheric pressure chemical ionization mass spectrometry [LC-(APCI)MS] was used to identify carotenoid esters in mandarin (*Citrus reticulata*) essential oil. A gradient of methanol and methyl-*tert*-butyl ether on a C-30 reversed phase column was employed. The polymeric C-30 stationary phase has demonstrated superior resolution for carotenoid separation in comparison to traditional C-18 columns. Alkaline saponification is usually performed before carotenoid extract analysis by HPLC, thus no information concerning carotenoid esters in natural samples can be obtained. Because carotenoid esters are known to be more stable than free carotenoids, we determined for the first time the main native carotenoid esters present in the non-volatile residue of mandarin essential oil, thus providing a carotenoid ester profile characteristic for this oil.

Inhibitory Effects of the Essential Oil from SuHeXiang Wan on the Central Nervous System after Inhalation

D-U. Lee¹, B-S. Koo², and S-I. Lee³

¹ Department of Biotechnology, College of Natural Science, Dongguk University, 780-714 Gyeongju, Korea; dulee@dongguk.ac.kr

² Department of Oriental Neuropsychiatry, College of Oriental Medicine, Dongguk University, 135-010 Seoul, Korea

³ School of Life and Health, Sorabol College, 780-711 Gyeongju, Korea

Abstract

The present study was performed to evaluate the central nervous system inhibitory effects of the essential oil from SuHeXiang Wan (Storax pill), a prescription usually used for treating epilepsy in traditional Chinese medicine, on fragrance inhalation (aroma therapy). Preinhalation of the fragrance oil markedly delayed the appearance of pentylenetetrazole-induced convulsion, but showed weak activities on picrotoxin- and strychnine-induced convulsions, which implies this drug may inhibit the convulsion by GABAergic neuromodulation.

This essential oil inhibited the binding of [³H]Ro15-1788, a selective antagonist for the benzodiazepine receptor and also the binding of [³H]flunitrazepam, a selective agonist for the receptor, in the presence of GABA and NaCl, showing a positive GABA shift, which suggested the strong possibility of the agonistic activity of the essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation inhibited the activity of γ -aminobutyric acid (GABA) transaminase as the inhalation period was lengthened. The GABA level was significantly increased and glutamate content was significantly decreased in mouse brain by preinhalation of the essential oil. The above results suggest that the anticonvulsive effect of this essential oil can also originate from the enhancement of GABA level in the mouse brain, because convulsion depends partially on GABA concentration which can be properly preserved by inhibiting GABA transaminase. Fragrance inhalation progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened and inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed; this also supported the above results, confirming the inhibitory effects of the essential oil of SuHeXiang Wan on the CNS *via* the GABAergic system.

Chemical Characteristics and Termiticidal Activity of Essential Oils from Root of Bogwood and Fresh Wood of *Cryptomeria japonica* D. Don

H. Narita¹, K. Hori¹, M. Yatagai¹ and T. Ohira²

¹ Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

² Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

Abstract

The essential oils from root of bogwood of sugi (*Cryptomeria japonica* D. Don), approximately 800 years buried in soil, was analyzed by GC/MS. *C. japonica*, Japanese native conifer tree, is one of the most commercially important trees as lumber and their board is used to make a barrel, which keeps brewed Japanese rice wine, sake, and add pleasant flavor to sake. The essential oils of 2 fresh wood of *C. japonica* were used as references. Large amounts of sesquiterpenes and small amounts of diterpenes were identified in each essential oil. The major identified components of the bogwood oils were *cis*-calamenene (4.6-10.0%), T-cadinol (2.6-9.5%), δ -cadinene (5.7-8.7%), cadalene (2.6-7.7%), and 1-*epi*-cubenol (3.1-7.5%), whereas those of the fresh wood oils were δ -cadinene (10.8-25.87%), 1-*epi*-cubenol (14.2-24.7%), cubenol (7.5-12.5%) and β -eudesmol (3.5-11.0%). T-Cadinol, cadalene, dihydroeudesmol, epizonarene, caryophyllenyl alcohol and amorphane-A were identified only in the bogwood oils. These results indicate that the essential oils in *C. japonica* had been gradually transformed to other terpenoids in a longer period of time. The termiticidal activities against Formosan subterranean termites, *Coptotermes formosanus* Shiraki, of the essential oils were examined by filter paper tests. The bogwood oils showed a little lower mortality than the fresh wood oils.

Analysis of Enantiomeric Ratios of Aroma Components in Flowers Using an Efficient GLC System with a Mixed Chiral Stationary Phase

M. Ishikawa¹, S. Tamogami¹, K-i. Awano¹, M. Amaike¹, Y. Takagi¹ and T. Kitahara²

¹ Technical Research Center, T. Hasegawa Co. Ltd., 335 Kariyado, Nakahara-ku, Kawasaki 211-0022, Japan

² Department of Applied Biological Chemistry, Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Abstract

Various types of GLC columns coated with cyclodextrin derivatives have been developed in recent years and applied to analyze the direct separation of optical isomers. However, all such columns have some limitations as to the capability of separating certain components, due to the particular characteristics of each chiral stationary phase.

In order to overcome these limitations of the methodology for the separation of enantiomers in GLC analysis, we attempted a novel approach to the preparation of a chiral stationary phase by mixing two or more those phases. As a result, a remarkable column named CHIRAMIXTM, coated with a mixture of two chiral stationary phases, heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)- β -cyclodextrin [2,6-Me-3-Pe- β -CD] and octakis-(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)- γ -cyclodextrin [2,6-Me-3-TFAC- γ -CD] has been successfully developed and proved to be greatly superior to those with single chiral phase for separating enantiomers of compounds having various functional groups, such as monoterpene hydrocarbons, oxygenated monoterpenes, γ -lactones and δ -lactones¹⁾.

Further applications for the analysis of enantiomeric ratios of aroma components in natural essential oils of several flowers, gardenia, osmanthus, etc, were carried out, again proving that most optical isomers of aroma components were easily separated using the CHIRAMIXTM column²⁾.

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Antibacterial Activity and Essential Oil Composition of *Grammosciadium platycarpum* Boiss. from Iran

A. Sonboli, M. Yousefzadi and M. Reza Kanani

Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, Po Box: 19835-389, Evin, Tehran, Iran

Abstract

The antibacterial activity of the essential oils has been recognized for a long time. There is an popularity and scientific interest to screen essential oils of plants in all over of the world. In the present study, the chemical composition and antibacterial activity of the essential oil (yeild: 1.3 %) obtained from the aerial parts of *Grammosciadium platycarpum* at fruiting stage were evaluated. The obtained oil was analyzed by GC and GC-MS. Sevethen compounds representing 98.8 % of total oil was identified. Two main components, Linalool (79%) and Limonene (10 %) comprised 89% of the oil. The antibacterial activity of the oil was evaluated against seven Gram (+) or (-) bacteria using disc diffusion method. It was found that the oil at 15 µl concentration exhibited gross inhibitory activity against tested bacteria, in which *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. aureus* and *Escherichia coli* were more sensitive strains.

Composition of the essential oils from leaves and cones of *Pinus* species

K. Kurose¹, D. Okamura¹, K. Hori¹ and M. Yatagai¹

¹ Department of Global Agricultural Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657 JAPAN; aa27128@mail.ecc.u-tokyo.ac.jp

Abstract

The essential oils from leaf and cone of *Pinus* species were investigated by GC/MS. The yield of the oils was 0.08-2.33 (w/w).

α -Pinene (0.2-51.8%) and β -pinene (0.1-38.2%) were contained in the leaf and cone oils of all *Pinus* species tested in this report.

The monoterpene fractions of *Pinus* leaf oils were comprised of pinanes and menthanes mainly. The amount of pinanes was more than that of menthanes in the leaf oils of *Pinus* species (except *P. petula*). One characteristic was that leaf of *P. pumila* contained caranes as a major structure, whereas that of *P. parvifolia* contained camphanes as a major structure.

As for sesquiterpenes in the leaf oils, caryophyllane and/or the cadinane group were the major structure types. Leaf oils of *P. merkusii*, *P. petula* and *P. rudis* contained larger amounts of caryophyllanes than those of the cadinane group. The sesquiterpenes of these three species were mainly caryophyllanes, while in the sesquiterpene fraction of other *Pinus* species the content of the cadinane group was larger than that of caryophyllanes.

Concerning cone oils, *P. rigida* and *P. taeda* cones mainly were composed of monoterpenes, while *P. densiflora* contained sesquiterpenes as main components.

Gas-Chromatographic Analysis of Citrus Essential Oils: Decoding of Complex Chromatograms by Fourier Analysis

M. C. Pietrogrande¹, L. Mondello² and Giovanni Dugo².

¹ Department of Chemistry, University of Ferrara, 44100 Ferrara; e-mail: mpc@unife.it

² Dipartimento Farmaco-chimico, University of Messina, Messina, Italia

Abstract

Natural matrices, as citrus essential oils, are multicomponent mixtures: they contain many components belonging to different chemical classes and present in a wide concentration range. Their Gas Chromatographic analysis yields a complex signal formed by a random series of peaks displaying a casual distribution of retention times and abundances. As a consequence, peak overlapping is present in a complex chromatogram, resulting in a significant loss of the analytical information contained in the chromatogram and hidden in it due its complexity. Therefore, the use of a signal processing procedure is practically mandatory to efficiently extract useful information from the raw chromatogram -- i.e. to decode the complex chromatogram to determine the number of components and the retention pattern [1].

In this work a chemometric approach based on the Fourier analysis is applied to complex chromatograms obtained from citrus essential oils: from the autocovariance function computed on the digitized chromatogram (EACF), the chromatographic parameters -- number of components, peak shape parameters, retention pattern -- can be estimated [2-5].

The procedure has been successfully applied to GC-MS chromatograms of the volatile fraction of Italian citrus essential oils: bergamot, lemon, mandarin, sweet and bitter orange [6]. The whole chromatogram as well as specific fractions of it -- corresponding to chemical classes of components, i.e., monoterpene hydrocarbons, sesquiterpene hydrocarbons and their oxygenated compounds.

Fundamental information on the mixture's chemical composition (number of components, m) and on the separation system performance (separation efficiency, σ) can be easily estimated [2].

Moreover, the ACF plot constitutes a simplified *fingerprinth* of the composition of the complex sample: it contains, in a simplified form, all the information on the retention pattern which can be related to the presence of specific molecular structures. The EACF plot shows some well-shaped positive peaks if peaks located at constant distances appear in the chromatogram. Information on quantitative composition of the samples can also be obtained, since the height of the EACF peaks is related to the abundance of repetitiveness in the chromatogram, i.e., the combination of the number of repeated peaks and their heights. The result is a high simplicity of the autocovariance function plot which makes it useful for the identification of a specific citrus oil. The method can also be applied for detecting adulteration of a essential oil by addition of cheaper vegetable oils.

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The Effect of Collection Region on the Essential Oil Yield and Composition of *Eucalyptus camaldulensis* Dehn. from Iran

M.M. Barazandeh¹, M.B.Rezaei¹ and H.Shaker²

¹ Research Institute of Forests and Rangelands, Tehran, Iran, P.O. Box: 13185-116
m_baraz@hotmail.com

² University of Shahid Beheshti, Faculty of Biology, Tehran, Iran

Abstract

In order to compare essential oils composition of *Eucalyptus camaldulensis* Dehn. from three locations, it was collected from Noor Abad (Fars province), Behshahr (Mazandaran province) and Tehran (Tehran province) in Feb. 2003 and hydrodistilled in the phytochemistry lab of Research Institute of Forests and Rangelands.

The oils were produced in light-yellow colors at the yields of 0.60%, 0.14% and 0.50%, respectively (based on fresh weights).

The oils were analyzed by CGC-FID and GC/MS .Thirty-four compounds were identified in each one among which 1,8-cineole (48.8%, 1.0% and 2.5%), p-cymene (16.3%, 35.5% and 23.9%) and spathulenol (3.7%, 2.7% and 15.4%) were the major constituents, respectively.

Essential Oils Composition of *Eucalyptus camaldulensis* Dehn. from Fars province of Iran during four seasons

M.M. Barazandeh, M.H. Asareh and M.B. Rezaei

Research Institute of Forests and Rangelands, Tehran, Iran, P.O. Box: 13185-116

Abstract

In order to compare essential oils composition of *Eucalyptus camaldulensis* Dehn. during four seasons, it was collected from Noor Abad (Fars province) in June, Sept. and Nov. 2003 and also in Feb. 2004 and hydrodistilled in the phytochemistry lab of Research Institute of Forests and Rangelands.

The oils were produced in light-yellow colors at the yields of 0.71%, 0.66%, 0.61% and 0.60% (based on dry leaves), respectively.

The oils were analyzed by CGC-FID and GC/MS. Thirty-four compounds were identified in each one among which 1,8-cineole (46.5%, 43.2%, 61.9%, 48.8%), γ -terpinene (18.4%, 8.9%, 12.3%, 2.3%) and p-cymene (7.6%, 16.2%, 0.8%, 16.

Essential Oil Composition of *Geranium rotundifolium* L. from Iran

M.M.Barazandeh

Research Institute of Forests and Rangelands, Tehran, Iran, P.O. Box: 13185-116
m_baraz@hotmail.com

Abstract

Geranium rotundifolium L. was collected from National Botanical Garden of Iran at full flowering stage in Jun 2003. Dry leaves of the plant were hydrodistilled and no significant amount of the oil was produced so it was separated from water by adding diethylether.

GC and GC/MS analyses of the oil revealed presence of 42 compounds among which α -terpinyl acetate (39.3%) and pulegone (27.7%) were the major constituents, respectively.

“Microwave Dry Distillation” of essential oil from aromatic plants: a rapid, clean and green approach

F. Chemat¹, M. E. Lucchesi¹, J. Smadja¹, L. Favretto², G. Colnaghi², F. Visinoni²

¹ Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments, Faculté des Sciences et Technologies, Université de la Réunion, 15 avenue René Cassin, B.P. 7151, F-97715 Saint Denis messag cedex 9, La Réunion, France D.O.M, smadja@univ-reunion.fr; fax: +262.262.93.81.83

² MILESTONE srl, Via Fratebenefratelli, 1/5, I-24010 Sorisole, Bergamo, Italy.

Abstract

A recent patent describes a new method relating to natural products extraction using microwave energy. The microwave dry distillation (**MDD**) apparatus is an original combination of microwave heating and dry distillation at atmospheric pressure. Microwave Dry Distillation is conceived for laboratory scale applications in the extraction of essential oils from different kind of aromatic plants. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without any added solvent or water. The internal heating of the *in situ* water within the plant material distends the plant cells and bursts out the glands and oleiferous receptacles. This process thus frees essential oil which is evaporated by the *in situ* water of the plant material. A cooling system outside the microwave oven condensed the distillate continuously, and the excess of water is refluxed to the extraction vessel. The MDD is neither a modified microwave assisted solvent extraction (MAE) which use organic solvents, nor a modified hydro-distillation (HD) which use a large quantity of water, and is time and energy consuming.



Microwave Dry Distillation

F. Chemat, M. Lucchesi, J. Smadja, **European Patent**, EP 03 001 183.7, **2003**.

Effect of location on the essential oil of Moroccan *Ormenis mixta*

S. Zrira¹; J. M. Bessiere²; C. Menut³ and B. Benjilali¹

¹ Institut Agronomique et Vétérinaire Hassan II, B.P 6202 Rabat Instituts, Rabat, Maroc.
s.zrira@iav.ac.ma

² Laboratoire de Chimie appliquée Ecole Nationale Supérieure de Chimie USTL Montpellier 8, Rue de l'Ecole Normale 34075 Montpellier France.

³ Laboratoire de Chimie organique Université Montpellier II Sciences et Techniques du Languedoc.
Place Eugène Bataillon Montpellier, 34095 Montpellier Cedex France

Abstract

The species *Ormenis mixta* belongs to the family of the synathéracées (synanthérées). It's an annual plant with much ramified swiveling root reaching 1 m in height. The flower heads are rubbed on the buttons to drain them and the small wounds to heal them. The wild chamomile is an endemic aromatic plant of Morocco, which is relatively abundant in the area of Gharb and the area of Kénitra. Essential oil known as wild chamomile of Morocco is a typically Moroccan product which is very required in perfumery. In the area of Gharb, the plant is collected by the local population during the period of flowering which extend from March to July. The goal of this work is to rationalize the exploitation of this plant by controlling the production and the commercial quality of its essential oil. In this direction, we carried out an exhaustive analysis of its essential oil, in the same way we examined the effect of location on the production and the chemical composition of this essential oil. Samples of wild chamomile of Morocco collected in the sites of Salé and Kenitra during the month of April 2003 were subjected to a hydrodistillation (Clevenger). Chromatographic analysis showed that essential oil of *Ormenis mixta* is low in monoterpenic hydrocarbons, it contains 3 particular alcohols: yomogi alcohol, santolina alcohol and artemisia alcohol. It also contains a multitude of sesquiterpenic compounds with relatively low contents. The chemical composition of the essential oil does not seem to depend on the location

Effect of Fibre Mulch Cultivation on Biomass and Essential Oil Production of *Melissa officinalis*, *Salvia officinalis* and *Thymus vulgaris*

J. Rohloff¹, G. Rakvåg¹, R. Mordal² and S. Dragland²

¹ The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway.

² The Norwegian Crop Research Institute (Planteforsk), Apelsvoll Research Centre Division Kise, N-2350 Nes på Hedmark, Norway.

Abstract

The project «Norwegian Herb Production (1993-98)» established the initial basis for the increasing production of herbs and medicinal plants cultivated in all parts of Norway today. Both the cold winter climate and short seasons might be limiting factors for a successful production of aromatic plants. Under the guidance of the the Norwegian Crop Research Institute, Apelsvoll Research Centre Division Kise, investigations with an all-year polypropylene plant cover (fibre mulch: Lutrasil®) in the production of lemon balm, sage and thyme were carried out at trial fields in Mid- and Southern Norway between 1999-2001, focusing on biomass production, essential oil content and composition.

The biomass production (d.w.) was distinctly increased under fibre cover with averagely 20, 70 and 13 % for lemon balm, sage and thyme in the 1. production year, and 72 % in the 2. year (lemon balm). Despite the decreased proportion of harvested leaf material from aerial plant parts (all species), the total leaf yield (d.w.) and thus, essential oil production of lemon balm was averagely increased by 67 % (1. and 2. year) as an effect of the fibre cover. In contrast, sage and thyme showed varying results with averagely 2 and 8 % increased oil yield, respectively. In general, the lemon balm oil was especially affected with an increase of neral and geranial, and decreased levels of citronellal under fibre mulch.

Chemical composition of the essential oil of *Annona senegalensis* Leaves from Burkina Faso

A. Bélanger¹, R. H. C. Nébié², R. T. Yaméogo² et F. S. Sib³

¹ Agriculture et Agroalimentaire Canada, Centre de R & D en horticulture, 430 Boul. Gouin, Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

² Centre National de la Recherche Scientifique et Technologique, Département Substances Naturelles, 03 BP 7047 Ouagadougou 03, Burkina Faso

³ Laboratoire de Chimie Organique: Structure et Réactivité; Département de Chimie, UFR-SEA, Université de Ouagadougou; 03 BP 7021; Burkina Faso

Abstract

Annona senegalensis Pers. (syn. *Annona chrysophylla* Boj.) which belongs to the Annonaceae family, is a shrub with height range 1 to 2 meters. Its leaves are large, oval and entire and they are fragrant when crushed. The flowers are yellowish, waxy, closely petal led and very fragrant.

In Africa, *A. senegalensis* can be found in semiarid areas between Senegal and Sudan down to the Guinea savannah (1). Different parts of *Annona senegalensis* are used in traditional medicinal to treat diseases such as respiratory problems, eye diseases and skin diseases. It is also used to treat diarrhoea, elevated blood pressure, dysentery and as antidote against snake and scorpion venom, while the bark is used as an insecticide.

The essential oil, obtained by steam distillation of air-dried leaves of *Annona senegalensis* growing in Burkina Faso was analyzed by GC and GC/MS. The oil was found to contain germacrene D (19.2%), β -caryophyllene (19.1%), γ -cadinene (11.1%) and α -humulene (9.7%) as major components.

Chemical composition of essential oils of *Vitex diversifolia* from Burkina Faso

A. Bélanger¹, R. H. C. Nébié², R. T. Yaméogo² and F. S. Sib³

¹ Agriculture et Agroalimentaire Canada, Centre de R & D en horticulture, 430 Boul. Gouin, Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

² Centre National de la Recherche Scientifique et Technologique, Département Substances Naturelles, 03 BP 7047 Ouagadougou 03, Burkina Faso

³ Laboratoire de Chimie Organique: Structure et Réactivité; Département de Chimie, UFR-SEA, Université de Ouagadougou; 03 BP 7021; Burkina Faso

Abstract

Vitex diversifolia Bak., which belongs to the Verbenaceae family, is a shrub or a tree whose height can reach 6 meters. The leaves are large, oval and entire. Young leaves, branches and buds are covered by fine fluffy bristle. In Africa, *Vitex* species are located in soudanien and guinea savannahs which belong to Ivory Coast, Central Africa and southern sahara. The main part of *Vitex diversifolia* is for medicinal uses, all parts of the plant are used to cure diseases. For example, bark extracts are used to cure tooth diseases, skin diseases, intestinal bilharzias, and headache, while the fruit are used to cure amoebiasis and the buds are use to clean the teeth.

The essential oils, obtained by steam distillation of air-dried leaves of *Vitex diversifolia* growing in Burkina Faso was analyzed by GC and GC/MS. The oil yield ranged between 0.31 and 0.55%. From the compounds detected, 43 have been identified representing up to 97% of the oil. The main constituents of the leaves oil were limonene (74.2%), humulene oxide (4.1%), caryophyllene oxide (3.2%) and α -humulene (2.8%).

Cloning the Diversity: a Multi-Functional Sesquiterpene Synthase as an Example of Product Maximization in *Citrus sinensis*.

A. Voster^{1,2}, C. M. Berteà^{1,2}, M. Maffei¹, H. J. Bouwmeester²

¹ Dipartimento di Biologia Vegetale Università degli Studi di Torino, Italy.

² Plant Research International, Wageningen, The Netherlands.

Abstract

The presence of multi-functional proteins significantly enhances the metabolic efficiencies of an organism. However sometimes enzymes evolve towards more specific and efficient catalysts especially when the product is profitable for the plant. Terpenoids are a large group of compounds that provide ecological and physiological benefit to the plant. Citrus fruits possess a unique blend of these terpenoid compounds that have important practical applications such as the use for flavours and fragrances. Here we report the isolation and functional expression of Cit3, a gene involved in the production of several sesquiterpene olefins that have been identified by gas chromatography-mass spectrometry (GC-MS). The recombinant protein was able to convert farnesyl diphosphate to germacrene A, 7-epi- α -selinene and the important aroma compound valencene. To better understand the Cit3 cyclisation mechanism enzyme assays were carried out under a range of biochemical conditions. A comparative analysis of Cit3 with CsTps1 (recently isolated by Sharon-Asa *et. al.* 2004) was used to provide additionally insight in the structure-function relationships of terpene synthases.

Composition and biological activity of essential oil from *Marrubium cuneatum* Soland. (LAMIACEAE)

D. Rigano¹, A. Grassia¹, F. Piozzi², A. Basile³ and F. Senatore¹

¹ Dipartimento di Chimica delle Sostanze Naturali, University of Naples “Federico II”, via D. Montesano 49, 80131 Naples, Italy; agrassia@unina.it

² Dipartimento di Chimica Organica, University of Palermo, Viale delle Scienze, Parco d’Orleans II-90128 Palermo, Italy.

³ Dipartimento di Biologia Vegetale, University of Naples “Federico II”, via Foria 223, 80139 Napoli, Italy

Abstract

The genus *Marrubium* (LAMIACEAE) consists of perennial plants, mostly growing along the Mediterranean Sea. Extracts of aerial parts from *M. vulgare* are used in treatment of cold, stomach disease and catarrhs of the respiratory tract. As chemical constituents of *Marrubium* sp., flavonoids and labdane diterpenoids have been reported. Our research group studies the essential oil from plants of Mediterranean zone, and now we report the chemical composition and biological activity of *Marrubium cuneatum* essential oil. Fresh aerial parts of the plant (20 g) were subjected to hydrodistillation according to the standard procedure (*European Pharmacopoeia*, 1997), to obtain oil in 0.2% yield. The oil was analysed by GC and GC/MS. Peak identification was accomplished by comparison of their mass spectra with those of NIST 98 and Wiley 5 Libraries and those reported in literature as well as by comparison of their retention indices with literature values. In this communication we also show the allelopathic and antibacteric activity of this essential oil. The allelopathic activity has been tested *in vitro* measuring the inhibition percentage on root growth of *Raphanus sativus* seedlings, while antibacterial activity has been performed *in vitro* on five Gram+ and Gram- bacterial strains.

Influence of Processing on the Chemical Composition of Valerian oil (*Valeriana officinalis* L.)

D. Lopes^{1,3}, H. Strobl² and P. P. Kolodziejczyk¹

¹ Olds College Centre for Innovation, 4500- 50th Street Olds-Alberta, Canada T4H 1R6

² Botanical Specialties, Cultus Lake, British Columbia, Canada V2R 5H6

³ Embrapa Food Technology, Av. das Américas 29501, Rio de Janeiro, Brazil 23020-470

Abstract

Valerian roots are used to prepare phytomedicines that can be taken as sedatives. Although the active components have not been fully characterized, they are generally believed to be the essential oil, the valepotriates and valerenic acid and its derivatives. The most active compounds within the oil in this respect were found in the oxygenated fraction. Valerian oil and its fractions were obtained by steam distillation of dried comminuted roots. The oil samples were analyzed by gas chromatography-mass spectrometry using two capillary columns (HP-5 and DB-Wax). The identification of single volatile component was performed by comparison of retention indices, mass spectra and co-injection of standards. The volatile oil, 0.50% (v/wt), contained borneol and its esters as the principal components, as well as free isovaleric acid (13.0%). Fresh root oil contained low amount of isovaleric acid (0.2%). Oil fractions were analyzed for the content of oxygenated components such as borneol and its esters, valeranone, valeranal, valerenic acid, cryptofauronol and alpha-kessyl alcohol.

Influence of Vacuum Distillation Parameters on the Chemical Composition of Lemon Concentrates

F. Torres¹, D. Lopes² and F. L. P. Pessoa³

¹ Instituto de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia Bl. A, Ilha do Fundão, Rio de Janeiro, Brasil

² Embrapa Food Technology, Av. das Américas, 29501, Rio de Janeiro, 23020-470, Brasil

³ Escola de Química, Universidade Federal do Rio de Janeiro, Centro de Tecnologia Bl.E, Ilha do Fundão, Rio de Janeiro, Brasil

Abstract

The chemical composition of 5-fold lemon oils, Sicilian type, prepared by vacuum distillation process has been investigated. Thirty-one components were identified by gas chromatography-mass spectrometry (GC-MS) representing greater than 99% of total volatiles. The experimental conditions applied were: temperature of 70-120°C, pressure from 5 to 10mmHg and with a reflux ratio in the range of 0.3 to 3. The quantitative composition of the obtained concentrates showed that it was possible to obtain high quality folded oils by just manipulating the operating variables in the vacuum fractionation process. The partially detersenized concentrates contained about 13.39-25.64 % of oxygenated compounds and 8.57-16.39% of aldehydes, compared to 2.51 and 1.60% found in single fold oil, respectively. The quality of the obtained concentrates and single fold oil was established by physico-chemical data and aldehyde content (expressed as citral).

Comprehensive GC (GCxGC) - for Citrus Volatile Fraction Analysis

A. Casilli¹, P. Q. Tranchida¹, M. Lo Presti¹, P. Dugo², Giovanni Dugo¹, G. Scala³, G. Errante⁴ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy

³ Avantech S.r.l., Via Crocefisso 75, 84012 Angri (SA), Italy

⁴ Shimadzu Italia, Gruppo Area Centro Sud, via Crocefisso 75, 84012 Angri (SA), Italy

Abstract

Citrus essential oils are complex mixtures obtained through distillation, solvent extraction or mechanical pressure. They are used mainly in food and perfume industries while several isolated components are also employed for their pharmacological properties. The volatile constituents are routinely analysed by conventional monodimensional GC methods using both polar and non-polar stationary phases. Component identification is generally carried out using MS information, linear retention indices (LRI) and co-injection with commercial standards. These well-established methods provide a great deal of information which is often fundamental for the assessment of quality and authenticity. However, it is well known that many 1D peaks are the result of 2 or more co-eluting compounds and cannot be resolved by any single stationary phase.

Comprehensive gas chromatography (GCxGC) is an adequate methodology for the separation and identification of very complex samples. It is based on the coupling of two capillary columns that each give a different but substantial contribution to the unprecedented resolving power of this technique. The 2D space chromatograms that derive from GCXGC analysis have a great potentiality for identification.

The scope of this research was to evaluate the effectiveness of 2D comprehensive GC in citrus essential oil analysis.

Compositional analysis of essential oil of *Lallemantia royleana*(Benth. In Wall.) Benth. From Iran

A. Ghannadi and B. Zolfaghari

Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, P.O. Box 81745-359, Iran

Abstract

Lallemantia royleana (Benth. in Wall.) Benth. Is an aromatic herb, distributed in different region of Iran, and has been of great interest to Iranian folk medicine because its seed used in the treatment of some various disorders such as some nervous, hepatic and renal disease.

The aerial parts of plant were collected, powdered and the volatile fraction was isolated by hydro distillation and analyzed by GC and GC-MS. Identification of components in the oil was based on GC retention indices relative to *n*- alkanes and computer matching with the Willey 275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature.

Forty-six components were characterized, representing 94.5% of the total oil components detected. The major constituents of the oil were verbenone (16.4%), *trans*-carveol (9.8%) and β -cubebene (8.9%). Many of the unidentified compounds were present in trace amounts.

Composition of the Essential Oil within Summer Savory (*Satureja hortensis* L., Lamiaceae) from Syria Based on SPME Single Gland Analysis

L. Merza-Bahoo, U. Mitteregger, J. Novak, C. Franz

Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Veterinärplatz 1; A-1210 Wien, Austria

Abstract

Summer savory (*Satureja hortensis*, Lamiaceae) is a well known medicinal and aromatic plant. It is used in folk medicine especially in the Eastern Mediterranean to treat various ailments based on the plants antispasmodic, anti-diarrhoeal, antioxidant, sedative and antimicrobial activities.

The essential oil of Lamiaceae is produced in oil glands on the leaf epidermis and stored in a subcuticular space of these specialised leaf structures. An interesting approach to better understand the variability of the essential oil composition within a plant is the analysis of single oil glands.

Oil glands from different positions on the plant (petals, calyces, young, medium and old leaves) were analysed by sampling the content of each oil gland with a SPME-fibre. The main compounds identified were γ -terpinene and carvacrol responsible for the major part (approx. 90%) of the total essential oil composition.

The portion of carvacrol was highest in the petals (85%). The calyx showed intermediate values of 75% while carvacrol was equal in all three leaf positions (young, medium and old leaf) with 58%. γ -Terpinene increased from 9% in the calyx to 30% in the leaves.

The lanceolate leaves were divided into four sections starting from the leaf base (no. 1) to the leaf top (no. 4). The content of carvacrol increased from 56% on sector no. 1 to 66% in sector no. 2 and decreased then steadily down to 53% in sector no. 4. γ -Terpinene showed the opposite trend with a decrease from 32% (sector no. 1) to 25% (sector no. 2) and a steady increase to 36% in sector no. 4.

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The effect of mycorrhization on qualitative and quantitative parameters of *Origanum* sp.

H. Vierheilig¹, J. Novak² and C. Franz²

¹ Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Gregor Mendel Straße 33, A-1180 Wien, Austria

² Institute for Applied Botany, Department of Public Health, University of Veterinary Medicine, Veterinärplatz 1; A-1210 Wien, Austria

Abstract

The arbuscular mycorrhizal symbiosis is a symbiotic association between plant roots and fungi. Arbuscular mycorrhizal fungi (AMF) colonize roots improving plant nutrition mainly by transferring phosphate (P) from the soil to the plant. This, in general, results in higher yield of the AM crop, especially in poor soils (Smith and Read, 1997). Experimental data on a wide range of agricultural plants are available, however, virtually nothing is known on the effect of mycorrhization on herbal plants. AMF do not only affect plant growth, but also the accumulation pattern of secondary plant compounds from different chemical groups, e.g. flavonoids (Larose et al. 2002), cyclohexanone derivatives (Maier et al. 1995) and glucosinolates (Vierheilig et al. 2000). In the production of essential oil crops two parameters are of importance, first the quality and content of the essential oil and secondly, the essential oil yield as the product of content and the production of biomass.

We tested the effect by the arbuscular mycorrhizal fungus *Glomus mosseae* on yield and essential oil composition of several species and genotypes of the herbal plant genus *Origanum* sp. comprising both main pathways in the genus, the sabinyl pathway (with the main compounds sabinene, *trans*- and *cis*-sabinene hydrate) and the cymyl-pathway (with the main compounds *para*-cymene, γ -terpinene, thymol, carvacrol).

Cuttings from five genotypes from two different species of *Origanum* were infected with AMF and potted in a mixture of sand:expanded clay:soil (v:v:v; 1:1:1). The plants were kept in the greenhouse and fertilized with a standard nutrient solution without phosphor (control group and AMF group) and a standard mix with phosphor to be able to compare any effect with the effect of phosphor fertilization.

Very soon, a more vigorous growth was observable for the AMF group that was even better than the growth of the phosphor group while the essential oil composition was not effected at all by the colonization of AMF or by the phosphor fertilization.

This first trial on *Origanum* demonstrates the positive effect of AMF on herb production on poor or problematic soils regarding the element phosphor. AMF could furthermore lead to an increase in productivity by lowering the costs for phosphor fertilization.

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Molecular Expression of Flavonoids Pathway Enzymes in Italian Bean Ecotypes.

A. Bonetti, I. Marotti, G. Trebbi, G. Dinelli and P. Catizone

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, V.le Fanin 44, 40127 Bologna, Italy.

Abstract

RT-PCR was applied to total RNA extracted from three Italian bean ecotypes (Sarconi bean, Zolfino bean and Lamoni bean) to verify expression of enzymes linked to flavonoids biosynthetic pathway.

Using Isoflavone synthase (IFS), Chalcone synthase (CHS) and Phenylalanine-ammonia-lyase (PAL) specific primers, an overexpression of IFS and CHS sequences was observed in Zolfino bean ecotypes. These findings are in agreement with results obtained by HPLC analysis about flavonoid content in these typical Italian beans, suggesting a possible usage destination of bean as functional food.

HPLC Characterization of Flavonoid Content in Three Italian Bean Ecotypes

A. Bonetti, I. Marotti, G. Trebbi, G. Dinelli and P. Catizone

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, V.le Fanin 44, 40127 Bologna, Italy.

Abstract

HPLC analysis has been a technique successfully applied for the detection of phytoestrogens from several matrices, such as plant tissues, raw and processed food. Three Italian bean ecotypes (Sarconi bean, Zolfino bean and Lamon bean) were analysed by HPLC for the determination of phytoestrogen content.

Soybean isoflavones chromatograms were compared to those of bean ecotypes. Results exhibited a phytoestrogen content in Zolfino beans different from soybean. Further analytical determinations permitted to identify compounds in Zolfino bean as different forms of kaempferol, a flavonoid with antioxidant properties.

Further contributions in the field of antimicrobial testings and gas chromatographic analyses of aroma chemicals

E. Schmidt¹, L. Jirovetz², G. Buchbauer², Z. Denkova³, A. Stoyanova³, I. Murgov³ and M. Geissler⁴

¹ Kurt Kitzing GmbH, Hinterm Alten Schloß, D-86757 Wallenstein, Germany

² Department of Pharmaceutical Chemistry, University of Vienna, Pharmacy-Center, Althanstrasse 14, A-1090 Vienna, Austria

³ Department of Essential Oils, University of Food Technologies, 26 Maritza Blvd., BG-4002 Plovdiv, Bulgaria

⁴ Shimadzu Germany, Department of GC and GC/MS, Albert-Hahn-Strasse 14, D-47269 Duisburg, Germany

Abstract

In continuation of our scientific work in the field of combined and systematical investigation of antimicrobial activities (by means of agar dilution and agar diffusion methods) and structural properties (using gas chromatographic analyses: GC and GC/MS with chiral and achiral columns) of different aroma compounds in various samples (pure chemicals, essential oils, extracts, etc.)¹⁻³, further odor-active hydrocarbons (2-carene, 3-carene, limonene and caryophyllene) and oxygenated mono- and sesquiterpenes (1,8-cineole, linalool, carvone and camphor as well as santalol) were used.

Only in the case of (+)-3-carene, (+)-carvone, (-)-linalool and racemic linalool antimicrobial activities were found against all used strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*).

Detailed results of both, antimicrobial activities and structural properties of the investigated aroma compounds will be presented in this paper.

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Preliminary Study on the Chemical Composition of Rosemary Grown in KwaZulu-Natal, South Africa

M. de Figueiredo

Department of Agriculture and Environmental Affairs, P/Bag X9059, Pietermaritzburg, 3200, South Africa

Abstract

The province of KwaZulu-Natal in South Africa has great climatic and economic potential for the production of essential oil crops. Rosemary is found in all of the different bio-climates that exist in this province. The economic potential for rosemary oil will be dependent on its quality and its compliance with international standards (ISO). Plant material from thirty two different growing areas of KwaZulu-Natal were distilled and analysed for chemical composition by gas chromatography. The following chemotypes were found:

α - pinene>camphor> α -terpineol>bornyl acetate (9 samples)

Camphor> α -terpineol. α -pinene>bornyl acetate (6 samples)

α -pinene> cineole> β -pinene>camphene>bornyl acetate (1)

α -pinene> α -terpineol>cineole>bornyl acetate (8)

Camphor>cineole> α -pinene>camphene (6)

Camphor> α -terpineol>bornyl acetate>cineole (2)

The main components found in the samples analysed were α -pinene, camphene, β -pinene, cineole, camphor, bornyl acetate, and α -terpineol and their percentages vary between 3.54 to 30.63, 1.48 to 11.31, 1.41 to 7.83, 6.19 to 18.74, 0.73 to 26.60, 0 to 16.45 and 0 to 22.58, respectively. No sample analysed, fully complied with the ISO 1342:2000(E).

GC-MS of Essential Oil of *Rhododendron anthopogon* and Biological Properties

G. Innocenti¹, S. Dall'Acqua¹, M. Carrara², A. Tubaro³, M. Barbera², K. Gurung⁴

¹ Dipartimento di Scienze Farmaceutiche, University of Padova, Via Marzolo 5, 35121 Padova, Italy.

² Dipartimento di Farmacologia ed Anestesiologia, University of Padova, Largo E. Meneghetti 2, 35121 Padova, Italy.

³ Dipartimento di Economia e Merceologia, University of Trieste, Via a. Valerio 6, Trieste, Italy

⁴ Siddhartha Herbal Industry, Kathmandu, Nepal

Abstract

Rhododendron anthopogon D. Don. (Ericaceae) is an evergreen shrubs growing at an altitude of 3000-4000-m and it is harvested in several regions in Nepal. This plant is a national symbol in Nepal. The leaves of this shrub are aromatic and used locally as an incense. *Rhododendron* can be used in gouty rheumatic conditions. The essential oil is a stimulant and affects fibrous tissue, bones and nervous system. Anthopogon oil, as it is usually referred to in Nepal, is obtained by steam distillation of the aerial parts of *R. anthopogon*. Also known as Sunpati oil, this oil is good natural source of sweet herbal, faint balsamic essence.

In this communication the chemical composition of a sample of the anthopogon oil was analyzed by means of GC and GC/MS.

Essential Oil from *Echinacea pallida* : Characterization and Biological Activity

S. Dall'Acqua¹, M. Carrara², M. Barbera² and G. Innocenti¹

¹ Dipartimento Scienze Farmaceutiche, University of Padova, Via Marzolo 5, 35121 Padova Italy

² Dipartimento di Farmacologia ed Anestesiologia, University of Padova, Largo E. Meneghetti 2, 35121 Padova, Italy

Abstract

Echinacea is a perennial plant native to North America. The root or aboveground part of the plant during the flowering growth phase is used in traditional medicine to relieve and ward off winter ills. Today, a great deal of research is still being carried out on the plant, as it is known to boost the body's natural defences. The mechanism for the immune-stimulating effects of *Echinacea* is not well understood, and it is still not known which constituents are the bioactive compounds. Components that have received the most attention include alkamides, polysaccharides, glycoproteines, polyacetylenes, and caffeic acid derivatives.

Essential oil of the roots of *E. pallida*, however, have been not investigated. Some studies were carried out on the essential oils of the aerial parts of *E. purpurea*, *E. angustifolia* and *E. pallida*.

In the present study we analysed by GC-MS the essential oil of *E. pallida* roots obtained by hydrodistillation. The roots contains 0.25 % of essential oil. The major components are two ketones.

Physical and Chemical Variations of *Pinus brutia* Essential Oils Based on Needles to Twigs Proportions

M. W. Ghosn¹, S. Talhouk², and N. A. Saliba¹

¹ Chemistry Department, American University of Beirut, Beirut, Lebanon

² Agriculture Department, American University of Beirut, Beirut, Lebanon

Abstract

Pinus brutia, exclusively localized in the eastern part of the Mediterranean, extends over 4 million hectares, from Greece to Lebanon and Iraq and is therefore of great economical and ecological interests. Studies dealing with the composition of many conifer essential oils obtained from needles and terminal twigs have shown variable differences. However, no such comparisons have been conducted on *Pinus brutia*. Hence, the composition of essential oils from needles and terminal twigs of *Pinus brutia* in Lebanon is investigated, using steam distillation as the extraction technique and GC-MS as the chromatographic and spectroscopic methods of separation and identification. Furthermore, the study explores the effect of the raw material, namely the relation in % w/w amounts of needles to terminal twigs, and the outcome of these parameters on the yield and the composition/quality of the distillate, described in terms of β -pinene, limonene and citronellol. Consequently, results will help to maximize and standardize the commercial product yield and composition from selected raw materials for economic efficiency and forests preservation.

Breeding of *Thymus x citriodorus* (Pers.) Schreb. and the Analysis of Essential Oils

B. Mikus-Plescher, I. Zobel, and S. Thust

PHARMAPLANT Medicinal - and Aromatic Plants, Research and Breeding, Ltd.; Am Westbahnhof 4, D-06556 Artern, Germany

Abstract

Controlled cross-breeding of the species *Thymus pulegiodes* and *Thymus vulgaris* aims at upright growing of winter hard thyme with lemon flavour to be used as a tea drug.

Starting point of the breeding process were plants from a *T. pulegiodes* line with an essential oil content of 0.24% and a contents of geraniol (31%), citral (17%), and citronellal (10%) as well as plants from a *T. vulgaris* line (*thymol-chemotype*) with an essential oil content ranging from 2.4% to 3.6%. In the first and second generation the plants were morphologically and sensory very different. After selection during two generations, mainly superior plants with upright growing and lemon flavour were obtained in the third generation.

Essential oil fractions from the leaves of 40 plants of the third generation in the first growing season were prepared by hydro-distillation and analysed by GC. In the different lines essential oil contents ranging from 0.49% to 2.2% could be obtained. The composition of the essential oil varied considerably. Plants with a high concentrations of geraniol (max. 83%), geranyl acetate (max. 48%), geranial (max. 23%), and neral (max. 18%) were analysed in detail.

In the following breeding steps it is intended to further improve the essential oil content, the concentration of geranial and neral (lemon flavour), and the morphological homogeneity of the plant population.

Synthesis and Olfactory Properties of 1-Isopropylbicyclo[2.2.2]-octane Derivatives

A. La Bella, M. Felici, I. Popolla, F. Leonelli, L. M. Migneco and R. Marini Bettolo

Istituto di Chimica Biomolecolare del CNR, Sezione di Roma and Dipartimento di Chimica Università degli Studi "La Sapienza", P.le Aldo Moro 5, BOX n. 34 ROMA 62, I-00185 Roma

Abstract

Some years ago, in the frame of studies directed to *patchouli alcohol* analogues,¹ a series of compounds having a bicyclo[2.2.2]octane structure have been synthesized. Nevertheless, 1-isopropylbicyclo[2.2.2]octane derivatives have not been yet described in the literature as well as their olfactory properties.

Thus, in connection with our studies in the field of natural products containing a bicyclo[2.2.2]octane moiety, we prepared, by the allylsilane Sakurai conjugate addition² and the Nagata intramolecular cyclization,³ a series of 1-isopropylbicyclo[2.2.2]octan-2-one and 1-isopropylbicyclo[2.2.2]octan-2-ol derivatives, which were fully characterized and whose olfactory properties were evaluated.^{4,5}

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- [5] We are indebted to Dr. Philipp Kraft and Mr. Jean-Jacques Rouge (Givaudan Schweiz AG, Fragrance Research, Duebendorf, **6**, 26(22), 4123.

New Preparation of 1,3,3-Trimethylbicyclo[2.2.2]octan-2,6-dione, a Never Isolated Key Intermediate in a Total Synthesis of (+)-Norpatchoulenol. A Formal Total Synthesis of (±)-Iso-Norpatchoulenol

A. La Bella, F. Leonelli, I. De Salve, L. M. Migneco and R. Marini Bettolo

Istituto di Chimica Biomolecolare del CNR, Sezione di Roma and Dipartimento di Chimica, Università degli Studi "La Sapienza", P.le Aldo Moro 5, BOX n. 34 ROMA 62, I-00185 Roma

Abstract

In the frame of a project directed to the synthesis of *patchouli alcohol*-like fragrances,¹ whose bicyclo[2.2.2]octane system we intend to construct by the intramolecular aldol condensation of 3-oxocyclohexanecetaldehydes,² a methodology not yet extended to the synthesis of this class of compounds, we prepared 1,3,3-trimethylbicyclo[2.2.2]octan-2,6-dione, a never isolated and apparently unstable key intermediate in the *Liu* and *Ralitsch* total synthesis of (+)-*norpatchoulenol*,³ a minor component of *patchouli oil* and the major responsible for the *patchouli* scent.⁴ Since 1,3,3-trimethylbicyclo[2.2.2]octan-2,6-dione is correlated to an intermediate in the *Monti* and co-workers' synthesis⁵ of (±)-*iso-norpatchoulenol*, its preparation constitutes also a formal total synthesis of the latter.

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- [1] This work was supported by Università degli Studi di Roma "La Sapienza" (Ateneo 60%) and MIUR (COFIN 2000 "Sintesi di Sostanze di Comunicazione Chirali" and COFIN 2002 "Aromi e Fragranze").
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Composition of the leaf, stem and root bark oils from *Isolona cooperi*

J. B. Boti,¹ G. Koukoua,² T. Yao N'Guessan,² A. Muselli,³ A-F. Bernardini³ and J. Casanova¹

¹ Université de Corse, Equipe « Chimie et Biomasse », UMR-CNRS 6134, Route des Sanguinaires, 20 000 Ajaccio, France.

² Université de Cocody-Abidjan, Laboratoire de chimie organique, UFR SSMT, Equipe des Produits Naturels, 22BP582, Abidjan 22, Côte d'Ivoire.

³ Université de Corse, Equipe Chimie des Produits Naturels, UMR-CNRS 6134, Campus Grossetti, 20250 Corte, France.

Abstract

Isolona cooperi (Cooper) Hutch. & Dalziel (Annonacea family) is a shrub up to 2 m high, growing wild in southern Côte d'Ivoire where it is used as a medical tonic. Essential oils, obtained from leaves, stems and root bark were analysed by combination of column chromatography (CC), GC/RI, GC/MS and ¹³C-NMR.

Leaf oil revealed a composition dominated by monoterpenes (22 components, 79,0%): (Z)- β -ocimene (29,6%), γ -terpinene (20,9%), α -terpinene (8,1%) and Δ^3 -carene (5,4%). They were accompanied by uncommon lactones (5 compounds, 14,7%): 5-[(Z)-hexylidene]-5H-furan-2-one (7,8%), massoia lactone (4,5%) and 5-[(E)-hexylidene]-5H-furan-2-one (2,3%). A total of 16 sesquiterpenes constituted 3,6% of the total oil.

Stem oils exhibited a similar composition: (Z)- β -ocimene (31.2%), γ -terpinene (18.4%), α -terpinene (7.4%) and Δ^3 -carene (5.1%) as well as 5-[(Z)-hexylidene]-5H-furan-2-one (7.6%) and 5-[(E)-hexylidene]-5H-furan-2-one (20.%). A total of 56 components representing 96.6 % of the oil were identified.

The composition of the essential oil obtained from root bark was quite different: beside (E)- β -caryophyllene (14.2%) and its oxide (6.7%), the major component was the unusual 5-prenylindole (24.7%)(56 compounds identified, 88.0% of the oil).

Composition and Antibacterial Activity of the Essential Oil from *Zanthoxylum hyemale*.

E. Simionatto, C. Porto, A. F. Morel, I. I. Dalcol

Departamento de Química, Núcleo de Pesquisa de Produtos Naturais, Universidade Federal de Santa Maria (UFSM), Campus Camobi, 97105-900 - Santa Maria RS - Brazil

Abstract

The essential oils from the aerial parts of young leaves (sample A), old leaves (sample B), fruits (sample C), and flowers (sample D) of *Zanthoxylum hyemale* from South of Brazil (Rio Grande do Sul), were analysed by GC, GC/MS, and chiral phase gas chromatography (CPGC). The identification of the chemical constituents was based on comparison of their relative retention times and mass spectra, with those obtained from authentic samples and/or Willey and NBA/NIST libraries and those published by Adams (1995). Thirty one compounds were identified from the essential oils representing *ca* 92.0, 91.0, 89.0 and 87.0% of the oils of samples A, B, C, and D, respectively. The major constituents of the essential oil of sample A was the sesquiterpene nerolidol (51%), absent in the oil of sample B. A unknown sesquiterpene (31%), with a humuleno skeleton, was the main component in the oil of sample B. In the oil of fruits (sample C), the main constituents were the monoterpenes β -pinene (25%) and α -pinene (10%), while in the oil of the flowers (sample D), the unknown sesquiterpene (22%) was the most abundant component. The antimicrobial activity of the oils was also available against Gram (+) and Gram (-) bacteria and yeasts.

Separation of chiral constituents of essential oils using a new selectively substituted cyclodextrin as stationary phase

I. I. Dalcol, E. M. Ethur, A. S. Mallmann, U. F. da Silva, E. Simionatto, and A. F. Morel

Departamento de Química, Universidade Federal de Santa Maria, Campus - Camobi, Santa Maria, RS, Brazil.

Abstract

Various chiral mono- and sesquiterpenes common to essential oils and another economically important products could be resolved on columns coated with per-O-alkylated cyclodextrins. In continuation of our chemical studies on a new selectively substituted cyclodextrins as stationary phases, in this work we describe the preparation of heptakis (6-O-isobutyryl-2,3-di-O-pentyl)- β -cyclodextrin as a new chiral stationary phases for the separation of enantiomers of volatile constituents of essential oils. The enantiomers of α - and β -pinene, limonene, α -tujene and various natural terpenoid alcohols as (+/-) menthol, (+/-) neo-menthol, (+/-)- linalol could be resolved in different essential oils. Synthetic alcohols as (+/-)-1-phenylethanol, (+/-)-1-phenylpropanol, (+/-)-3-buten-2-ol, (+/-)-3-butyn-2-ol and (+/-)- 2,6,6-trimethyl-2,4-cycloheptadien-1-ol, were also analysed.

Composition and antimicrobial activity of essential oils from *Poiretia bahiana* C. Muller (Papilionoideae-Leguminosae)

F. M. Araújo¹, M. G. V. M. Passos², E. de O. Lima³, N. F. Roque¹, D. Martins¹, M. L. S. Guedes⁴, F. Guaré Cruz¹

¹ Grupo de Estudos de Substâncias Naturais Orgânicas (GESNAT), Instituto de Química, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

² Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

³ Laboratório de Micologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, 58.059-900, João Pessoa, Paraíba, Brasil

⁴ Instituto de Biologia, Universidade Federal da Bahia, Campus de Ondina, 40.190-270, Salvador, Bahia, Brasil

Abstract

Poiretia bahiana C. Muller (Papilionoideae-Leguminosae) is a new species found in Brazil. This species is endemic from Chapada Diamantina, Bahia, Brazil, where is known as “arruda da serra” or “arruda brava”. The aerial parts of this plant are traditionally used to treat sinusitis and to dead flea. This work reports the composition of the volatile oils from aerial parts of five specimens and essential oil from fruits of one specimen of *P. bahiana* that was harvested in four different places of Chapada Diamantina, Bahia, northeastern region of Brazil. One sample of the aerial parts oil and one sample of fruits oil had its antimicrobial properties tested against six bacteria, two yeasts and five filamentous fungi.

The essential oils were obtained by hydrodistillation and analysed by capillary GC and GC-MS. It has been observed that Umbellulone (as a rule 61%) is the major volatile constituent in the essential oil of *P. bahiana*, followed by Sabinene (as a rule 15%).

To evaluate antimicrobial activity, assays were performed in duplicate using the agar diffusion method. The *in vitro* results demonstrated that essential oils exhibit antimicrobial activity against *Escherichia coli*, *Salmonella choleraesuis*, *Proteus mirabilis*, *Cladosporium herbarum*, *Penicillium notatum*, *Staphylococcus aureus*, *Staphylococcus aureus* methicilin resistant (MRSA), *Microsporum canis*, *Trichophyton rubrum* and *Candida albicans*, and did not present activity against *Pseudomonas aeruginosa*, *Aspergillus fumigatus* and *Penicillium notatum*. Due to the increased number of infections caused by MRSA over the last years, the fact that the samples presented greater inhibition zones to this microorganism than to methicilin susceptible *S. aureus*, deserves more investigation with special attention to the mechanism of action of the bioactive components present at *Poiretia bahiana*

Monoterpene Patterns of industrially produced Needle Tree Oils

J. Rohloff¹, B. O. Langleite² and R. Hafstad²

¹ The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway.

² Norske Eteriske Oljer AS (*Norwegian Essential Oils*), N-2560 Alvdal, Norway.

Abstract

In the period from 1997–2002, essential oils (EO) from species of the *Pinaceae* and *Cupressaceae* family were produced at a distillation plant at Alvdal in Central Norway. The EO yield from needles and branches ranged from 4.3 (*Picea*), 4.8 (*Pinus*) to 10.0 ml/kg f.w (*Abies*). In contrast to members of the genus *Larix* with 2.2 ml/kg f.w., *Cupressaceae* species such as Common juniper, Port Orford cedar and Northern White cedar were also rich in EO (on average 10.6 ml/kg f.w.).

Important monoterpenes of the *Pinus* species were pinene, 3-carene, limonene and β -phellandrene. *Picea* and *Abies* species were dominated by pinene, camphene, 3-carene, limonene, β -phellandrene and bornyl acetate with higher amounts of β -pinene in *Abies* sp. and respectively, β -myrcene and camphor in *Picea* sp. EOs rich in bornyl acetate (over 30 %) were restricted to these genera. High amounts of 3-carene (over 40 %) could be detected in *Larix* species, whereas distinct levels of sabinene were especially found in *Juniper* needles. The monoterpene content reached levels above 83 % in all EO samples except for the cedar oils (*Chamaecyparis*, *Thuja*) showing high levels of sesquiterpenes. Sesquiterpene contents over 6 % were found in pines, whilst *Picea* and *Abies* species showed concentrations up to 4 %. The EO yield and monoterpene profiles of 29 coniferous species are presented and discussed.

Essential Oils from Seeds and Pods of *Aframomum corrorima* and Rhizomes of *Zingiber officinale* from Ethiopia

J Rohloff, A. Hymete and T-H. Iversen

The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway. E-mail: jens.rohloff@bio.ntnu.no

Abstract

Zingiberaceae essential oil (EO) from seeds and pods of *Aframomum corrorima* (Braun) Jansen and rhizomes of *Zingiber officinale* Roscoe were obtained by steam distillation and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Over 60 compounds could be detected and identified in the pod oil of *A. corrorima*, in contrast to the less complex seed oil (>40 compounds). The seeds, which are widely applied in herbal medicine, contained mainly monoterpenes such as 1,8-cineole (44.3 %), sabinene (17.3 %), β -pinene (7.2 %) and α -terpinyl acetate (6.0 %). Despite the many monoterpenes in the pod EO with γ -terpinene as the main compound (4.9 %), this oil was dominated by sesquiterpenic structures such as *E*-nerolidol (17.2 %), β -caryophyllene (9.7 %) and its oxide (6.9 %), cyperene (6.4 %) and eudes-7(11)-en-4-ol (5.9 %). The EO yield was 3.8 and 0.27 % (v/w) for seeds and pods, respectively.

More than 50 compounds were detected and identified in the rhizome EO of Ethiopian *Zingiber officinale*. The main EO constituents were the characteristic α -curcumene (17.4 %) and relative high amounts of α -zingiberene (14.2 %), β -sesquiphellandrene (12.0 %) and β -bisabolene (8.3 %). The EO of *Z. officinale* rhizomes was observed to be rich in sesquiterpenic structures, whereas the total amount of monoterpenes comprised only 9 %.

Innovative Analytical Methods To Characterize Citrus By-Products For Their Possible Use As Ingredients Of Functional Foods

C. Corradini¹, R. Galanti², I. Nicoletti², S. Padula¹

¹ Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università di Parma, Italy

² Istituto di Metodologie Chimiche, C.N.R. – Area della Ricerca di Roma 1 - Italy

Abstract

Oranges and lemon are one of the main products of the Sicilian region and the processing of citrus produces a series of by-products. For instance, once the juice has been extracted from oranges, peels and rags is left behind, they are equivalent to approximately 50% of the processed fruit's weight.

Orange peel has been reported to contain appreciable quantities of pectins which are a group of complex polysaccharides found in the cell walls of higher plants. It is well known that pectins are extracted from orange peels and that they can be used to form aqueous gels, and this properties is commonly exploited by the food industry [1]. Moreover, pectins exhibit prebiotic properties.

Prebiotics, according to the definition of Gibson and Roberfroid [2], are non digestible carbohydrates that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve the host health. Pectins are not degraded or adsorbed in the stomach or in the small intestine and reach the colon intact. Here they are fermented by some resident bacterial groups. They can be considered prebiotics because promote the proliferation of bifidobacteria, which constitute a significant portion of the intestinal microflora and are claimed to have several beneficial effects on the host. Beneficial effects of prebiotics are mediated by short chain fatty acids (SCFAs), the outcome of carbohydrate microbial fermentation in the colon [11]. In order to characterize more deeply citrus peel as prebiotics, an innovative method to analyze SCFAs in faecal cultures, based on capillary electrophoresis, was developed. Capillary electrophoresis (CE) with indirect UV detection is a valuable detection method for non-UV-absorbing low-molecular-mass ions and reversing the electroosmotic flow (EOF) is essential to achieve rapid CE separations of anionic analytes. In our approach, the dynamic coating of the bare fused-silica capillary with hexadimethrine bromate was performed prior the electrophoretic separations which were conducted using mixtures of benzoic acid-methanol as the running electrolyte. Prior prebiotic evaluation pectins extracted from dried orange and lemon by-products, which were obtained by an industrial drying process, were characterized by diffuse reflectance FT-IR (Fourier transform infrared spectroscopy), which has advantages in the analysis of pectin derivatives compared with other techniques. This infrared technique has been successfully applied to record and analyse spectra of dried powder pectins for the quality control of commercial samples.

Furthermore, recent approaches to the development of products with increased dietary benefits from citrus peel have placed emphasis not only on the recovery of pectins, but also associated with a wide variety of phytonutrient compounds. These phytonutrients include *citrus flavanones* (types of flavonoids that include the molecules *hesperetin* and *naringenin*), *anthocyanins*, *hydroxycinnamic acids*, and a variety of *polyphenols*.

For this reason the characterisation of dried citrus by-product was extended to the determination of flavonoids and antocyanin.

Two innovative reverse-phase high-performance liquid chromatographic methods (RP-HPLC) for the determination of flavonoids and anthocyanins in citrus by-products and were developed and validated.

Our investigation was focused on flavanones, such as hesperidin, hesperetin, and naringin, which are naturally occurring citrus flavonoids and have recently received considerable attention, with

particular interest in the use of these phenolic compounds as hypocholesterolemic [4] as well as anti-cancer compounds [5].

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Essential oil composition of sea fennel (*Crithmum maritimum* L.) from Italy and Portugal

R. Piccaglia, M. Marotti, S. Grandi and M. T. Amaducci

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, viale Fanin, 44 40127 Bologna, Italy

Abstract

The sea fennel (*Crithmum maritimum* L.) belonging to the *Apiaceae* family, is a typical perennial herb of the maritime rocks of the Atlantic and Mediterranean coasts used as condiment, salad ingredient and in folk medicine with tonic, diuretic and anti scorbatic properties

In our work, four types of sea fennel growing wild along the coast of the Adriatic sea of the Middle Italy and that of the Atlantic ocean (South Portugal and Madeira Island) were collected at full flowering stage to evaluate their essential oil yields and compositions. GC-MS analyses evidenced important qualitative differences among the accessions also when they belonged to very close areas.

In fact, the oils from Italy showed, as main components, β -phellandrene and γ -terpinene but only one was characterized by the presence of thymol methyl ether in relevant amounts. This finding confirms the existence, in spontaneous flora, of numerous chemotypes representing important resources which can be exploited in different fields of application.

Composition of the Essential Oils of *Phlomis rigida* Labill. and *P. samia* L.

B. Demirci¹, K. H. C. Bařer¹ and M. Y. Dadandi²

¹ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskiřehir, Turkey

² Department of Biology, Faculty of Science and Letters, Erciyes University, 38039 Kayseri, Turkey

Abstract

The essential oils of *Phlomis rigida* Labill. and *P. samia* L. (Lamiaceae) obtained from the aerial parts by hydro distillation were subsequently analyzed by GC and GC/MS. *P. rigida* (albino and normal form) oils were found to be rich in β -caryophyllene (31.2% and 38.7%) and β -selinene (13.1% and 15.1%, respectively). *Phlomis samia* oil contained germacrene D (33.8%) and β -caryophyllene (6.4%), as major components.

Marigold (*Tagetes* spp.) as source of thiophenes

M. Marotti, R. Piccaglia, I. Marotti and G. Venturi

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, viale Fanin, 44
40127 Bologna, Italy

Abstract

Marigold (*Tagetes* spp.) is a worldwide spread ornamental plant belonging to *Asteraceae* family, that, in recent years, has been revaluated as a source of different classes of secondary metabolites with interesting biological activities. The roots accumulate thiophenes, polyacetylenic sulphur compounds having a strong recognized biocide activity mainly against nematodes.

In this work, six *Tagetes* species (*T. erecta*, *T. filifolia*, *T. lucida*, *T. minuta*, *T. patula* and *T. tenuifolia*) cultivated in North Italy, were evaluated for the yields and the contents of thiophenes in roots, leaves and flowers. The thiophenes, extracted with ethanol and characterized by HPLC, were found mainly in the roots with relevant differences among the species (from 12 to 230 mg 100g⁻¹ of fresh matter). *T. lucida* resulted the richest one and the bithienyl BBT was the main component. Leaves and flowers showed low concentrations of thiophenes.

The presence of these compounds biologically active allows to employ marigold in intercrop and rotation systems as natural pesticide reducing the use of the synthetic ones.

Characterization and biological activity of essential oils of several aromatic plants

I. Stefanini, M. Marotti, R. Piccaglia and B. Biavati

Dipartimento di Scienze e Tecnologie Agroambientali, Università di Bologna, viale Fanin, 44
40127 Bologna, Italy

Abstract

Seven aromatic species: myrtle (*Myrtus communis* L.) and eucalyptus (*Eucalyptus citriodora* L.) of *Myrtaceae*, spearmint (*Mentha spicata* L.), winter savory (*Satureja montana* L.) and calamint (*Calamintha nepeta* L.) of *Lamiaceae*, lemon verbena (*Lippia citriodora* (Ort) HBK) of *Verbenaceae* and St.John's wort (*Hypericum perforatum* L.) of *Guttiferae* family were grown in field. Their essential oils, obtained by steam distillation from inflorescences or leaves, were characterized by GC-MS. The biological activity, towards 31 strains belong to several species of microorganisms, and the antioxidant property were evaluated. Our data referred to the antimicrobial activity were obtained using M.I.C. (minimal inhibitory concentration) in the range 200-2000 ppm.

The essential oils showed antimicrobial properties with different degrees of effectiveness on the tested strains. Winter savory was the most active.

Antioxidant analysis was performed measuring the zone of colour retention in agar plate added with linoleic acid and β carotene. Eucalyptus showed the highest antioxidant activity.

These antimicrobial and antioxidant properties could suggest a lot of applications of essential oils in different areas.

Effect of Extraction Methods on the Chemical Composition and Antimicrobial Activity of *Nigella sativa* Seed Essential Oils

L. Kokoska¹, J. Havlik¹, I. Valterova², H. Sovova³ and V. Rada¹

¹ Czech University of Agriculture Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic, E-mail address: kokoska@itsz.czu.cz

² Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10 Prague 6, Czech Republic

³ Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Rozvojova 2, 165 02 Prague 6-Suchdol, Czech Republic

Abstract

Nigella sativa Linn. (Ranunculaceae) is used in folk medicine all over the world for the treatment of a number of diseases [1]. Its seed essential oil (EO) has previously demonstrated a wide range of biological activities, including antimicrobial and antifungal effects [2].

In our study, we aimed to determine the influence of three different extraction methods on the chemical composition and antimicrobial activity of *N. sativa* seed EOs.

EOs were extracted by hydrodistillation (H) of seeds and by steam distillation (SD) of crude oils obtained by solvent extraction (SE) or supercritical fluid extraction (SFE) and then analysed by gas chromatography-mass spectrometry. Antimicrobial activities were expressed as minimum inhibitory concentration (MIC) values using the broth microdilution method [3].

The results showed that EOs tested differ markedly in their chemical compositions and antimicrobial activities. EO obtained by H was dominated by p-cymene (46.2%), whereas the major constituent identified in both EOs obtained by SD was thymoquinone (60.3% for SE and 46.3% for SFE). EO obtained by H showed lower antimicrobial activity (MICs \geq 2000 μ g/ml) than both EOs obtained by SD (MICs \geq 63 μ g/ml).

Acknowledgement

This work was supported by grant GA CR 525/02/0257.

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Separation and analysis of water-soluble components from selected essential oil

H. Krüger

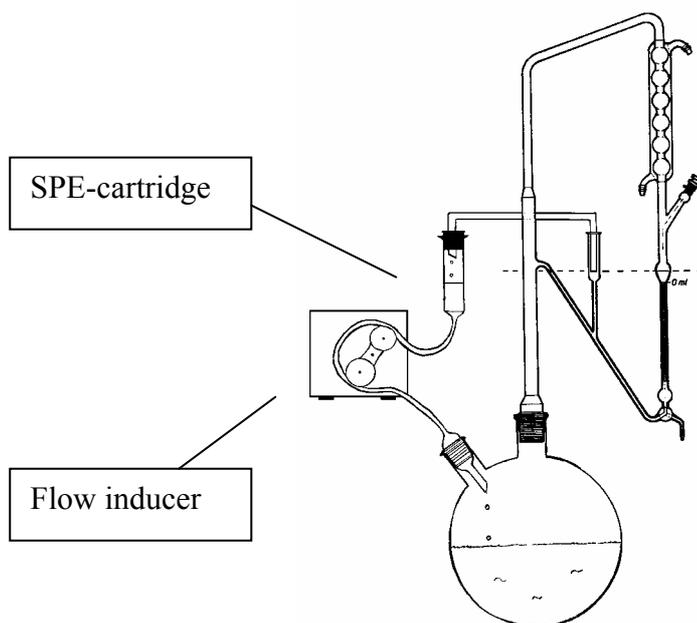
Federal Centre for Breeding Research on Cultivated Plants, Institute of Plant Analysis, Neuer Weg 22-23, D-06484 Quedlinburg, Germany,

Abstract

The extraction of essential oils usually takes place via hydrodistillation or steam distillation. Hydrodistillations are accomplished often including reflux of the distillation water (cohobation). In steam distillations (e.g. in container distillations) the distillation water is usually drained off. This has some technological advantages, however, this procedure causes high oil losses, because the flowing off water separates water-soluble terpenes from the essential oil.

The dimension of the losses was examined with a special apparatus (Fig.1). In the equipment of figure 1 the distilled water with the solved essential oil components is pumped off by a flow inducer at the refill fitting of a distillation apparatus. Water and solved compounds are led over an RP18-SPE-cartridge. The solid phase retains the oil components and only pure water flows back into the flask. This is like a steam distillation where constantly fresh water is supplied.

The distillation water contains above all the physiologically active terpene alcohols and phenols. These adsorbed substances are eluted and analyzed by GC [1].



The oil loss is surprisingly high. It depends not only on the technology, but also on the variety and the chemotype of the investigated plants. By the example of numerous different medicinal and spice plants it is shown that the losses can be higher than 30 % of the total oil quantity.

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Composition and antimicrobial activity of *Bupleurum plantagineum* Desf. essential oil

H. Laouer¹, A. N. Chaker¹, G. Ferretti², C. Reynaud⁵, G. de Billerbeck⁵, L. Haddioui⁵, H. Belkhelfa⁵

¹ Natural Biological Resources Valorization Laboratory (NBRVL), University Ferhat Abbas of Setif, 19000, Algeria.

² Dipartimento di Scienze Chimiche, Università degli Studi di Camerino - Via S. Agostino n. 1 62032 Camerino, Italy.

⁵ Université Paul SABATIER, Toulouse.

Abstract

The essential oil of the aerial parts of *Bupleurum plantagineum* Desf., were endemic in Algeria, was obtained by hydrodistillation. The oil composition was analysed by GC and GC/MS and 62 compounds were identified. The main components were chrysanthenyl acetate (33,4%), α -pinene (16,7%), thymol (16,6%), myrcene (7,4%) and limonene (5,2%). The antimicrobial activity of *B. plantagineum* oil was studied using the agar diffusion test on eight strains of bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Serratia marcescens*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *mosprunorum*) and against fungus (*Aspergillus niger*) and yeast (*Candida albicans*). The oil does not show antimicrobial activity.

Key word index:

Bupleurum plantagineum, Apiaceae, essential oil composition, chrysanthenyl acetate, α -pinene, thymol, myrcene and limonene, antimicrobial activity.

Essential Oils of New Citrus Fruits

G. Ruberto¹, D. M. Biondi¹, A. Renda¹, G. Russo² and P. Rapisarda²

¹ Istituto del C.N.R. di Chimica Biomolecolare – Sezione di Catania, Via del Santuario 110, 95028 Valverde CT, Italy

² Istituto Sperimentale per l'Agrumicoltura, Corso Savoia 190, 95024 Acireale CT, Italy

Abstract

A *Citrus* peel essential oil is specific for an individual *Citrus* taxa representing a sort of fingerprint for these plant species. With this in mind we are carrying out a systematic study of new *Citrus* fruits whose aim is the qualitative characterisation of the essential oils of these new fruits, the evaluation of using these oils profiles as 'fingerprints' in the prosecution of the breeding programme and the identification of new flavour components or new fragrances for industrial uses.

A new breeding approach is the production of hybrids by cross between a diploid female and a tetraploid male parent whose result is the production of seedless triploid hybrids ($2n = 3x = 27$), this being due to the unbalanced chromosomic number.

An overview of the essential oils of new diploid and triploid hybrids, coming from the cross-breeding of grapefruit and clementine, lemon and clementine, and blood oranges (Tarocco, Sanguinello and Moro cvv.) with clementine and mandarin, will be reported.

Composition of *Xylopi*a *aethiopi*ca Dunal from Cameroon and identification of a minor diterpene: ent-13-epi manoyl oxide

F. Noudjou¹, H. Kouninki², T. Hance², E. Haubruge¹, L. Ngamo³, P. M. Maponmestsem³, M. Ngassoum³, F. Malaisse¹, G. Lognay¹

¹ Faculté Universitaire des sciences agronomiques, Passage des Dépoetés, 2,B-5030 Gembloux, Belgique

² Unité d'écologie et de biogéographie, 4-5 place Croix du Sud, 1348, Louvain-la-neuve, Belgique

³ Université de Ngaoundéré, BP 455, Ngaoundéré, Cameroun

Abstract

*Xylopi*a *aethiopi*ca (Dunal) A. Rich. is an aromatic tree widespread in the tropical and humid forest zones of west africa. Traditionally used in cooking, folk medicine and even in food storage, it has been studied since some years for its insecticidal, termite antifeedant and microbiological properties. This work present a view of the chemical composition by GCMS analysis of *Xylopi*a *aethiopi*ca essential oil from Cameroon in relation with its insecticidal properties. The essential oil of different parts of the fruits: grains, fibres and integument were found identical. Main components were β -pinene (17.73% \pm 2.88), terpinen-4-ol (15.78% \pm 2.19) and β -phellandrene (10.72% \pm 1.06). Samples from different markets of the country: Bafoussam, Douala, Ngaoundere, and Yaounde were also investigated. They yield 0.87% , 6.96%, 4,.5% and 6.09% (w/w) respectively. β -phellandrene (31.42%) was the mojour compound follows by β -pinene(8.22%) and 9% of oxygenated compounds in the oil from Bafoussam. Whereas the other samples showed some similarities with β -pinene (39-44%) as major compound and 7-12% of oxygenated compounds. A kinetic study of hydrodistillation was done by collecting and analysing 8 fractions of the essential oil produced (every 30 min) during 4 hours period. The results showed that β -pinene, terpinen-4-ol and 9-epi-(E)-caryophyllene represent respectively 33.25%, 7.22% and 1.47% % and after 30min of distillation in the first fraction. They represent 15.15%, 4.12% and 3.5% in the last fraction produced between 110 and 240min. One minor diterpenes (M^+ = 290 uma) has been detected in all samples from north Cameroon. Further solvant extraction, chromatographic purification and spectroscopic investigations allowed to identified ent-13-epi manoyl oxide which is reported for the first time in *Xylopi*a *aethiopi*ca essential oil.

A Simple Method to Obtain Essential Oils from *Salvia triloba* L. and *Laurus nobilis* L. by Using Microwave-Assisted Hydrodistillation

Z. Tunalier, M. Kosar, T. Ozek, M. Kürkcüoğlu, K. H. C. Baser

Faculty Pharmacy, Department of Pharmacognosy, Anadolu University, 26470, Eskisehir, Turkey.

Abstract

A microwave-assisted hydrodistillation protocol was modified to extract essential oils from leaves of *Salvia triloba* L. and *Laurus nobilis* L.. The essential oils of these plants are generally obtained by hydrodistillation or steamdistillation. The volatile compounds obtained by microwave- assisted hydrodistillation and hydrodistillation methods of these plants were analyzed by GC and GC/MS. Both distillation methods and analytical results were compared.

1,8-cineole (46.8-54.2 %) was the main component in the leaf oils of both samples. Although the distillation was accomplished in a shorter time, oil yields and 1,8-cineole contents were higher in the microwave-assisted hydrodistillation compared to usual hydrodistillation. Microwave-assisted hydrodistillation appears to be an effective method for the production of essential oils.

Microwave-Assisted Hydro-Distillation of Essential Oils

M. Lo Presti¹, M. L. Crupi¹, P. Dugo², A. Trozzi³, S. Ragusa³, G. Colnaghi⁴, F. Visinoni⁴, Giovanni Dugo¹ and L. Mondello¹

¹ Dipartimento Farmaco-Chimico, Facoltà di Farmacia, University of Messina, Viale Annunziata, 98168 Messina, Italy

² Dipartimento di Chimica Organica e Biologica, Facoltà di Scienze MM.NN.FF., University of Messina, 98166 Messina, Italy

³ Dipartimento di Scienze Farmacobiologiche, Facoltà di Farmacia, Università di Catanzaro, 88021 - Roccelletta di Borgia, Catanzaro, Italy

⁴ Milestones S.r.l., Via Fatebenefratelli, 1/5 – 24010 Sorisole (BG), Italy

Abstract

Essential oils are traditionally isolated by steam distillation (SD) from herbs and spices. The present investigation is based on the comparative extraction of essential oils through conventional hydro-distillation and microwave-assisted hydro-distillation. The latter, achieves the in-situ heating of the molecules of water present in the sample and consequently the rupture of the glands containing the molecules of the essential oils that is distilled together with the water in a Clevenger modified apparatus. The qualitative/quantitative composition of the derived essential oils were achieved through gas chromatography and gas chromatography coupled to mass spectroscopy (GC-MS). Microwave extraction demonstrated a series of major advantages in respect to the conventional technique: low costs, the greatly reduced extraction time (18-30 min against 4-6 hours) and the attainment of high quality essential oil as the risks linked to thermal degradation are avoided due to the less time of extraction. Moreover, the absence of environmental impact of this innovative technique must be emphasized. The excellent results obtained will hopefully lead to a more widespread use of this promising approach.

Comparison of the essential oils of *Xanthogalum purpurascens* Lallemand obtained by different extraction techniques

G. Özek¹, T. Özek¹, K.H. C. Baser¹, A. Duran² and M. Sagiroglu²

¹ Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University 26470-Eskisehir, Turkey

² Faculty of Letters and Sciences, Department of Biology, Kirikkale University, 71451-Kirikkale, Turkey

Abstract

The essential oils of *Xanthogalum purpurascens* Lallemand were obtained by hydrodistillation (HD), microdistillation (MD), micro steam-distilled solid phase micro extraction (MSD-SPME) techniques. The oils were then analyzed by GC and GC/MS. The composition of oils depended on the extraction method employed. The main components of the hydrodistilled oil were found to be α -phellandrene (32%), β -phellandrene (23%), isopropyl hexanoate (6%), limonene (5.3%), p-cymene (3.7%), α -pinene (3.2%), bicyclogermacrene (3%).

Composition of the Essential Oil of *Centaurea dichroa* Boiss. & Heldr.

A. Altintas¹, Y.B. Kose², E. Yücel³, B. Demirci¹, K.H. C. Baser¹

¹ Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, 26470 Eskisehir, Turkey; aaltinta@anadolu.edu.tr

² Faculty of Pharmacy, Department of Pharmaceutical Botany, Anadolu University, 26470 Eskisehir, Turkey

³ Faculty of Science, Department of Biology, Anadolu University, 26470 Eskisehir, Turkey

Abstract

Composition of the water-distilled oil of *Centaurea dichroa* Boiss. & Heldr., endemic in Turkey, was analysed by GC and GC-MS. Hexadecanoic acid (11.8%), caryophyllene oxide (9.8%) and spathulenol (5.8%) were the main constituents in the oil.

New Terpenoid Lactones: Synthesis and Odour Characteristics

R. Obara¹, A. Wzorek¹, J. Kula², C. Wawrzeńczyk³

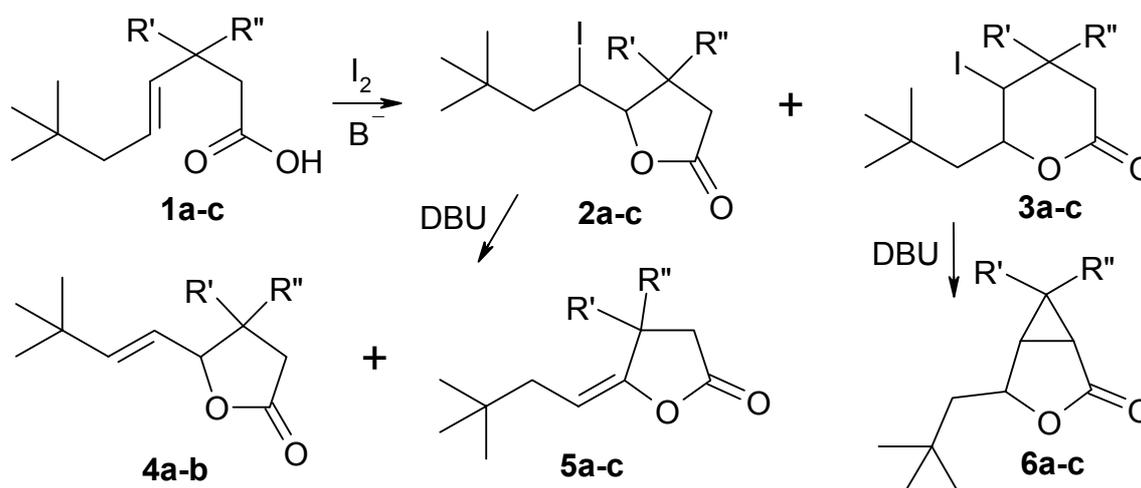
¹ Institute of Chemistry, Świątokrzyska Academy, ul. Chęcińska 5, 25-020 Kielce, Poland

² Institute of General Food Chemistry, Technical University of Łódź, Stefanowskiego 4/10, 90-924 Łódź, Poland

³ Department of Chemistry, Agricultural University of Wrocław, Norwida 25, 50-375 Wrocław, Poland

Abstract

New lactones **4-6** were obtained in two-step synthesis from γ,δ -unsaturated acids. The sequence of two reactions: halolactonization and dehydrohalogenation were applied. Dehydrohalogenation of iodolactones **2** led to typical unsaturated compounds **4**, **5**. We have found, that the γ -halo- δ -lactones **3** undergo, in the same conditions, the 1,3-elimination-cyclization to bicyclic lactones **6** with cyclopropane moiety. This is a lucky phenomenon because the odor sensory activity of cyclopropane ring is homothetic to the double bond. With reason to their chemical stability on atmospheric factors cyclopropane compounds are valuable group of fragrances.



a: $R'=R''=H$; **b:** $R'=H, R''=Me$; **c:** $R'=R''=Me$

Lactones obtained possess interesting odoriferous properties. Considerable differences of odor sensation between each of group of homologues (**4**, **5** and **6**) were observed. Dramatic differences in intensity of scent between **c**, **b** and **a** type of homologues were also noted.

Effect of a spray-dried *Opuntia Ficus Indica* extract on prostate cancer cells (DU145) *in vitro*

A. Panico¹, V. Cardile², F. Garuffi¹, C. Puglia¹, F. Bonina¹ and A. Saija³

¹ Dipartimento di Scienze Farmaceutiche, Università di Catania V.le A.Doria 6, 95125 Catania, Italia

² Dipartimento di Scienze Fisiologiche, Università di Catania, V.le A.Doria 6, 95125 Catania, Italia

³ Dipartimento Farmaco-Biologico, Università di Messina, Contrada Annunziata, 98168 Messina, Italia

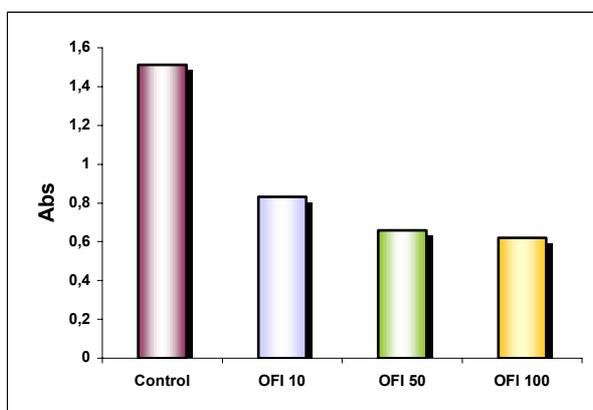
Abstract

Many studies describe the protective role of natural substances against cancer development and in the treatment of established cancer.

The extracts of *Opuntia ficus indica* cladodes, mainly used in folk medicine for their antiulcer and wound healing activities, contain interesting polysaccharidic species characterized by high molecular weight. Furthermore these compounds have a chemical structure similar to the polisaccharidic species contained in different varieties of *Aloe* (*saponaria*, *vera*, ecc.), which are widely studied for their antiproliferative activity in different *in vitro* models.

This work was designed to determine the antiproliferative effect of a spray-dried extract from the cladodes of *Opuntia Ficus Indica* (**OFI**) using human prostate cancer cell lines (DU145).

DU145 cells were cultured in the presence of **OFI** extract at different concentrations (10, 50, 100 µg/ml) for 72 h and the percentage of cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. **OFI** showed a dose-dependent inhibition of cell proliferation. These investigations suggested that this extract had strong antiproliferative activity.



Evaluation of antimicrobial activity of Citrus essential oils

M. A. Cannatelli¹, F. Procopio¹, S. Ragusa², V. Tullio³, G. Di Giacomo⁴, N. Mandras³, A. Marino¹,
A. Nostro¹

¹ Dipartimento Farmaco-Biologico, University of Messina, viale Annunziata, 98168 Messina, Italy.

² Dipartimento di Scienze Farmacobiologiche, University of Catanzaro, Roccelletta di Borgia, 88021 Catanzaro, Italy

³ Dipartimento di Sanità Pubblica e Microbiologia, University of Torino, via Santena, 10126, Torino, Italy.

⁴ Citrus Vita S.p.A., ZIR Diramazione Viaria d, 98040 Giammoro, Messina, Italy.

Abstract

The effect of eight essential oils derived from the Citrus fruit processing (*Citrus limon* (L.) Burm. f. cv. "Femminello", *Citrus sinensis* (L.) Osbek cv. "Sanguinello" and *Citrus deliciosa* Ten. cv. "Avana") on growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis* and mycetes was reported. The antimicrobial activity was evaluated by standard methods - disc diffusion test and broth microdilution method - as well as vapour contact assay. Among the compounds examined, *Citrus limon* exhibited the best results against Gram positive bacteria and mycetes, having the largest inhibitory zone (15-30 diameters in mm) and the best minimum inhibitory concentration (0.5-1% v/v in liquid state and 0.03-0.25 % v/v in vapour phase). *Citrus sinensis* and *Citrus deliciosa* oils, did not show antibacterial activity at the highest concentration tested (1%, v/v). However, when assayed as volatiles, determined inhibition of *Aspergillus niger* growth verified by reduction of colony diameter as compared with control; at the concentration of 0.5 % (v/v) a growth reduction of approximatively 60% was observed.

Comparative in vitro activity of essential oils against dermatophytes and other filamentous fungi

A. Nostro¹, V. Tullio², P. Dugo³, M. A. Cannatelli¹, N. Mandras², A. M. Cuffini², N. Carlone², V. Alonzo¹.

¹ Dipartimento Farmaco-Biologico, University of Messina, viale Annunziata, 98168 Messina, Italy.

² Dipartimento di Sanità Pubblica e Microbiologia, University of Torino, via Santena, 10126, Torino, Italy

³ Dipartimento di Chimica Organica e Biologica, University of Messina, 98166 Messina, Italy.

Abstract

The increasing incidence and severity of fungal infections, particularly in patients with impaired immune system, have stimulated the search for natural drugs as a possible alternative to chemical substances. The essential oils, known since ancient times in popular medicine, have recently been recognized for their potential antimicrobial role. The aim of the present study was to evaluate the in vitro activity of some essential oils (fennel, clove, pine, lavender, sage, thyme, lemon balm) against dermatophytes and other environmental and clinical filamentous fungi. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were assayed in RPMI 1640 medium by microdilution method recommended by the NCCLS and by vapour contact assay. The composition of oils was analyzed by gas-chromatography (GC) and gas-chromatography/mass spectrometry (GC/MS). The results showed that the antifungal activity of oils depended on the assay method used. The inhibiting effects of essential oil in vapour phase were generally higher than that in liquid state. Thyme oil was the overall best growth inhibitor followed by fennel and clove; on the contrary pine oil had limited effects.

Bioactivity of selected essential oils for the intensive livestock industries

J.D. Brooker¹, K.P. Svoboda², G. Ruzickova¹ and M. Ritter¹

¹ Avian Science Research Centre.

² Dpt of Life Sciences , Auchincruive campus, SAC, West Mains Rd, Edinburgh, Scotland.

Abstract

Livestock production systems in the EU, particularly the intensive industries, suffer significantly higher risk of disease because of the ban on prophylactic use of antibiotics. There is therefore increasing interest in identifying alternative natural compounds that may lessen the risk, along with changes to management strategies.

In the poultry industry, one of the most common, and economically significant, diseases is necrotic enteritis (NE), both from the standpoint of bird death through acute clinical disease, and production losses due to sub-clinical disease. The causative agent of NE is type A or C strains of toxigenic *Clostridium perfringens*, although there are other complicating factors. We have examined the effect of *Backhousia citriodora*, *Leptospermum petersonii*, *Eucalyptus citriodora* and *Myrica gale* essential oils (compositions confirmed by GCMS) on the growth and fermentative activity of *C. perfringens* derived from NE-infected chickens, using a novel semi-micro gas pressure analysis.

We have compared the bacteriocidal and bacteriostatic effects of these essential oils on *C. perfringens* with their effect on the commensal gut bacteria, *Lactobacillus fermentum* and *L. acidophilus*, and the potential for the development of resistance. The results demonstrate the differential effect of essential oils on individual bacterial species and the susceptibility of *C. perfringens*. These data indicate a possible natural alternative to in-feed antibiotics.

Essential Oils from *Elaeoselinum fontanesii* Boiss (Umbelliferae)

M. Akssira¹, J. Anaya², F. El Hanbali¹, M. Grande², M. C. Grande², C. Raposo², P. Torres³.

¹ Laboratoire de Chimie Bioorganique et Analytique, UFR C35/ 97 F.S.T Université Hassan II-Mohammedia, 20800 Mohammedia, Morocco.

² Departamentos de Q. Orgánica y de Q. Farmacéutica, Universidad de Salamanca, E-37008 Salamanca, Spain. mgrande@usal.es.

³ UNED, Facultad de Ciencias, Candalix s/n, E-03202, Elche, Spain.

Abstract

Elaeoselinum fontanesii Boiss (= *Laserpitium thapsioides* Desf.), is an Umbelliferae growing on North Africa different from other five species described around the Mediterranean region¹ but the botanical description is not as clear as it should be and these plants can be confused with other *Elaeoselinum*.

In the course of our investigations of essential oils from Apiaceae we have analyzed by GC, GC/MS techniques the essential oils of plants allegedly assigned as *E. fontanesii* collected in different places of Morocco (Chichaoua, Taliquin and Safi). Main constituents of the investigated oils are terpene hydrocarbons and oxygenated monoterpenes while sesquiterpene derivatives were found only in small amounts. A comparison between these essential oils and those of other better-known *Elaeoselinum* species was made in order to establish the differences between these plants.

In the three collected samples, the root essential oil main components were alpha-pinene (from 65 to 81%) and sabinene (from 10 to 22%), being the sum of both of them between 87 and 91%.

More different were the data obtained from the leaves and the green fruits (umbels). In this case the main components were alpha pinene, sabinene and limonene. The samples from Chichaoua and Taliquin present almost the same ratios pinene vs sabinene but in the samples collected in Safi, the amount of sabinene is strongly reduced and the amount of limonene increases from a small 1-2% to values ranging the 40%. The E.O. composition let us clearly differentiate the *E. fontanesii* from other *Elaeoselinum* species previously studied.²

The essential oils from the Safi samples have shown moderate antibacterial activity.

¹ F. García-Martín, S. Silvestre, *Lagasalia*, **13**, 205(1985).

² M. J. Macías, M. Grande, P. Torres, K.-H. Kubeczka, W. Schultze and C. Vollmann. 24th ISEO. Berlin (1993).

***In vitro* inhibition of the growth of *Helicobacter pylori* by Bergamot essential oil.**

F.C. Pizzimenti¹, A. Nostro¹, A. Marino¹, G. Crisafi¹, F. Procopio¹, L. Mondello², D. Monteleone³, V. Alonzo¹

¹ Dipartimento Farmaco-Biologico, University of Messina, Viale Annunziata 98168 Messina, Italy.

² Dipartimento Farmaco-Chimico, University of Messina, Viale Annunziata 98168 Messina, Italy.

³ Collaboratore Farmacista, Ministero della salute, Direzione generale della sanità veterinaria e degli alimenti.

Abstract

Helicobacter pylori, not only is an important cause of peptic ulceration, chronic gastritis but the infected populations have a high risk of gastric cancer. *Helicobacter pylori* is sensitive to several agents in vitro but the eradication of infection in vivo by using several drugs including colloidal bismuth subcitrate (CBS), together with antibiotics, such as amoxicillin, clarithromycin and metronidazole is not effective.

The essential oil of “Citrus bergamia” Risso e Poiteau (bergamot) possesses antimicrobial activities. The aim of the study was to evaluate the inhibitory effect of essential oil on *Helicobacter pylori* growth in vitro.

Activity of essential oil against five clinical isolates and ATCC *H.pylori* was evaluated by using the diffusion susceptibility test at various concentrations using dimethylsulphoxide (DMSO) as diluent and measuring the diameter (mm) of inhibition zone. Pure DMSO was used as control. The results obtained by the disc diffusion method were good. The MIC values obtained with agar dilutions were around 104,7 µg/ml. This oil could be utilized in the prevention or treatment of *H. pylori* infections and is worth further extensive evaluation.

“Peratoner” Accelerates Wound Healing In Rat

M.R. Mondello, M. Giampà, M.F. Taviano, G. Contartese, G.F. Ciancio, E.M. Galati.

Dipartimento Farmaco-Biologico, Università di Messina, Messina, Italy.

Abstract

Several literature data report wound healing activity of plant extracts, which have been used also in traditional medicine.

The aim of the present paper is to study, in rat, the cicatrizant activity of an ointment containing “peratoner”. “Peratoner” is obtained from the distillation of residues and recycled water of the cold-pressing treatment of the fruits of “*Citrus bergamia* Risso.”

The rats were depiled on the back where we produced a standard wound. The wound was treated with a base cream containing 50% of “peratoner”. The treatment was carried out for 5 days. The wounds were observed daily and measured. At the 5th day the animals were sacrificed under ether anaesthesia and biopsies were prepared for histology. The results were evaluated with respect to control rats treated with a commercial preparation.

On the 5th day of treatment with “peratoner”, healing was at an advanced stage.

The skin of healed rats has a normal organization of all layers, with epidermal proliferation and clear signs of the cytomorphous process; a compact corneal stratum is evident. The infiltrating elements are reduced and neo-formation in the vascular network is remarkable.

Data suggest that the treatment accelerates wound healing and it is active in promoting the cutaneous “restitutio ad integrum”.

Composition of the Essential Oil of *Tanacetum paradoxum* Bornm., from Iran

T. Biniyaz, T. Ghodrati, Z. Habibi

Department of Chemistry, Shahid Beheshti University, Tehran, Iran

Abstract

The genus *Tanacetum* comprises 26 species occurring in Iran, from which *T. paradoxum* Bornm. is endemic to Iran.

T. paradoxum plants were collected during the flowering period, from Fasa in Fars province in June 2003. Air dried aerial parts of the plants were grounded and submitted to hydrodistillation, for 4 hours, using a Clevenger-Type apparatus. The oil was pale greenish and had a pleasant odour, with a yield of 0.2% (w/w). The oil was analysed by GC-MS. Eighteen components representing 93% of the oil were characterized, of which camphor (23.78%), lavandulol (15.89%) and 1,8-cineol (13.20%) were the main compounds.

A Comparative Investigation of the Antimicrobial Properties of Indigenous South African and Popular Commercially Available Essential Oils

S. van Vuuren and A. Viljoen

Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York rd, Parktown 2193, Johannesburg, South Africa

Abstract

Essential oils have been accepted and recognized as having several therapeutic applications. Popular commercial oils such as Lavender, Rosemary, Tea tree, Thyme and Peppermint have been used extensively in aromatherapy and as a treatment regimen against bacterial and fungal infections. Extensive studies on indigenous essential oils used in South African traditional healing rites indicate efficacy against a number of pathogens. By means of comparatively determining the MIC of these five most popular commercial oils against the essential oils of five indigenous plants (*Myrothamnus flabellifolius*, *Osmitopsis asteriscoides*, *Heteropyxis natalensis*, *Artemisia afra* and *Lippia javanica*), efficacy was determined against eight bacterial reference strains and two yeast reference strains. The laboratory conditions and inoculum were standardized to ensure all ten (commercial and indigenous) essential oils were evaluated under identical criteria. The main compounds of the essential oils were determined by GC-MS. Where MIC data indicated coinciding values between commercial and indigenous oils, The MIC was further refined to narrow the increments. To comparatively demonstrate the time kill efficacy, commercial and indigenous essential oils were exposed to *Staphylococcus aureus* and other pathogens, and the bactericidal efficacy plotted with time against the logarithm of viable colonies. Results indicate that South African indigenous essential oils compare favorably, and with some pathogens even better than the commercial oils.

The Essential Oil of *Ferula Szowitsiana* DC. From Iran

Z. Habibi and H. R. Aghaei

Dept. of Chemistry, Shahid Beheshti University, Tehran, Iran

Abstract

The genus *Ferula* with 30 species is widespread in Iran. The aerial parts of *F. Szowitsiana* DC. were collected during The flowering period from Semnane Province, in center of Iran, in April 2003.

The dried aerial parts of the plants were hydrodistilled for 4 hours, using a Clevenger-Type apparatus to yield 0.3% (w/w) of oil. The oil was yellowish and had a pleasant odour.

The oil was analysed by GC. MS.

The identification of the compounds was carried out by comparison of their Mass spectra with those of known compounds together with the relative retention indices Twenty Three. Components representing 97% of the oil were characterized that α -pinene (12.56%), β -pinene (10.09%) and germacrene D (12.48%) were the main compounds. Monoterpenes and sesquiterpenes percentage in the oil were 37% and 60% respectively.

Antifungal activities of essential oils of *Artemisia afra*, *Pteronia incana*, *Lavandula officinalis* and *Rosmarinus officinalis* on selected dermatophyte fungi and yeasts associated with dandruff and related scalp disorders

N.Y.O. Muyima and L. Nkata

The Environmental and Natural Products Biotechnology Research Group, Biochemistry and Microbiology Programme Unit, Faculty of Science and Technology, University of Fort Hare, Alice 5700, South Africa

Abstract

The Eastern Cape region of South Africa has, arguably, the richest composition of indigenous aromatic plants in the whole of South Africa. Essential oils could find a wide range of applications in the pharmaceutical/medical, food and cosmetic industries. This study aimed at determining the antifungal properties of *Artemisia afra*, *Rosmarinus officinalis*, *Lavandula officinalis* and *Pteronia incana* oils from the Eastern Cape region of South Africa on eight dermatophyte fungi and two yeast strains. The test organisms were from the genera *Epidermophyton*, *Microsporum*, *Trichophyton*, and *Malassezia* respectively. These were selected based on their association with dandruff and other scalp disorders while the oils were selected for their potential economic value. The agar diffusion assay was performed on Modified Leeming and Notman agar for *Malassezia* strains and on Sabouraud dextrose agar or Malt extract agar for *Epidermophyton*, *Microsporum* and *Trichophyton* strains. All four oils exhibited strong antifungal activities against the test organisms used. *Artemisia afra* oil displayed the highest inhibitory effect and *Pteronia incana* oil, the lowest effect. Considering the overall antifungal activity on dermatophytes associated with dandruff and other related scalp disorders, the above essential oils could be recommended as candidate additives for preparations aimed at improving hair and scalp hygiene.

Composition of the Essential Oil of *Lagochillus aucheri* Boiss., from Iran

A. Laleh, R. Ghahremanzadeh, Z. Habibi

Dept. of Chemistry, Shahid Beheshti University, Tehran, Iran

Abstract

The genus *Lagochillus* comprises 5 species occurring in Iran, from which *L. aucheri* Boiss. is endemic to Iran.

The aerial parts of *L. aucheri* were collected during the flowering period at Sanandaj in the Kordestane Province, in the West of Iran, in June 2003.

The dried aerial parts of the plants were hydrodistilled for 4 hours, using a Clevenger-Type apparatus to yield 0.3% (w/w) of oil. The oil was yellowish and had a pleasant odour.

The oil was analysed by GC-MS. Fifteen components, representing 95% of the total oil, were identified of which di-(2-ethyl hexyl) phthalate (33.84%), germacrene D (20.63%) and hexadecanoic acid (14.04%) were the main constituents.

On the mechanisms of the antimicrobial activity of four monoterpenes of oregano essential oils

D. Trombetta, V. Venuti, M. Cristiani, M. D'Arrigo, G. Bisignano, A. Saija

Department Farmaco-Biologico, University of Messina, Italy

Abstract

Several papers reported in literature are focussed on the antimicrobial properties of essential oils and of the main monoterpenes found in them. In fact many terpenes are known to be active against a wide variety of microorganisms, including gram-positive and gram-negative bacteria and fungi. Toxic effects on membrane structure and function have been generally used to explain the antimicrobial action of essential oils and of their monoterpenoid components; however the specific mechanisms involved in this antimicrobial activity remain poorly characterized.

We have investigated if the antimicrobial efficacy of four monoterpenes (carvacrol, *p*-cymene, thymol and γ -terpinene), which are the main components of oregano essential oils, against the gram(+) bacterium *Staphylococcus aureus* ATCC 6538P and the yeast *Candida albicans* ATCC 10231 is related to their capability to affect membrane permeability [evaluated by monitoring the release of carboxyfluorescein (CF) from phosphatidylcholine (PC) unilamellar vesicles (LUVs)] and to their relative lipophilicity (measured by RP-HPTLC). Among the monoterpenes tested, *p*-cymene showed the strongest toxic activity against *S. aureus*, according with its high lipophilicity and good capability to evoke CF leakage from PC LUVs. Conversely, γ -terpinene appears completely unable to affect CF release from PC LUVs, although it possesses a modest microbicidal activity; thus other unknown mechanisms, different from membrane permeabilization, may contribute to the antimicrobial efficacy of monoterpenes.

Frequent sources of error when determining specific gravities and refractive indices

P. Wyss

Mettler-Toledo GmbH, Business Unit Analytical, Sonnenbergstrasse 74, 8603 Schwerzenbach, Switzerland; peter.wyss@mt.com

Abstract

The determination of the specific gravity and the refractive index are an important stage when testing essential oils. These two physical properties are helpful to detect an adulteration of the products with foreign substances. Modern digital density meters and refractometers allow the corresponding measurements to be performed quickly and easily, but such instruments alone do not guarantee accurate measurements.

A number of important requirements must be fulfilled to ensure correct results: The absolute accuracy of the instrument must be ensured, the measuring cell must contain only the sample to be measured and the nature of the sample must be such that an accurate measurement is possible. If the samples are transferred manually into the measuring cells of the instruments, it is the operator's responsibility to take the possible sources of error into account. Modern fully automatic measuring systems reduce the risk of erroneous results by minimizing operator related effects. Such automated systems cannot offer 100% security that measuring errors are ruled out either, but they may automatically detect most of the sources of error and warn the operator accordingly.

Interspecific Hybridization Effect on the Content and Enantiomeric Distribution of Monoterpenes (α -And β -Pinene, Limonene) in Essential Oils of Eucalyptus Grown in Morocco

A. Farah¹, M. Fechtal², A. Chaouch³ B. Satrani²

¹ Université Sidi Mohamed Ben Abdellah, Fès. Institut National des Plantes Médicinales et Aromatique, BP: 7048, Fès – Ezhour, Morocco. E-mail: farah117@caramail.com, Hybride_farah@hotmail.com

² Centre National de la Recherche Forestières BP 763, Rabat - Agdal, 10050, Morocco,

³ Dépt. Chimie, Laboratoire de chimie appliquée et de contrôle de la qualité, Faculté des Sciences, Université Ibn Tofaïl, BP. 133. Kénitra, Morocco.

Abstract

In this work, hybridization effect on the content and enantiomeric distribution of monoterpenes such as α -pinene, β -pinene and limonene were studied. The α -pinene is detected in leaves essential oils of the crossing *E. tereticornis* x *E. grandis* with a content of 13% and an enantiomeric excess (e. e) of 86% in favour of the R form. When *E. tereticornis* is substituted by *E. rudis* female species, this compound is obtained with a content of 7.5% and an enantiomeric excess of 52%. For β -pinene and limonene, essential oils of the two crossings presented different enantiomeric distributions. Essential oil of the crossing *E. camaldulensis* x *E. tereticornis* reveal an α -pinene with a content of 8.9% and enantiomeric excess of 82% while the reciprocal crossing shows a content of 7.7% and enantiomeric excess of 46% of the R isomer. In essential oils of the reciprocal crossing *E. tereticornis* x *E. camaldulensis*, the forms: (R)-(+)- β -pinene (60%) and (S)-(-)-limonene (81%) are prevalent. With an amount of 3.8%, a racemic mixture of limonene is shown in essential oils of the crossing *E. rudis* x *E. grandis*. A similar phenomenon is observed in essential oils of the male specie *E. grandis*. In general, the contents and the enantiomeric distribution of monoterpenes are intermediate in comparison with those of their parental species.

Key-words:

Eucalyptus hybrids, essential oils, Gas chromatography on chiral phase, enantiomeric separation of monoterpenes.

Investigation of the essential oils from three *Cephalotaxus* species

W. Cisowski¹, I. Mażol², M. Gleńsk²

¹ Department of Pharmacognosy, Medical University Gen. J. Hallera Street 107, 80-416 Gdańsk, Poland

² Department of Pharmacognosy, Medical University of Medicine, 50-149 Wrocław pl. Nankiera 1, Poland, Fax.: ++ 48 7840218

Abstract

The genus *Cephalotaxus* is the only one in the *Cephalotaxaceae* family and eight species belongs to this genus. All of them are trees or shrubs found from the eastern Himalayas to Japan. In Europe they are cultivated in areas with a warmer climate as an ornamental plants.

The main constituents present in these species are tropolone alkaloids mainly derivatives of cephalotaxine and homoerythrina showing significant antitumour activity.

So far the essential oil was investigated in one species of the genus *Cephalotaxus* (*Cephalotaxus griffithii* Hook.). In our research we have investigated the essential oils in three species and one subspecies of the genus *Cephalotaxus*: *Cephalotaxus fortunei* Hook., *Cephalotaxus harringtonia* K. Koch., *Cephalotaxus sinensis* Li. and *Cephalotaxus harringtonia* var. *drupacea* Koidz. which were cultivated in Poland.

To obtain essential oils plant materials were distilled with water vapour in Deryng apparatus according to the method described in the Polish Pharmacopeia. In the investigated species the essential oils content was very low ranging from 0,01-0,22 %. In all investigated species the presence of α -pinene varying from 18,4% to 35,1% as well as sesquiterpene hydrocarbone β -caryophyllene up to 22% at *Cephalotaxus fortunei* were revealed as the main components. Generally in all investigated essential oils 47 components were detected. The chemical compositions of the volatile oils from *Cephalotaxus* species cultivated in Poland were similar to the chemical composition of the *Cephalotaxus griffithii* essential oil from Thailand, there were however quantitative differences.

Components qualitative and quantitative analysis was carried out using gas chromatography coupled with mass spectrophotometry. The resulting spectra were compared with previously published MS data (Terpene Library of Finningan MAT, Terpenoids and Related Constituents of Essential Oils).

High speed & High resolution GC-FID analysis of Geranium and Limette essential oils

H.-U. Baier

Shimadzu Deutschland GmbH, Albert-Hahn Str. 6-10, Duisburg, Germany

Abstract

Modern narrow bore columns allow reliable high quality separation in routine work. The GC analysis of essential oils is rather complex due to the large number of peaks observed in such chromatograms. Therefore resolution is very important. To operate narrow bore columns at optimum separation power a proper set of instrument parameters has to be selected. Here the essential oils geranium and limette are analyzed using a carbowax column of 10 m, 0.1 mm inner diameter and 0.2 μm film thickness. As carrier gas hydrogen was taken with a constant linear velocity of 60 cm/s over the entire temperature program (40 °C to 220 °C/min with 50 °C/min). As the sample capacity of narrow bore columns is reduced compared to standard ones the split ratio was selected to be 400:1 with 1 μl injection.

The GC detector need to be fast enough to follow rapid signal changes observed in fast GC. The 2 parameters are the filter time constant and sampling frequency which was selected to be 4 ms and 250 Hz of the FID-2010, respectively. With this set of parameters up to 31 ingredients were separated in less than 5 minutes.

The Determination of Allergenes in Fragrance Products

Fast GCMS with narrow bore columns

H.-U. Baier

Shimadzu Deutschland GmbH, Albert-Hahn Str. 6-10

Abstract

At least 24 Allergens have to be determined in cosmetic products. The analytical method of choice is GCMS. However the analysis is very complex and requests a good separation together with a large dynamic range of the detector. Fast GC and GCMS using narrow bore columns is a powerful tool to increase analysis efficiency by maintaining the resolution. The instrument hardware has to fulfill some needs like for the GC it should be able to maintain the mean linear velocity of the carrier gas at the different temperatures to operate the columns at optimum separation efficiency as well as it should have a sufficient dynamic pressure range, high possible split ratios, large linear heating ramps and rapid cooling. Regarding the detector part the system must be able to follow rapid changes of signals as the peak width is expected to be around 0.5 s. For a quadrupole GCMS system this means a high number of scans per seconds which in turn needs both a high scan speed (up to 10000 amu/s) due to the mass range scanned and also in addition a small interscan deadtime. The results obtained with a RTX-5 (Restek) 10 m , 0.1 mm ID, 0.1 μm column with standards and perfumes (20 % in acetone) indicate that the resolution as is a least as good as in the standard analysis by a speed gain of a factor of about 11 and a retention time of less than 7 min for benzyl cinnamate. The spectra quality under these conditions gave high similarity indices up to 96 % at the front, top and the tail of the peaks indicating no scwing effect even for sharp peaks obtained with the GCMS QP2010. The data achieved demonstrate the practical use of this fast method on real samples.

The Determination of Allergenes in Fragrance Products Fast GCMS with narrow bore columns

H.-U. Baier,

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Abstract

At least 24 Allergens have to be determined in cosmetic products. The analytical method of choice is GCMS. However the analysis is very complex and requests a good separation together with a large dynamic range of the detector. Fast GC and GCMS using narrow bore columns is a powerful tool to increase analysis efficiency by maintaining the resolution. The instrument hardware has to fulfill some needs like for the GC it should be able to maintain the mean linear velocity of the carrier gas at the different temperatures to operate the columns at optimum separation efficiency as well as it should have a sufficient dynamic pressure range, high possible split ratios, large linear heating ramps and rapid cooling. Regarding the detector part the system must be able to follow rapid changes of signals as the peak width is expected to be around 0.5 s. For a quadrupole GCMS system this means a high number of scans per seconds which in turn needs both a high scan speed (up to 10000 amu/s) due to the mass range scanned and also in addition a small interscan deadtime. The results obtained with a RTX-5 (Restek) 10 m , 0.1 mm ID, 0.1 μm column with standards and perfumes (20 % in acetone) indicate that the resolution as is a least as good as in the standard analysis by a speed gain of a factor of about 11 and a retention time of less than 7 min for benzyl cinnamate. The spectra quality under these conditions gave high similarity indices up to 96 % at the front, top and the tail of the peaks indicating no skewing effect even for sharp peaks obtained with the GCMS QP2010. The data achieved demonstrate the practical use of this fast method on real samples.

A New Chemotype of *Foeniculum vulgare* Mill. (Umbelliferae)

J. Anaya¹, M. Grande¹, A. Pujadas-Salvà², C. Raposo¹, P. Torres³, E. Triano-Muñoz⁴

¹ Departamento de Química Orgánica, Universidad de Salamanca, E-37008 Salamanca, Spain. mgrande@usal.es.

² Dpto. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, E-14004 Córdoba, Spain. cr1pusaa@uco.es.

³ UNED, Facultad de Ciencias, Candalix s/n, E-03202, Elche, Spain. ptorres@elx.uned.es.

⁴ Majadilla, 25, E-14810, Carcabuey, Córdoba, Spain. litodoro@teleline.es

Abstract

Fennel (*Foeniculum vulgare* Mill.), is a well known plant belonging to the Umbelliferae family, widely dispersed around the W and S of Europe, Macaronesian islands, Anatolia, Caucasian region, and W and central Asia, for which many studies on the essential oil composition have been published. Fennel shows a morphological variability and it is commonly accepted the subsp. *piperitum* (Ucria) Beg. Also under the typical subspecies *vulgare*, several taxons (weakly consistent) have been recognized: var. *azoricum* (Mill.) Thell., var. *dulce* (Mill.) P. Fourn. and var. *sativum* C. Presl.¹

We have recently found in the Sierra de Grazalema (Cádiz, Spain) several populations of fennel which main morphological characteristic is the reddish color of the inflorescences and fruits (and for this reason we will call it from now on "*rubrum*"). In the same places there are also populations of the subsp. *piperitum* with yellow inflorescences. Even though we have tried to find plants with combined characteristics we have not been able to locate them. Up to our knowledge there is not any report on the existence of red fennels.²

In order to know if there are some noteworthy differences in the chemical metabolites of the *rubrum* plants and other *Foeniculum*, we have done a comparative study of the essential oils.

In the analysis of the components of the essential oil from the roots it is verified that the *rubrum* plants have a high level of α -phellandrene (>50%) not detected in the roots of other fennel plants, and terpinolene.³ It is also noticeable the low content of phenylpropanoid derivatives: myristicine and apiol are not observed an only dillapiol is present in a small percentage. The low levels or the absence of anethol, eugenol and pinenes in the roots differs with de high amount of these substances usually found in the aerial parts of some varieties of fennel.

¹ Badoc A., Lamarti A., Bourgeois G., Carde J.P. & Deffieux G. *Bull. Soc. Pharm. Bordeaux* **1995** 134: 107-126.

² C. Aedo, personal communication.

³ E. Stahl-Biskup, A. M. Wichtmann *Flavour Frag. J.*, **1991**, 6, 249

Essential Oils from *Eryngium dilatatum*, *E. bourgatii* and *E. aquifolium* s.l. (Umbelliferae)

J. Anaya¹, M. Grande¹, A. Pujadas-Salvà², C. Raposo¹, P. Torres³, E. Triano-Muñoz⁴

¹ Departamento de Química Orgánica, Universidad de Salamanca, E-37008 Salamanca, Spain. mgrande@usal.es.

² Dpto. de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, E-14004 Córdoba. Spain. cr1pusaa@uco.es.

³ UNED, Facultad de Ciencias, Candalix s/n, E-03202, Elche, Spain. ptorres@elx.uned.es.

⁴ Majadilla, 25, E-14810, Carcabuey, Córdoba, Spain. litodoro@teleline.es.

Abstract

The essential oil from the aerial parts of *Eryngium dilatatum* Lam. collected at Villarrodrigo (Jaén, Spain) are composed mainly by monoterpene (α -pinene, β -mircene, limonene) and sesquiterpene hydrocarbons (β -cariophyllene, germacrene D and B) and the roots are characterized by the presence of γ -decanolactone, β -bisabolene and α -farnesene as the main components.

The aerial parts of *Eryngium bourgatii* var. *viridescens* É. Rev., harvested at Sierra María (Almería, Spain), contain as the main component of the essential oil germacrene D (22-44%) as well as duraldehyde, α -pinene, β - and γ -elemenes. The roots are also rich in germacrene D, β -sesquiphellandrene and γ -decanolactone.

Eryngium aquifolium Cav. samples were collected from two morphologically different populations at Grazalema (Cádiz, Spain) and Cabra (Córdoba, Spain). The first population fits with typical *E. aquifolium*, but Cabra population are quite distinct mainly by the number and consistency of bract spinules, but also by general color, flower number, bract length/capitulum ratio... This population resemble *E. huteri* Porta, but leaf venation bring up to *E. aquifolium*.

In fact we found significant differences between the essential oils of both populations. The aerial parts of samples from Grazalema have a high proportion of germacrene D (44-46%) together with $\alpha + \beta$ -pinenes (18-25%) and 3,7(11)-selinene (3-9%) but the aerial parts of Cabra populations contains mainly β -sesquiphellandrene (28-31%) and falcarinol (22-25%). The components of the roots are also quite different: selinenes in the roots of the Grazalema samples accounts for more than 55% of the essential oils but the Cabra samples contains mainly β -sesquiphellandrene (26%) and falcarinol (53%). These data corroborates the need for a detailed botanical study of these populations, and its possible relationships with the Moroccan *E. caespitiferum* Font Quer & Pau (cf. Nieto Feliner, 2001, *Anales Jard. Bot. Madrid* 58, 367-371).

Synthesis and Odour Characteristics of some Compounds with Trimethylcyclohexane System.

K. Winska¹, C. Wawrzenczyk¹ and S. Lochynski²

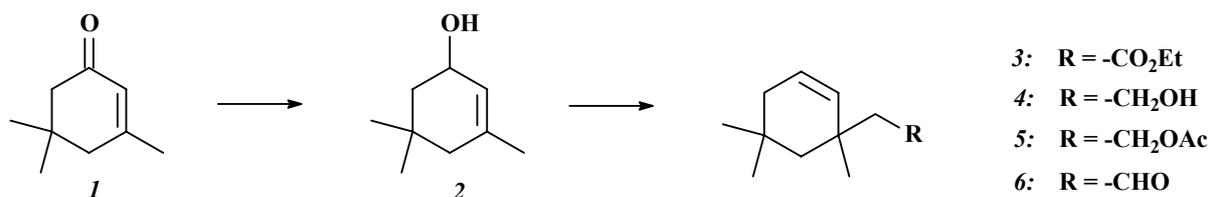
¹ Department of Chemistry, Agricultural University, C. Norwida 25, 50-375 Wrocław, Poland

² Institute of Organic Chemistry, Biochemistry and Biotechnology, Wrocław University of Technology, W. Wyspińskiego 27, 50-370 Wrocław, Poland

Abstract

In our previous papers we reported the synthesis of odoriferous compounds with *gem*-dimethylcyclohexane, *gem*-dimethylbicyclo[3.1.0]hexane and trimethylbicyclo[3.1.0]hexane system, starting from (+)-3-carene, a major constituent of turpentine from some species of Pine (in Poland from *Pinus sylvestris* L.)

Searching for the new synthetic chiral odorants, now we present further syntheses of trimethylcyclohexane derivatives, using isophorone **1** as a starting material. Reduction of this enone **1** afforded allylic alcohol **2** as an enantiomeric mixture. The racemic alcohol and after separation its (-)- and (+)-enantiomers were subjected to the Claisen rearrangement (ortoacetate modification) giving appropriate γ,δ -unsaturated esters **3**. Reduction of esters **3** gave primary alcohols **4**, which in the reaction of esterification with acetyl chloride were transformed to acetates **5**. Oxidation of alcohols **4** led to desired aldehydes **6**.



All newly obtained compounds possess interesting olfactory properties. Details of synthesis and odour characteristics will be presented with special emphasis on structure-activity relationship.

Antifungal activity of the thymol/carvacrol chemotype of *Thymus pulegioides*

L. R. Salgueiro¹, E. Pinto², M. J. Gonçalves¹, S. Costa-de-Oliveira³, C. Pina-Vaz³, A. G. Rodrigues³, A. Palmeira², C. Cavaleiro¹, C. Tavares³, J. Martinez-de-Oliveira⁴

¹ Lab. Pharmacognosy, Faculty of Pharmacy / CEF, 3000 Coimbra, Portugal; ligia@ff.uc.pt

² Dept. Microbiology, School of Pharmacy/CEQOFF, 4050 Porto, Portugal.

³ Dept. Microbiology School of Medicine, 4200 Porto, Portugal.

⁴ Dept. Obstetrics/Gynaecology, School of Medicine, 4200 Porto, Portugal.

Abstract

The antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte strains was studied in order to support its use as antifungal agent. The composition of the essential oil was investigated by GC and GC-MS. The oil was characterized by high amounts of thymol (26.0%), carvacrol (21.0%) and its biogenetic precursors, γ -terpinene (8.8%) and *p*-cymene (7.8%) (thymol/carvacrol chemotype).

The minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) were used to evaluate the antifungal activity against *Candida* spp. (7 clinical isolates and 3 ATCC type strains), *Aspergillus* strains (5 clinical isolates, 2 ATCC and 2 CECT strains) and dermatophyte clinical strains (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*). The oil exhibited significant antifungal activity against all the tested microorganisms, particularly against dermatophyte and *Aspergillus* strains, with MIC values ranging from 0.16-0.32 μ l/ml. To clarify its mechanism of action, flow cytometry assays were performed on *Candida* strains. Great morphological changes were seen on scattergram after 1 hour incubation with MIC values. Using propidium iodide, a fluorescent probe that only penetrates cells with severe lesions of the membrane, most cells were stained.

The thymol/carvacrol chemotype of *Thymus pulegioides* shows a fungicidal activity against *Candida* spp, *Aspergillus* and dermatophyte, producing severe morphological changes and lesion on the cell membrane.

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Anticandidal Activity of Three *Heracleum* Essential Oils

G. Iscan¹, T. Ozek¹, G. Ozek¹, A. Duran² and K. H. C. Baser¹

¹ Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskisehir, Turkey, fax: +90-222-3350750

² Kirikkale University, Faculty of Sciences and Letters, Department of Biology, 71451, Kirikkale, Turkey

Abstract

Fruits of *Heracleum crenatifolium* Boiss, *Heracleum sphondylium* L. subsp. *ternatum* (Velen.) Brummit and *Heracleum platytaenium* Boiss (Umbelliferae) were hydrodistilled to obtain essential oils that were then analysed by GC and GC/MS. Major component was identified as octyl acetate (93.7%, 87.6% and %31.6 respectively). Octyl butyrate was also characterized as the main component in *H. platytaenium* oil. Furthermore, anticandidal activity of the oils was evaluated using microdilution broth method. All the oils showed good inhibitory effects against *C. glabrata*.

Comparison of the essential oils of three endemic Turkish *Heracleum* species obtained by different isolation techniques

T. Özek¹, G. Özek¹, K. H. C. Baser¹ and A. Duran²

¹ Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University 26470-Eskisehir, Turkey

² Faculty of Letters and Sciences, Department of Biology, Kirikkale University, 71451-Kirikkale, Turkey

Abstract

The essential oils of three Turkish endemic *Heracleum* species, *H. crenatifolium* Boiss., *H. platytaenium* Boiss. and *H. sphondylium* L. subsp. *ternatum* (Velen.) Brummitt were obtained using hydrodistillation (HD), microwave-assisted hydrodistillation (MWHD), microdistillation (MD), micro-steam distillation – solid-phase microextraction (MSD-SPME) techniques and analysed by GC and GC/MS. Compositions of the oils isolated by different methods showed some differences. The main components in all the oils were found to be octyl acetate (19 – 95 %), octanol (0.6-5.0 %). Octyl isovalerate (0.8 – 6.4%) and decyl acetate (0.5 – 4.3%) were the next most abundant constituents in the oil of *H. crenatifolium*. Octyl hexanoate (3.0 – 4.7%), (*Z*)-4-octenyl acetate (1.6 – 2.1%), octanol (0.7 – 1.0%), and decanal (0.7 – 1.2%) were the next major constituents in the oil of *H. platytaenium*. Octyl butyrate (34.6 – 41.0%), apiole (4.7 – 20.4%), (*Z*)-4-octenyl acetate (2.2 – 4.4 %), (*Z*)-4-octenyl butyrate (2.6 – 3.0%) were found as other main constituents in the oil of *H. sphondylium* subsp. *ternatum*.

Composition of the Essential Oil of *Phlomis nissolii* L.

N. Kirimer, K. H. C. Baser and M. Kürkçüoğlu

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470-Eskisehir, Turkey
fax: +90-222-3350750

Abstract

Composition of the water-distilled oil of *Phlomis nissolii* L., endemic in Turkey, was analyzed by GC and GC/MS. Eighteen compounds were identified representing 92.6% of the total oil. Germacrene-D (33.9%), bicyclogermacrene (15.3%) and (*Z*)- β -farnesene (10.7%) were the main constituents in the oil.

Comparison of Microwave-Assisted Hydrodistillation and Hydrodistillation Methods for the Analysis of Volatile Secondary Metabolites

M. Kosar, T. Ozek, F. Goger, M. Kürkcüoğlu, K. H. C. Baser

Faculty of Pharmacy, Department of Pharmacognosy, Anadolu University, 26470 Eskisehir, Turkey

Abstract

Microwave-assisted hydrodistillation (MWHD) and hydrodistillation (HD) were carried out for the analysis of volatile components in whole and ground fruits of *Anethum graveolens* L. (Dill seed) and *Coriandrum sativum* L. (Coriander seed). Fruits were distilled using a microwave oven modified to fit a Clevenger apparatus. The effect of microwave energy on the yield and composition of the essential oil was investigated against the classical hydrodistillation. Essential oils of all samples were analyzed by GC-FID and GC-MS. In the whole dillseed oil, the amounts of *cis*-isohydrocarvone (from 7.49 to 10.89%) and carvone (from 45.71 to 69.27%) were increased by microwave energy while limonene (from 17.86 to 4.67%), myristicine (from 8.73 to 5.69%) and dill apiole (from 14.14 to 6.16%) were decreased. Grinding also increased the amounts of main components except for myristicine and dill apiole. A decrease in the linalool content of the Coriander oil (from 80.0 to 75.5%) was observed in the microwave-distilled ground fruits. Microwave-assisted distillation appeared to increase the amounts of fatty acids, e.g. tetradecanoic acid (from 2.8 to 8.8%) and hexadecanoic acid (from 1.9 to 6.0%) in Coriander oil.

Essential Oil Composition of Different *Tagetes lucida* Cav. ssp. *lucida* Accessions

I. Göhler¹, C. Franz¹, A. Orellana²

¹ Institute for Applied Botany, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria

² Institute of Agricultural Research and Technology, ICTA/Chimatenango

Abstract

Tagetes lucida Cav.ssp.*lucida* Cav. (Asteraceae) is a common perennial herb growing wild in the highlands of Central and Southern America.

The infusion derived from the flowering herb with its strong coumarin and anise taste is widely used as a drink and is of great significance in traditional medicine where it is primarily utilized for the treatment of gastrointestinal complaints such as stomach cramps and diarrhea. Of lesser importance is its use as seasoning herb.

Although the essential oil derived from *Tagetes* has not yet played a role in aroma therapy it is an uncontested fact that the essential oil composition is a value-determining factor.

The major essential oil components of *Tagetes lucida* that have been reported so far, are anethole, methyleugenol and estragole.

This report describes the composition of essential oils derived from the flowering herb of different accessions of *Tagetes lucida* that were all cultivated at the same location in order to exclude environmental influence.

Six different accessions of *Tagetes lucida* were cultivated on an experimental field in Guatemala.

The dried leaves and flowers of 60 individual plants from each population were harvested at full blooming stage and distilled with a Clevenger distillation unit. Peak assignment and analysis of the GC chromatograms are based on prior identification of the essential oil components using GC-MS*. The essential oil composition varied strongly between as well as within accessions. Presence of the three reported main components of the volatile oil at the tested samples of different origin is so divergent that typing in e.g. “anethol type” and “estragol type” respectively is possible.

* References: C. Bicchi, M. Fresia, P. Rubiolo, D. Monti, Ch. Franz, I.Göhler, *Falavour Fragr. J.*, 12, 47-52 (1997)

Evaluation of Thymol and Palmarosa Oil as Biofumigant for Control of Bacterial Wilt of Tomato Under Field Conditions

M. T. Momol¹, P. Ji¹, S. M. Olson¹, P. M. Pradhanang¹ and J. B. Jones²

¹ North Florida Research and Education Center, University of Florida, Quincy, FL, USA; ²Plant Pathology Department, University of Florida, Gainesville, FL, USA

Abstract

Thymol (a fraction of thyme or oregano oil) and palmarosa oil, were evaluated under field conditions for control of bacterial wilt of tomato caused by *Ralstonia solanacearum* (race 1, biovar 1). Field soils that were artificially infested with the pathogen were treated with the essential oils for 3 or 6 days under plastic mulch, and tomato seedlings were transplanted into the field 10 or 13 days after the field was infested. In fall of 2002, both thymol and palmarosa oil reduced bacterial wilt incidence and increased tomato yield significantly on the susceptible tomato cultivar Equinox when used at a concentration of 0.7%. Thymol was more effective than palmarosa oil since 33.1% and 48.1% of plants wilted in thymol and palmarosa oil treated plots, respectively. In 2003 field trial, thymol significantly reduced bacterial wilt incidence on the susceptible cultivar Solar Set. Disease incidence in untreated plots reached 65.5%, while in thymol treated plots, only 12% of plants wilted. Thymol treatment also increased tomato yield significantly. These results indicated that thymol provided effective control of bacterial wilt of tomato under field conditions when used as pre-transplanting treatment of soils. Because of its volatile property and broad-spectrum efficacy, thymol shows the potential to be used as a soil biofumigant in the integrated management of various plant pathogens.

Biotransformation of (+)-(1*R*,2*S*)-Fenchol by the Larvae of Common Cutworm (*Spodoptera litura*)

M. Miyazawa

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, 3-4-1 Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan

Abstract

The biotransformation of monoterpenoids are field of investigation that gain a growing interest: these reactions are performed by bacteria, fungi, yeasts and even algae. However, there are few reports in the literature on the biotransformation of terpenoids by lepidoptera in insects. In the present study, the biotransformation of terpenoids was attempted by the larvae of common cutworm (*Spodoptera litura*). The reasons for using the larvae of *S. litura* as a biological catalyst are as followed: lepidopteran larvae feed on plants contained terpenoids as their diet and therefore possess a high level of enzymatic activity against terpenoids; the worm consumes a large amount of plants, making it possible to obtain more metabolites; and the worm is easy to rear on a laboratory scale.

(+)-(1*R*,2*S*)-Fenchol (**1**), bicyclic monoterpene alcohol, is known to be an important and widespread terpene, has a camphoraceous odor, and one of the oldest known organic compounds. Compound **1** is a typical bicyclic monoterpene and is contained in various plants. It is widely used to flavor foods and oral pharmaceutical preparation. There are, however, no report in which the metabolism of **1** in biocatalysts has been investigated.

In the present paper, the main four metabolites (three new) were formed from **1** after biotransformed by the larvae of *S. litura* and the metabolic pathway of **1**.

Biotransformation of (+)-(1*R*,2*S*)-fenchol in *Spodoptera litura* larvae has been investigated. (+)-fenchol was hydroxylated, regioselective transformation, (+)-(1*R*,2*S*)-10-hydroxyfenchol (new compound), (+)-(1*R*,2*R*,3*S*)-8-hydroxyfenchol (new compound), (-)-(1*S*,2*S*,6*S*)-6-*exo*-hydroxyfenchol (new compound) and (-)-(1*R*,2*R*,3*R*)-9-hydroxyfenchol. Intestinal bacteria from the frass of larvae did not participate in the metabolism of (+)-fenchol.

Keywords: *Spodoptera litura*; biotransformation; regioselective; hydroxylation; (+)-(1*R*,2*S*)-10-hydroxyfenchol; (+)-(1*R*,2*R*,3*S*)-8-hydroxyfenchol; (-)-(1*S*,2*S*,6*S*)-6-*exo*-hydroxyfenchol; (-)-(1*R*,2*R*,3*R*)-9-hydroxyfenchol

*Author to whom correspondence should be addressed (telephone +81-6-6721-2332; fax +81-6-6727-4301; e-mail: miyazawa@apch.kindai.ac.jp).

1. Introduction

Terpenoids are known as not only raw materials for flavor and fragrance but also biologically active substances. A great majority of biologically active terpenoids are produced as plant secondary metabolites, and these terpenoids have been shown to have biological activity against plants, microorganisms and insects. Various attempts have been made to search for new biologically active terpenoids. However, it is difficult that these active compounds were produced by organic synthesis. Biotransformation is the biologically synthetic process that using enzymes in the living body as biocatalysts. The characters of biotransformation are as follows: regio and stereoselective reaction under mild condition and produced optical active compounds easily. These points suggested that the biotransformation is a one of the way to produce the biologically active terpenoids. Previously, it biotransformed using mainly the mammals and microorganisms.

The biotransformation of monoterpenoids are field of investigation that gain a growing interest: these reactions are performed by bacteria, fungi, yeasts and even algae. However, there are few reports in the literature on the biotransformation of terpenoids by lepidoptera in insects. In the present study, the biotransformation of terpenoids was attempted by the larvae of common cutworm (*Spodoptera litura*). The reasons for using the larvae of *S. litura* as a biological catalyst are as followed: lepidopteran larvae feed on plants contained terpenoids as their diet and therefore possess a high level of enzymatic activity against terpenoids; the worm consumes a large amount of plants, making it possible to obtain more metabolites; and the worm is easy to rear on a laboratory scale.

(+)-(1*R*,2*S*)-Fenchol (**1**), bicyclic monoterpene alcohol, is known to be an important and widespread terpene, has a camphoraceous odor, and one of the oldest known organic compounds. Compound **1** is a typical bicyclic monoterpene and is contained in various plants. It is widely used to flavor foods and oral pharmaceutical preparation. There are, however, no report in which the metabolism of **1** in biocatalysts has been investigated.

In the present paper, the main four metabolites (three new) were formed from **1** after biotransformed by the larvae of *S. litura* and the metabolic pathway of **1**.

Essential oil composition of *Stachys spectabilis* Choisy ex DC. from Iran

A. R. Fakhari Zavareh^{1,2}, A. Sonboli², F. Sefidkon³, N. Masoodian¹

¹ Department of Chemistry, Science Faculty, Shahid Beheshti University, 1983963113, Evin, Tehran, Iran; a-zavareh@sbu.ir

² Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, P.O. Box 19835-389, Evin, Tehran, Iran

³ Research Institute of Forests and Rangeland, PO Box 13185-116, Tehran, Iran

Abstract

Stachys spectabilis Choisy ex DC. Belongs to the family of Lamiaceae growing wild in different parts of Iran. The plant was collected at full flowering stage on July 2003 from west Azerbaijan Province, Takab. The essential oil of aerial parts was obtained by hydrodistillation and analyzed by GC and GC-MS. Forty-two compounds accounting for 93% of total oil identified. The predominant fraction of the oil was sesquiterpens representing 75% of total oil with germacrene D (27%), *Z*- β -farnesene (15%) and epi- α -bisabolol (10%) as the major constituents. Pulegon (5%), isoverbenyl acetate (3%) and benzaldehyde (2.5%) were the principal components of monoterpens with 25% of the oil.

Reference

Sajjadi, S.E., Mehregan, I.; 2003, Composition of the Essential Oil of *Stachys laxa* Boiss. & Buhse, Iranian Journal of Pharmaceutical Research, 2, 57-58.

Linalool Content in the Essential Oils from the Leaves of 23 Germplasm Samples of *Croton cajucara* Benth.

H. R. Bizzo¹, F. C. M. Chaves², P. C. S. Angelo², J. J. B. N. Xavier²

¹ Embrapa Food Technology – Av. das Américas, 29501 Rio de Janeiro, RJ, 23020-470 Brazil

² Embrapa Amazonia Ocidental – Rod. AM-010, km 29 Manaus, AM, 69011-970 Brazil

Abstract

The leaves and bark of *C. cajucara* Benth., locally known as "sacaca" have been used in Amazonian folk medicine for the treatment of diabetes, malaria, gastrointestinal and liver disorders. The essential oil from the leaves is rich in linalool and this species is being considered to be a sustainable alternative to the oil of *Aniba roseaodora* var. *amazonica*. A germplasm collection is maintained at Embrapa Amazonia Ocidental since 1996. The essential oils from the leaves of 23 germplasms were obtained by hydrodistillation in a Clevenger-type apparatus. The analysis by GC and GC/MS showed a high variation in the linalool content, ranging from 6 to 41%. It was possible to arrange the plants in two groups: one with linalool content from 20 to 40%, which also has nerolidol (10-17%); the other one, with linalool from 10 to 20%, but the major compound being 5-hydroxi-calamenene (27-33%).

Essential Oil of a Commercial *Mentha* Genotype from Brazil

H. R. Bizzo¹, R. F. Vieira²

¹ Embrapa Food Technology – Av. das Américas, 29501 Rio de Janeiro, RJ, 23020-470 Brazil

² Embrapa Genetic Resources and Biotechnology - CP 02372 Brasilia, DF, 70970-900 Brazil

Abstract

As part of a study to evaluate the introduction of 25 genotypes of *Mentha sp.* in the Brazilian savannah ("cerrados"), the agronomic parameters and the essential oil composition of a local commercial species (*Mentha spicata* L.) was analysed by gas chromatography/mass spectrometry. The essential oil was obtained by hydrodistillation of aerial parts in a Clevenger-type apparatus for 2h. Analyses were performed in an Agilent 5973N MDS coupled to a 6890 gas chromatograph fitted with a HP5 MS capillary column (30m X 0.25mm X 0.25µm). The oil yield was 1,4%, corresponding to 16,2L/ha. Forty-three compounds were identified, accounting for 98.5% of the oil composition. Carvone was the major component (67.5%), followed by limonene (10.3%). The essential oil composition resembled that from U.S. spearmint oil.

Composition and antifungal activity of the essential oil from cultivated and wild Ecotypes of *Cymbopogon parkeri* (stapf), from Iran

D. Hadian¹, S. M. F. Tabatabaei¹, P. Salehi², A. Khalighi³

¹ Department of Horticultural Science, Faculty of Agriculture, Tehran University, Iran

² Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, Tehran Iran

³ Department of Horticultural, Tehran Research Institute, Islamic Azad University

Abstract

Cymbopogon is one of the most important aromatic plants among poaceae, which its cultivation dates back to about half century ago in the world specially South East Asia. Various essential oils of the species of this genus are used in perfumery, cosmetic and pharmaceutical preparation. One of this genus *C.parkeri* with some traditional medicinal uses, has been extensively distributed in tropical and sub-tropical regions in the south parts of Iran. The aerial parts of *C.parkeri* were collected from field and two natural habitats. the essential oils obtained by hydrodistillation was studied by GC and GC / MS. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. The main constituent in all samples was **piperiton** and they formed the same chemotype. Other identified compounds were: germacrene-D, elemol, α -eudesmol, α -terpineol. Furthermore, the antifungal activity of the essential oil in different dilution was tested against five fungal. Results of the bioassays showed that the high concentration of the oil inhibited the mycelia growth of the all examined fungal.

Composition and Anticandidal Activity of The Essential Oil of *Chaerophyllum byzantinum* Boiss

M. Kurkcuoglu¹, K. H. C. Bařer¹, G. Iscan¹, H. Malyer², G. Kaynak²

¹ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskiřehir, Turkey.

² Department of Biology, Faculty of Science & Letters, Uludag University, Bursa, Turkey.

Abstract

Water-distilled essential oil of *Chaerophyllum byzantinum* Boiss., collected from Bursa: Uludag in Turkey, was analyzed by GC and GC/MS. Sixty five components were identified representing 94.6 % of the oil. Sabinene (30.0 %), p-cymen-8-ol (16.0 %) and terpinolene (11.5 %) were characterized as main constituents. The oil was tested on five *Candida* species and two strains of *Candida albicans* and showed good to moderate activity. The activity of the oil against *C. glabrata* was twice as better than Ketoconazole.

GC-MS characterization of “*Ocimum basilicum* var. *Gigante Napoletano*”. A preliminary study of the volatile fraction in function of different controlled atmosphere storage conditions.

S. De Luca¹, M. Quinto¹, G. Colelli², D. Centonze¹, M. L. Amodio²

¹ Dipartimento di Scienze Agroambientali, Chimica e Difesa Vegetale, Università di Foggia, via Napoli 25, 71100 Foggia, Italy. e-mail: m.quinto@unifg.it

² Dipartimento di Scienze delle Produzioni, dell'Ingegneria, della Meccanica e dell'Economia applicate ai Sistemi Agro-zootecnici, Università di Foggia, via Napoli 25, 71100 Foggia, Italy

Abstract

Basil (*Ocimum basilicum* L.) is an important officinal plant used not only in food field, but also in medicine because of its therapeutic power. The volatile fraction of some cultivars of basil have been already studied¹, highlighting the presence of a great number of molecules that give the distinctive flavour to this plant. The composition of such species can change in function of the cultivar, the environment and the storage conditions. In this study, through the Purge and Trap technique hyphenated with GC-MS, a characterization of the volatile fraction of *Ocimum Basilicum* var. *Gigante Napoletano* has been carried out, to the best of our knowledge, for the first time. Furthermore, the same technique has been successfully used with samples stored in controlled atmosphere, in order to establish the post-harvest storing conditions that better preserve this plant. In particular, two different mixtures have been used: 2% O₂ + 3% CO₂ in N₂, and 2% O₂ in N₂, together with samples maintained in air (check samples). Sampling have been carried out at 6, 13 and 20 days. The Principal Component Analysis of the obtained results demonstrated that the volatile fraction of check samples resulted significantly different from samples stored in the other conditions (that showed no appreciable differences between themselves), in particular for 20 day old samples.

¹ Jirovetz, L., Buchabauer, G., Shaf, M. P., Kaniampady, M. M., “Chemotaxonomical anaysis of the essential oil aroma compounds of four different *Ocimum* species from southen India”; Eur. Food Res. Techol; (2003), 217, 120-124; Briphy, J. J., Jogia, M. K.,”Essential oils from Fijian *Ocimum basilicum* L.”; Flavour and Fragrance J. (1986), 1, 53-55.

Chemical composition of essential oils from two medicinal plants growing in the south-east of Tunisia: *Thymus capitatus* and *Rosmarinus officinalis*

A. Akrou¹, I. Chrif², M. Hammami², and M. Neffati¹

¹ Institut des Régions Arides, 4119 Medenine, Tunisia; Ahmed.Akrou¹@ira.rnrt.rn.

² Faculté de Médecine, Monastir, Tunisia

Abstract

Thymus capitatus and *Rosmarinus officinalis* were two spontaneous medicinal plants growing in the Matmata mountain range in the south east of Tunisia. These plants were used in folk medicine against respiratory diseases (bronchitis, cough, influenza, etc.). The essential oil of these plants (airial part) was isolated using a modified Clavenger apparatus, and was investigated by GC/SM. The oil isolated from *Thymus capitatus* (4.37% v/w) was found to be mainly composed of carvacrol (68.8%), p-cymène (11.1%) and gamma-terpinene (8.1%). The oil extracted from *Rosmarinus officinalis* (2.95% v/w) was found to be mainly composed of 1,8-cineole (22.3%), camphor (22.8%), camphene (11.1%) and alpha-pinene (12.0%). The yield and the composition of these oils was found to be different from the same plants growing in other countries.

Essential oil compositions of the roots, rhizomes and the aerial parts of *Geum kokanicum*

H. R. Monsef-Esfahani¹, M. A. Faramarzi², M. Moghimi¹ and A. R. Shahverdi²

¹ Department of Pharmacognosy

² Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, P. O. Box 14155-6451, Tehran, Iran; E-mail: monsefes@sina.tums.ac.ir

Abstract

Geum kokanicum Regel et Schmath, a member of Rosaceae family, is an endemic herbaceous wild plant of Iran. It grows spontaneously mostly at mountainous parts of the country. In folk medicine, the roots and rhizomes have been used as astringent and tonic in the treatment of chronic diarrhea. The preliminary studies indicated the high concentration of tannins in the radix and the presence of volatile compounds in all parts of the plant.

The purpose of this investigation was to analyse the essential oil of *Geum kokanicum* obtained by water distillation method. The plant was collected from Esfarayen (Khorasan province) during June 2002. The essential oils of the roots, rhizomes and the aerial parts were separately subjected to GC/MS analyses after extraction by hydrodistillation method using a Clevenger-type apparatus.

Eugenol (33.5%) and thymol (7.4%) and carvacrol (6.2%) were the main components among the seventy-two constituents characterized in the oil of the aerial parts representing 87% of total components characterized. Seventeen compounds were identified in the oil of the root representing 95% of the total oil with eugenol (80.9%) and myrtenol (5.2%) as major constituents.

Biological activities of the essential oils of *Anethum graveolens* and *Levisticum officinale* hairy roots

P.A. Santos¹, M.G. Miguel², S. Gomes², M.L. Faleiro², A.C. Figueiredo¹, J.G. Barroso¹, L.G. Pedro¹, S.G. Deans³, J.J.C. Scheffer⁴

¹ Centro de Biotecnologia Vegetal, Dep. de Biologia Vegetal, FCUL, C2, Campo Grande, 1749-016 Lisbon, Portugal

² Universidade do Algarve, FERN, Campus de Gambelas, 8005-139 Faro, Portugal

³ Dept. of Pharmaceutical Sciences, University of Strathclyde, Glasgow G4 0NR, Scotland, UK

⁴ LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands.

Abstract

Antimicrobial and antioxidant activities of the essential oils isolated from *Anethum graveolens* and *Levisticum officinale* hairy root cultures maintained in different growth media were evaluated. For the antimicrobial tests, three Gram-negative bacteria, three Gram-positive bacteria and one fungus were used.

The antimicrobial activity of the essential oils was determined by agar overlay technique method. Three microliters of each oil were used and water, chloramphenicol and cyclohexamide were used as controls. The essential oil of the *A. graveolens* hairy roots did not show antimicrobial activity, but the oil of the *L. officinale* hairy roots was effective against the Gram-positive bacteria, but not against the Gram-negative ones.

The antioxidant activity of the oils was determined by the modified TBARS assay using egg yolk, either in the absence or in the presence of ABAP. The antioxidant activities were compared with those of α -tocopherol, BHA and BHT. The *L. officinale* oil showed a homogeneous activity regardless the tested concentrations (61-72%) in the absence of ABAP. In the presence of ABAP the percentages ranged from 61% to 81%. The antioxidant activity of the *A. graveolens* oil was clearly dependent on the oil concentration, namely from 29% to 62%.

Acknowledgements

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Essential oil of *Dittrichia viscosa* subsp. *revoluta* (Hoffmans & Link) a possible new tool for combating *Helicobacter pylori* infection

M.L. Faleiro¹, M.G. Miguel¹, C. Cavaleiro², L.R. Salgueiro², J. Casanova³

¹ Universidade do Algarve-FERN Campus de Gambelas Faro, Portugal

² Faculdade de Farmácia, Universidade de Coimbra, Coimbra, Portugal

³ Université de Corse, Équipe Chimie et Biomasse, UMR CNRS 6134, Route des Sanguinaires, 20000 Ajaccio, France.

Abstract

D. viscosa subsp. *revoluta* is an endemic species of Algarve (Portugal). The antimicrobial activity against *H. pylori* and the chemical composition of the essential oils were evaluated. The crude essential oil was obtained by hydrodistillation. This oil was submitted to fractionation by column chromatography on silica gel. This separation allowed isolation of oxygenated compounds (DV4-DV8) from hydrocarbons. Anti-helicobacter activity of crude essential oil and oxygenated fractions were evaluated against *H. pylori* strain (CCUG 15818) used as laboratory strain and three different isolates (28/02, 30/00 and 40/00). Essential oils were tested by incorporation in Columbia agar at different concentrations. Crude essential oil was used at concentrations 0.033, 0.066, 0.1 and 0.133 $\mu\text{l.ml}^{-1}$. Concentrations of 0.005, 0.01, 0.02, 0.025, 0.05, 0.060, 0.07, 0.075, 0.1 and 0.2 $\mu\text{l.ml}^{-1}$ in the same medium were used for testing oxygenated fractions (DV4, 5, 6 and 7). *H. pylori* strain (CCUG 15818) demonstrated to be susceptible to the crude essential oil. At concentration of 0.033 $\mu\text{l.ml}^{-1}$ the initial population was reduced for about 1 log. Susceptibility to all the four oxygenated fractions was also observed. Fraction DV5 presented the highest activity followed by fraction DV7. With the fraction DV5 at the concentration of 0.025 $\mu\text{l.ml}^{-1}$ no growth was registered; at this concentration, fraction DV7 reduced the initial population for 5 log. The three *H. pylori* isolates were more resistant to fraction DV5 than strain CCUG 15818. At concentration of 0.025 $\mu\text{l.ml}^{-1}$ the initial population of 28/02 isolate was reduced for 2 log, whereas for 30/00 and 40/00 isolates was reduced 1log. *H. pylori* strains are susceptible to either crude essential or oxygenated fractions. Susceptibility of *H. pylori* strains to DV5 fraction varies among strains. Crude oil activity may be due to the oxygen-containing compounds of the DV 5 and DV7 fractions.

The Essential Oil of *Meristotropis xanthioides*

M. Tajbakhsh², M. A. Khalilzadeh¹, J. Ghanaat²

¹ Department of Chemistry; I.A.University; Ghaemshahr campus

² Department of Chemistry; I.A.University; Ayatollah Amoli campus; Amol; Iran

Abstract

The genus *Meristotropis* (Fam. Papilionaceae) is represented in the flora of Iran by only one species: *M. xanthioides* Vassiles (Syn: *Glycyrrhiza triphylla* Fisch and C.A. Mey) [1,2]. Our study deals with the analysis of the volatile oil of *Meristotropis xanthioides* Vassiles grown wild in Iran. The aerial parts of the genus *Meristotropis* was collected from Ashkhane, province of Khorassan, Iran, in July 2002, at full flowering stage. The water distilled volatile oil from aerial parts of *Meristotropis xanthioides* was analyzed by GC and GC/MS. Some of the important components have been identified by using of Kovats indices and fragmentation of the components from mass spectra and GC chromatogram. Twenty two components were identified that approximately constitute more than 94% of the oil. The main constituents of the essential oil were Myrcene(20.6%), Limonene(18.9%), and β -Caryophyllene (11.8%). Due to our literature searches, the essential oil of *Meristotropis xanthioides* has not been the subject of previous study.

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Chemical Composition of the Essential Oil of *Allium jectianum* Boiss. From Iran

H. Amiri¹, R. A. Khavari-Nejad², A. Rustaiyan³

¹ Biology Department, University of Lorestan, Khoramabad, Iran

² Biology Department, University to Teachers Education, Tehran, Iran

³ Department of Chemistry, Science and Research Campus, I. A. University, Tehran, Iran

Abstract

Allium jectianum Boiss. belongs to Liliaceae family, which grows wildly in West and Northwest of Iran. The aerial parts were collected in flowering stage. The essential oil from epigeous parts, which was obtained by Hydrodistillation, was subjected to analysis by GC and GC/MS. The major components were found to be: Trisulfide dimethyl (22.34%), Hexadecanoic acid (21.03%), Phytol (15.82%), Alpha-cedrene epoxide (15.82%), 2-Pentadecanone (6.36%), Curzerene (9.62%), Decanal (5.32%).

Fumigant Activity of Six Essential Oils from Cameroon on Cowpea Pest *Callosobruchus maculatus* (Coleoptera: Bruchidae)

B. M. Ngassoum¹, J. Fonjio¹, L. T. Ngamo², P. M. Maponmetsem², G. C. Lognay³, E. Haubruge³, F. Malaisse³ and T. Hance⁴

¹ Département de Chimie Appliquée, ENSAI, Université de Ngaoundéré, BP 455, Ngaoundere, Cameroun: e-mail: ngassoum@yahoo.fr

² Département de Biologie, Faculté des Sciences, Université de Ngaoundéré, BP 455, Ngaoundere, Cameroun

³ Faculté universitaire des Sciences agronomiques, Passage des Déportés, 2, B-5030 Gembloux, Belgique

⁴ Unité d'Écologie et de Biogéographie, 4-5 place Croix du Sud, 1348, Louvain-la-Neuve, Belgique.

Abstract

The bruchid *Callosobruchus maculatus* (F.) causes major losses during the storage of seeds of *Vigna unguiculata* (Walp.). Methyl bromide, the most widely used fumigant, is responsible for 10% of all the factors causing the depletion of the ozone layer. Its deregistration is scheduled for 2005. An alternative strategy is the use of natural fumigants of plant origin such as essential oils. In Cameroon, farmers often introduce aromatic plants into granaries at harvest time. Essential oils were extracted from six common local plants, *Annona senegalensis* Pers., *Xylopiya aethiopica* Dunal (Annonaceae), *Lippia rugosa* L. (Verbenaceae), *Ocimum gratissimum* L. (Lamiaceae), *Eucalyptus citriodora* HOOK and *Eucalyptus saligna* Sm. (Myrtaceae). The major components of these essential oils were : limonene, myrcene and linalool in *A. senegalensis*, β -pinene, β -phellandrene, terpinene-4-ol in *X. aethiopica*, linalool, geraniol and geranial in *L. rugosa*, thymol in *O. gratissimum*, 1,8-cinéole in *E. saligna* and, geranial and neral in *E. citriodora*. Weevils were reared on cowpea seeds and newly emerged males and females were deposited on uninfected seeds. Two hours bioassays - fumigation with various quantities (10, 20 and 40 μ l) of pure essential oils were carried out on adults. Twenty four hours after fumigation, adult mortality were evaluated. The more active oils were *A. senegalensis* (100% mortality with 10 and 40 μ l) followed by *L. rugosa* (97% and 100% mortality resp. with 10 and 40 μ l), *E. citriodora* (83% and 96% resp.), *O. gratissimum* (50% and 100% resp.). The lower active oils were *X. aethiopica* (5% and 30% resp.) *E. saligna* (30 and 68% resp.). Our results suggest that essential oils from *A. senegalensis* and *L. rugosa* can be used as an alternative to synthetic fumigant insecticides.

Keys words : Cowpea, *Callosobruchus maculatus*, fumigation, essential oil, insecticides

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Volatile Constituents of Benzoin Gums: Siam and Sumatra

C. Castel¹, X. Fernandez¹, L. Lizzani-Cuvelier¹, A-M. Loiseau¹, J-F. Arnaudo², C. Delbecque², C. Perichet²

¹ *Laboratoire Arômes, Synthèses, Interactions*, Faculté des sciences de Nice Sophia-Antipolis, Parc Valrose, 06108 Nice Cedex 2, France. E-mail: castel@unice.fr

² CHARABOT, *Research Natural Products*, 10, Av. Yves-Emmanuel Baudoin, 06130 Grasse, France

Abstract

Benzoin gum is a balsam obtained from *Styracaceae* trees. It is mainly produced in South-East Asia. Two types of benzoin gums exist in the trade, Siam benzoin from *Styrax Tonkinensis* and Sumatra benzoin from *Styrax Benzoin*; each are graded according to the size of the pieces. The first one presents a sweet-balsamic odour with a distinct note of vanilla, while the other one has a strong styrene-like and more spicy odour.

Even if these resins are widely used in flavours and fragrances, only few studies have been done on the chemical composition of *Siam* or *Sumatra* benzoin gums, essentially composed of aromatic acids or esters.¹

We have recently presented the chemical composition of the volatile extracts obtained from *Siam* and *Sumatra* benzoin gums and volatile compounds of oils and crushed benzoin gums by SPME.²

Three objectives will be presented in this communication:

- Study of the headspace composition of several varieties of Siam and Sumatra benzoin gums using three extraction techniques (Static-Headspace, SPME and Headspace Sorptive Extraction) to identify most of volatile compounds from these gums.³
- Adjustment of a fast method to distinguish varieties and grades using electronic noses: FMS (Fingerprint Mass Spectrometry) and SAS (Sensor Array System).
- Study of vanillin content in different benzoin gums.

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Comparison of the Volatile Composition of three *Stachys* species Oils Obtained by Hydrodistillation

M. Khanavi¹, H. Monsef¹, A. Hadjiakhoondi¹, A. Vasheghani Farahani¹, A. Shafiee² and A. Rustaiyan³

¹ Department of Pharmacognosy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, khanavim@sina.tums.ac.ir

² Department of Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

³ Department of Chemistry, Sciences and Research campus, Islamic Azad University, Tehran, Iran.

Abstract

The genus *stachys* (lamiaceae), consists of about 200 species widespread throughout the world. In Iran 34 species exist, among which 13 are endemic.

The oils obtained by hydrodistillation of the aerial parts of *Stachys inflata* Benth., *Stachys setifera* C. A. Mey. and *Stachys turcomanica* Trautv. were analyzed by GC and GC/MS. More than 90% of each oil was identified. The essential oil obtained by hydrodistillation of the aerial parts of *S. inflata* and *S. turcomanica* were characterized by a high amount of monoterpene hydrocarbons (42.7% and 43.4%) and sesquiterpene hydrocarbons (23.7% and 37.4%) of which α -pinene (14.2% and 28.8%), germacrene D (10.4% and 24.4%) were the major constituents, whereas water distilled essential oil of the aerial parts of *S. setifera*, was rich in fatty acids (36.6%) of which hexadecanoic acid (24.6%) and linoleic acid (12.0%) were the major constituents.

Effects Of Harvest Time On Essential Oil Of Anis Fruits (Pimpinella Anisum L.)

M. Pirali-Hamedani ¹, A. Hadjiakhoondi ² and E. Esmaelii ²

¹ Department of Pharmaceutical Chemistry, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran

² Department of Pharmacognosy, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran, hadjiakhoondi@yahoo.com

Abstract

Anise (*Pimpinella anisum* L.) is an annual, herbaceous plant belonging to the Apiaceae family. It is used in medicinal, food and cosmetics industries. In this investigation, the effects of harvest time of anise fruits on the essential oil content and its constituents were investigated. Seeds were cultivated on the mid of March (11th of March) and carried out in the farm of agriculture, Tarbiat Modarres university. The plants irrigated. Hoeing and mechanical weeding took place when needed. We harvested the fruits at unripe and ripe stages. The collected fruits were dried in the shade. The essential oil of each treatment was extracted by hydrodistillation by Clevenger apparatus. The essential oil constitute was analysed by GC/MS method. According to the results harvest time had significant effect on the essential oil contents and its constituents. The fruits harvested at unripe stage, accumulated highest amount of essential oil (5.53%) than the ripe fruits (3.4%). The essential oil of unripe fruits contained the highest amount of trans-anethol (90.35%) than the other treatment (80.7%).

Chemical Constituents and Biological Study of the Essential Oil of *Polyphium Involucratum* (pall.) Boiss

M. Pirali-Hamedani¹, A. Hadjiakhoondi² and E. Esmaelii²

¹ Department of Pharmaceutical Chemistry, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran

² Department of Pharmacognosy, School of Pharmacy; Tehran University of Medical Sciences (TUMS), Tehran, Iran

Abstract

Polyphium involucratum (umbelliferae) is an endemic plant of Iran. The seeds of this plant have a strong odor. The chemical constituents of the essential oil and the biological properties of *Polyphium involucratum* was investigated and showed containing 3.8% essential oil. The constituents were identified by GC and GC/MS. The major components were limonene (60.39%), aldehyde and alcohol derivatives of limonene (32.45%). The antivectorial effect of its essential oil was also investigated on Anophel Stephensi and Colex Pipiens larva. LD₅₀ of the essential oil for 10, 20 and 30 minutes were achieved 8.01, 10.74 and 13.59 mg on Aophel larva and also the related LD₅₀ on Colex larva were achieved as 3.68, 4.75 and 5.13 mg respectively at same times. These results can be considered in *P.involucratum* antivectorial effects and also can be used for its properties in prevention of malaria .

Essential oil composition of *Citrus aurantium* L. volatile oil and the hydrolat

Z. Alhani, H. R. Monsef-Esfahani, Y. Amanzade, M. A. Faramarzi

Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran; E-mail: niaalhani@yahoo.com

Abstract

The genus *Citrus* (Rutaseae) comprises various species, varieties and hybrids, most of them are found in the north, south and south-eastern parts of Iran. Among them, *Citrus aurantium* L. is the unique specie with pharmacologically applications which the hydrolat of its flowers has been used in traditional medicine as a remedy for treatment of mild depression, sedation and as a heart tonic.

The flowers of *Citrus aurantium* were collected from Darab (Fars province) during April 2003. The neroli oil and hydrolat were obtained from air-dried flowers by hydrodistillation method. GC/MS analyses were performed on the samples followed by computer library and Kovats index for identification of the compounds.

The major compounds in the neroli oil were linalool (19.5%), geraniol (19.3%) and 2 α -pinen (8.9%). Geraniol (26.6%), α -terpineol (20.7%) and linalool (15.4%) were the main constituents in the respected hydrolat.

Chemotypical characterization of the Essential oil of *Rhodiola rosea* L from Mongolia

S. Shatar, S. Altantsetseg and O. Sarangerel

Institute of Chemistry and Chemical Technology of the Mongolian Academy of Science. Ulaanbaatar-51, Mongolia

Abstract

The *Rhodiola rosea* L (Crassulaceae) has been reported that it was as food product and also used in folk traditional Mongolian and Tibetan medicine [1,2].

The result of the Gas Chromatographic and Mass-spectroscopic analysis of the essential oil from *Rhodiola rosea* L is discussed in Table I.

Table I. Comparative Study of the Essential Oils of *Rhodiola rosea* L in Mongolia (%)

	Rhodiola rosea [3]		Rhodiola crenulata [4]	Rhodiola fastigiata [4]	Rhodiola yunnanesis [5]
	Mongolia	Norway	Tibet	Tibet	China
geraniol	25.93-32.33	12.49	53.3	45.3	21.92
n-octanol	-	2.79	13.4	12.3	-
1-octanol	13.43-13.73	-	-	-	28.25
Myrtenol	14.94-15.71	+	3.00	-	4.66
Linalool	-	-	-	-	-
Trans-pinocarveol	11.0-11.63	+	-	-	-
n-decanol	-	30.38	-	-	4.09
α -pinene	-	4.69	-	-	-
Limonene	-	4.61	-	-	-
total	65.30	59.96	69.7	57.6	58.92

Most of these compounds are oxygenated monoterpenes (65.30%-73.40%). The main percentage of Essential oil occurs in the oxygenated compounds such as geraniol (25.93%-32.33%), 1-octanol (13.43-13.73%), myrtenol (14.94-15.71%) and trans-pinocarveol (11.0-11.63%) respectively.

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Trigeminal Chemosensation and its Relation to Physiological Odor Effects

E. Heuberger¹, C. Springsics, R. Meddeb and G. Buchbauer

¹ Dept. of Pharmaceutical Chemistry, University of Vienna, Althanstr. 14, A-1090 Vienna Austria

Abstract

Besides the olfactory system the nasal trigeminal system is the second sensory system which decodes quality aspects of chemosensory stimuli. In mammals, trigeminal chemosensation is biologically significant since it serves as an alarm system in the presence of potentially harmful smells, and the nasal trigeminal system is connected anatomically with brain regions regulating the arousal state, e.g. the ascending reticular activation system (ARAS). Although almost every odor possesses trigeminal components (1) trigeminal stimulation is an often neglected feature of volatile substances. Nevertheless it may be a crucial factor to the effects of odorants like essential oils on humans. In previous work (2, 3) we found that East Indian Sandalwood oil and α -santalol increased the arousal level of healthy human subjects after inhalation whereas they decreased it after transdermal absorption. In the present investigation we tested the propensity of these odorants to stimulate the trigeminal system in comparison to a strong nasal irritant (NH₃) and a “pure odorant” (2-PEA) by means of psychophysical procedures.

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Composition and antimicrobial activity of the essential oil of *Saccocalyx satureioides* Coss. et Dur.

H. Laouer¹, N. Chaker¹, G. Ferretti², C. Reynaud³, G. de Billerbeck³, L. Haddioui³, H. Belkhef³

¹ Natural Biological Resources Valorization Laboratory (NBRVL), Faculty of Sciences, University Ferhat Abbas of Setif, 19000, Algeria.

² Dipartimento di Scienze Chimiche, Università degli Studi di Camerino-Via S. Agostino n. 1 62032 Camerino, Italy.

³ Université Paul SABATIER, Toulouse, France

Abstract

The water-distilled essential oil from dried aerial part of *Saccocalyx satureioides* Coss. et Dur., was endemic in Algeria, was analysed by GC and GC/MS. Borneol (58,6%), α -terpineol (7,9%), thymol (11,54%), and p-cymene (2,6%) were the most abundant components. The antimicrobial activity of *S. satureioides* oil was studied using the agar diffusion test on 4 strains of bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae*). The two-fold oil solution showed a stronger antimicrobial activity against all microbes, except on *P. aeruginosa* AT 27853 (the most resistant organism).

Key word index: *Saccocalyx satureioides*, Lamiaceae, essential oil composition, borneol, α -terpineol, thymol, and p-cymene, antimicrobial activity.