Revista Virtual de Química
ISSN 1984-6835

8º Simpósio Brasileiro de Óleos Essenciais e Simpósio Internacional de Óleos Essenciais

Palavras-chave: Óleos essenciais; Produtos Naturais; Biodiversidade; Aroma de alimentos; Perfumes; Fitofármacos.

O Simpósio Brasileiro de Óleos Essenciais (SBOE) é um evento científico itinerante que acontece a cada dois anos. Nele reúnem-se pesquisadores e estudantes que desenvolvem seus trabalhos nas diferentes disciplinas envolvidas na prospecção, uso e aplicação de óleos essenciais no país. A multidisciplinaridade é uma das características das sessões do SBOE, bem como o alto percentual de participação de estudantes de graduação e pós-graduação. A estrutura básica do simpósio inclui a apresentação de conferências plenárias e conferências curtas de pesquisadores com sólida contribuição ao estudo de óleos essenciais e substâncias voláteis de plantas. Também há espaço para as comunicações orais de jovens cientistas e alunos de pós-graduação, além das sessões de pôsteres. O 8º SBOE foi, pela primeira vez, um evento de caráter internacional, na forma de simpósio conjunto (*joint symposium*) com o *International Symposium on Essential Oils*, o principal evento da área de óleos essenciais, que ocorre anualmente no continente europeu desde 1969. Além de um elevado número de conferencistas estrangeiros, na programação foi previsto um dia dedicado somente a apresentações sobre óleos essenciais de plantas nativas e cultivadas nas Américas, o 1st *Pan American Symposium on Essential Oils*, realizado também pela primeira vez, tendo como objetivo atrair e integrar os pesquisadores da área no continente americano. O escopo do simpósio foi limitado aos óleos essenciais e substâncias voláteis de plantas e os tópicos previstos na programação incluem recentes abordagens na análise, metabolômica e atividade biológica de óleos essenciais e substâncias voláteis, bem como a aplicação destas substâncias em estudos taxonômicos, seu papel nas interações planta-inseto e nos estudos de melhoramento e cultivo de plantas aromáticas. Registraram-se 346 participantes, dos quais 285 do Brasil e 61 estrangeiros, de 15 nacionalidades. Cerca de metade do número de inscritos era de profissionais de universidades, institutos de pesquisa e indústrias. A outra metade era composta de estudantes de graduação (19 %) e pós-graduação (32 %). Foram apresentadas 7 conferências plenárias, 14 conferências curtas ou *keynotes* e 10 apresentações orais, sendo 6 destas na sessão para jovens cientistas, além da apresentação de 219 trabalhos sob a forma de pôster.

Agradecimentos: Agilent Technologies, IFF, Waters, Natura, Chromaleont, CNPq, FAPERJ, Leco, CAPES, Jardim Botânico do Rio de Janeiro,

Realização: Embrapa, UFRJ, Infobibos.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

8th Brazilian Symposium on Essential Oils and International Symposium on Essential Oils

Keywords: Essential oils; Natural Products; Biodiversity; Food flavour; Perfumes; Phytomedicines.

The Brazilian Symposium on Essential Oils (SBOE) is an itinerant scientific event that takes place every two years. It is the meeting spot for researchers and students who develop their work in the different disciplines involved in the exploration, use and application of essential oils in Brazil. A multidisciplinary approach is one of the characteristics of SBOE sessions as well as the high percentage of participation of undergraduate and graduate students. The symposium basic structure includes the presentation of plenary lectures and short conference from researchers with solid contribution to the study of essential oils and volatile substances from plants. There is also room for oral presentations of young scientists and graduate students, in addition to poster sessions. The 8th SBOE was be, for the first time, an international event, in the form of a joint symposium with the International Symposium on Essential Oils, the main event of the essential oils field, which takes place every year in Europe since 1969. In addition to a large number of foreign lecturers, the program included a day dedicated only to presentations on essential oils from native and cultivated plants from the Americas, the 1st Pan American Symposium on Essential Oils, held also for the first time, and aimed to attract and integrate researchers from the Americas. The scope of the symposium was limited to essential oils and volatile substances from plants, including recent approaches in the analysis, metabolomics and biological activity of essential oils and volatile substances, and the application of these substances in taxonomic studies, their role in insect-plant interactions and studies of breeding and cultivation of aromatic plants. Three hundred and forty six participants were registered, with 285 Brazilians and 61 foreigners from 16 nationalities. Half of the assistance was composed by professionals from universities, research institutes and industries. The other half comprised under graduated students (19 % of the total registered) and graduated students (32 % of the total). A total of 7 plenary lectures, 14 keynotes and 10 short oral communications were presented, including 6 presented by young scientists, as well as 219 posters.

Acknowledgements: Agilent Technologies, IFF, Waters, Natura, Chromaleont, CNPq, FAPERJ, Leco, CAPES, Jardim Botânico do Rio de Janeiro,

Realization: Embrapa, UFRJ, Infobibos.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Identification and Accumulation of Chemical Substances from Aerial Parts of *Baccharis trimera* (Less.) DC. var. CPQBA-1 under Organic Fertilizer Levels

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Keywords: Essential oil; brazilian medicinal plant; agronomic factors.

Baccharis trimera is a popular Brazilian medicinal plant long used by indigenous people for a number of purposes. Despite the importance of phytotherapy, this still lacks agronomic studies in order to improve the supply of raw material in quantity and quality adequate, mainly about natural essential oil. This study aimed to investigate the identification and accumulation of chemical substances from aerial parts of *B. trimera* (Less.) DC. var. CPQBA-1 cultivated under different organic fertilizer levels and 2 harvests (120 and 242 DAT). This study was conducted in Botucatu - São Paulo State, Brazil. The experimental design was randomized blocks with four replicates and five levels of organic fertilizer: 10, 20, 30, 40 and 50 ton ha^{-1} and control (0 ton ha^{-1}), with spacing 0.6 X 0.6 m among plants. Aerial parts were harvest at 120 and 242 DAT. The plants were dried in an artificial dryer at 38 °C for 36 h and distilled in Clevenger type apparatus to obtain essential oil. Analysis was carried out in a GC/MS (Shimadzu QP-5000) at 70 eV, fitted with a fused silica capillary column DB-5 (30 m X 0.25 mm X 0.25 µm), helium as carrier gas (1.7 mL min^{-1}), injector at 240 °C and the following oven temperature program: 60-95 °C (3 °C min^{-1}); 95-130 °C (8 °C min^{-1}); 130-190 °C (3 °C min^{-1}); 190-240 °C (10 °C min^{-1}); split at 1:20; flow: 1 mL min^{-1}. Identification of chemical constituents was performed by comparison of the mass spectra of the substances with the database of GC/MS system (NIST 62.lib), literature and retention index. Retention indices (RI) of the compounds were obtained by injection of a standard mixture of *n*-alkanes applying the equation of Van den Dool and Kratz.[1] Quantification of the compounds was made in a GC/FID (detector at 230 °C) operating under the same conditions of the GC/MS system. Means were submitted to analysis of variance and averages compared by *Tukey* test at 5 % probability. Twenty-five substances were identified on essential oil samples. The major chemical substances and accumulations (coefficient of variation, level of organic fertilization and DAT) were: *trans*-caryophyllene 15.8 (9.7 %, 50 t ha^{-1}, 120 DAT); germacrene D 15.3 (14.0 %, 50 t ha^{-1}, 120 DAT); bicyclogermacrene 23.9 (13.9 %, 30 t ha^{-1}, 120 DAT); spathulenol 25.2 (53.3 %, 20 t ha^{-1}, 242 DAT) and caryophyllene oxide 7.0 (36.7 %, 20 t ha^{-1}, 242 DAT). These are considered promise in the aerial part of *B. trimera* and it can be useful to next studies about natural products from *B. trimera* essential oil.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Identification of Essential Oil of *Piper capense* (Piperaceae) from Limpopo Province, South Africa

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Keywords: *Piper capense*; Piperaceae; essential oil; South Africa.

'Wild Pepper' (*Piper capense)* is a straggling shrub or small tree that is known as Mulilwe in the Venda sub-region of Limpopo Province, South Africa. Our interest in its essential oil components arose mainly because it is used by local traditional healers for treatment of various ailments *inter alia* sore throat, tongue sores and venereal diseases.[1,2] Plant materials were collected from Zoutspansberg mountains and voucher specimens were deposited at the Thohoyandou Botanical Gardens (Venda Herbarium). Fresh leaves (300 g) and stem (300 g) were separately subjected to hydrodistillation in a modified Pyrex® Barett distilling receiver apparatus for approximately 3 h. The oil yields (% w/w) were 0.15 % and 0.03 %, respectively. The essential oils were analyzed using a Hewlett Packard 6890 GC/MS system (HP-5MS column) and mass spectra was recorded by a HP 5937 series mass selective detector (MSD). Identification of oil components was achieved based on their retention indices and by comparison of their mass spectral fragmentation patterns (NIST database/ ChemStation data system).The leaf oil contains δ-3-carene (16.0 %), citronellyl acetate (1.4 %), *o*-cymene (12.0 %), limonene (40.0 %), linalool (6.2 %), *p*-menth-2-en-1-ol (1.7 %), *cis*-β-ocimene (8.6 %), *trans*-β-ocimene (1.7 %), α-phellandrene (10.2 %) and α-terpinolene(2.2 %), while the stem oil contains δ-3-carene (36.1 %), citronellyl *n*-butyrate (2.0 %), *o*-cymene (9.1 %), limonene (25.8 %), linalool (10.9 %), *p*-menth-2-en-1-ol (1.2 %), *cis*-β-ocimene (12.1 %), α-phellandrene (1.7 %) and piperitone (1.2 %). Citronellyl acetate, *trans*-β-ocimene and α-terpinolene are only found in the leaf oil whereas citronellyl *n*-butyrate and piperitone are only found in the stem oil. A comparison of essential oil components of *P. capense* with those of different geographical origin will also be given.

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Acknowledgements: SA-NRF, Univen, Forthare (GC/MS analysis), UCT.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Synergistic Potential of Dillapiole Oil for Synthetic Pyrethroid Insecticides against the Fall Armyworm.

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Keywords: *Piper aduncum*; essential oil; methylenedioxyphenyl compounds; brazilian Amazonia.

Spodoptera frugiperda (J.E. Smith) is a serious lepidopterous pest of several economically important crops. Control of the fall armyworm depends exclusively on insecticides, developed resistance to the major classes of insecticides. There has been mounting interest in the use of synergism to reduce this resistance by combined application of insecticide. It was shown that piperonylbutoxide (PBO) and others methylenedioxyphenyl compounds inhibited the microsomal oxidation of many insecticides and other xenobiotics in a number of insect species. *Piper aduncum* L. is a widespread tropical shrub, known as an invading plant in Amazon areas deforested after timber exploitation. The leaves and stems of *P. aduncum* contain an essential oil composed mainly of dillapiole (5-allyl-6,7-dimethoxy-1,3-benzodioxole),[1] which has demonstrated to have synergistic effects with several pesticides. The objective of this study was to evaluate the synergy and response homogeneity of the *S. frugiperda* larvae population to the essential oil of *P. aduncum* in combination with pyrethroid insecticides: α-cypermethrin, β-cypermethrin, fenpropathrin and γ-cyhalothrin, compared with piperonylbutoxide (PBO positive controls). By the ratio of the LC_{50} and LD_{50} of the insecticides taken singly and their respective synergistic combinations with essential oil and (PBO), the synergism (FS) factors for comparison with each other were obtained. The slope of the dose/concentration-mortality curves was used to establish the relative toxicity increase promoted by synergism and to determine the response homogeneity. Residual contact revealed a significant potentiation for commercial insecticides formulated with β-cypermethrin (FS= 9.05-0.5), fenpropathrin (FS= 34.05-49.77), when combined with the essential oil of *P. aduncum*. In the topical contact, there occurred significant potentiation only for the α-cypermethrin (FS= 7.55-3.68), fenpropathrin (FS= 3.37-1.21) and γ-cyhalothrin (FS= 5.79-10.48) insecticides when combined with the essential oil. Except fenpropathrin and γ-cyhalothrin, other synergistic combinations presented homogeneous response by topical contact as well as residual, for at least a synergistic combination with the essential oil of *P. aduncum*. The FS significance values of combinations of the *P. aduncum* essential oil with insecticides α-cypermethrin, β-cypermethrin, fenpropathrin and γ-cyhalothrin, may indicate that this essential oil as an alternative to PBO.

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Acknowledgements: Embrapa, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Preliminary Investigation of Antimicrobial Capacity from Essential Oil of *Liquidambar styraciflua* L., Altingiaceae

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Keywords: *Liquidambar styraciflua*; antimicrobial activity; diffusion method.

The genus *Liquidambar* is the best known of the family Altingiaceae Horan and one of the species of this genus, *Liquidambar styraciflua.*[1] Its antimicrobial activity from the essential oil is well known, so we carried its evaluation through the technique of diffusion in solid medium from the hole, with some modifications.[2] The leaves of *L. styraciflua* were collected at Embrapa forests in Curitiba-PR. A voucher specimen was sent to the herbarium of the Federal University of Paraná. The extraction of the essential oil from the dried leaves of *L. styraciflua* was performed by hydrodistillation, using the Clevenger apparatus. To achieve the antimicrobial tests were selected eight standard strains of gram-positive and gram-negative and five standard yeast strains. The essential oil was analyzed at four different concentrations by serial dilution procedure (1:2, 1:4, 1:8, 1:16), using dimethylsulfoxide as solvent. The positive controls used were ketoconazole and terbinafine solutions (50 µg and 20 μL^{-1}) for yeast, and standard antibiotic disks of NEWPROV company (Ampicillin 10 µg, Erythromycin 15 µg, Gentamycin 10 µg, Penicillin 10 µg, Vancomycin 30 µg and Tetracycline 30 µg) for bacteria, applying the methodology of the disk diffusion.[3] The results were analysis using the statistical program SISVAR. The essential oil, in its lower dilution (1:2), showed antibacterial capacity of all tested strains except for *E. faecalis* (ATCC 29212) and *S. pyogenes* (ATCC 19615). *S. aureus* (ATCC 25923) was the most sensitive to the antimicrobial action of the essential oil. In the data analysis, it´s possible to see that the action of the essential oil, in all dilutions, has not overcome the action of antibiotics standards, but there are similarities between some. The greatest inhibition capacity of the essential oil was to for *S. aureus* and *B. subtilis* (ATCC 6633), presenting a similar action to the standard Vancomycin, Penicillin, Ampicillin and Gentamicin ($p < 0.01$). Antifungal activity was observed only against *C. albicans* and *C. tropicalis*, which is the most sensitive to the action of the essential oil, but lower than the reference standards used.

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Acknowledgements: Embrapa, UFPR, REUNI.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Analysis of Plant and Marine Products by Using (Low-)Flow Modulated Comprehensive 2D Gas Chromatography-Mass Spectrometry

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Keywords: fish lipids; Artemisia essential oils; GC×GC-MS flow modulation.

One of the major limitations of current-day flow-modulated comprehensive two-dimensional gas chromatography (FM GC×GC) is the generation of high gas flows (e.g., 20 mL min^{-1}) in the second analytical dimension. Even though such high flows are necessary to efficiently flush the content of the modulator onto the second dimension, they also greatly restrict the employment of mass spectrometry (MS). One way to enable the use of MS systems, in FM applications, is to divert a substantial part of the second-dimension flow to waste. It is obvious that such a choice has a negative impact on sensitivity.The present contribution is focused on the development of high sensitivity methods using flow-modulated comprehensive two-dimensional gas chromatography-mass spectrometry. Specifically, an FM GC×GC-MS approach was developed in which the flows necessary to efficiently flush the modulator were greatly reduced. Consequently, there was no need to divert flow to waste. The efficiency of the set-up is demonstrated on fish oil fatty acids and a sample of Artemisia essential oil.

Acknowledgements: This work has been carried out within the framework of the Research Project "PRIN 2012": assessment of quality and safety of Mediterranean seafoods by "omics" sciences, supported by the Italian Ministry of University and Scientific Research, nr. 2012TLC44W.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oils Synergism in Biocompound for Treatment of Cracks in Diabetic Patients' Feet

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Keywords: *Syzygium aromaticum;Melaleuca alternifolia;* diabetic foot.

Essential oils are products with great therapeutic and pharmacological potential.[1] The main constituents of essential oil of cloves (*Syzygium aromaticum*) are eugenol and β-caryophyllene. Eugenol has antiviral, anti-ulcer, anesthetic, anti-inflammatory and antimicrobial properties, while β-caryophyllene reduces edema and is an anti-inflammatory.[2] The essential oil of tea tree (*Melaleuca alternifolia*), or tea tree oil, has healing and anti-infectious properties.[3] Its main constituents are the monoterpenes. The purpose of this study was to analyze the chemical constituents of "healing biocompound" given the results obtained in the treatment of cracks in the feet of diabetic patients. It is a product made from essential and carrier oils, registered on the health regulatory agency (ANVISA), with antiseptic and healing properties, and empowering and revitalizing the skin, produced by Magia da Mata Cosméticos Ltda. Study population: 47 diabetic patients type 2 volunteers, adults of both sexes, registered in the Association of Diabetics of Nova Friburgo, RJ (ADINF), which had cracks in the feet.They were divided into 2 groups: Study group - 25 patients treated with biocompound; Control group - 22 patients treated with 10 % urea cream. Evaluation: weekly visits by 119 days, with a photograph of the lesions measured and assessment of complications, healing of fissures and therapeutic efficacy (decreased as the lesion, absence of complications - pain, bleeding, itching, and increase lesion areas). Results: Study group: 72% were cured and 28 % showed improvement. All reported improved local pain, itching and swelling. Chromatographic analysis of essential oil of *Syzygium aromaticum*: main eugenol (84 %) and β-caryophyllene (6 %), components with important properties for the study (antimicrobial, anti-inflammatory, anesthetic and inhibiting edema); of essential oil of *Melaleuca alternifolia*: monoterpenes (42 %) (anti-infective and analgesic); the biocompound: presence of these key components. The effectiveness of the biocompound was possibly due to the presence of eugenol, β-caryophyllene and monoterpenes, properties whose combined acted to reduce the inflammatory process and allow healing of the lesions.

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Acknowledgements: FAPERJ, ADINF, FINEP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Diversity in Essential Oil of *Eplingiella fruticosa* (Salzm. ex. Benth.) Harley & J.F.B. Pastore Genotypes

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Keywords: *Hyptis fruticosa*; medicinal plant; active germplasm bank.

Eplingiella fruticosa (syn *Hyptis fruticosa*) is a native Lamiaceae plant from Brazil who has wide use in folk medicine as an analgesic and anticonvulsant. Recent studies show great variability in the chemical composition of the essential oil from *E. fruticosa*, related different conditions to soil and climatic.[1,2] The aim of this study was to characterize the composition and the chemical diversity of the essential oils from 12 *E. fruticosa* genotypes kept in active germplasm bank (BAG) deployed in the State University of Feira de Santana. About 100 g of leaves per replicate of each genotype were used for hydrodistillation with Clevenger type apparatus for 3 h. The identification of the compounds and their contents was performed by GC/FID (Agilent 6890N) and GC/MS (Agilent 5973N), both with HP-5 fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1 mL min^{-1}. Oven temperature from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated at 70 eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. We use 15 major compounds data in diversity analysis. Cluster analysis and canonical variables were made, using as dissimilarity measure the Mahalanobis distance (D^2). Identified compounds were classified in two major classes of terpenes, mono and sesquiterpenes, with higher for the latter percentage ranging from 49.8 % to 68.7 %, while the monoterpenes showed a percentage ranging from 24.0 % to 38.7 %. The cluster analysis result classify the genotypes in four clusters: 1 - formed by genotypes EF001, EF006, EF007, EF008, EF010, EF011 and EF012 with (*E*)-caryophyllene and bicyclogermacrene as major compounds; 2 - EF002 and EF003 genotypes showed that the same, however, percentage with average about 30 % higher; 3 - EF004 and EF005 genotypes that showed a greater production of (*E*)- caryophyllene; and 4 - with EF009 genotype, forming a single group to present α-pinene as balanced majority and percentage among the rest. This result was confirmed by canonical variables, which explained 76 % of the variation. The bicyclogermacrene, 1,8-cineole, α-copaene and spathulenol compounds were the most important.

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Acknowledgements: Fapesb, CAPES, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Effects of Drying Kinetics on Essential Oil Yield from *Piper aduncum* L. Leaves

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Keywords: *Piper aduncum* L.; essential oil; Amazon; yield

Piper aduncum L., better known as "pimenta-de-macaco", is one of the most important natural resources of dillapiole, a chemical compound with great potential to be used in the insecticide industry. The yield of the essential oil of this plant, however, is directly affected by the conditions applied before extraction. This work intends to evaluate the effects of natural air-drying on the yield of essential oil of leaves from *P. aduncum* L. To perform the tests, plants of this species were collected and cleaned at Embrapa Amazônia Ocidental in December, 2014. The drying process was performed at the same location during an 8-day period, divided into 5 groups with 5 repetitions each. The effective diffusivity coefficient was determined based on Fick's law and the drying curve obtained from moisture ratio and time data was fitted to five different mathematical models. The drying of *P. aduncum* L. leaves took place only in the falling rate period, since the constant rate period was absent in the drying curve. Among the models investigated, the two terms model was found to best fit the behavior of the drying curve, showing the highest value of R^2 = 0.9946 and low values of χ^2 = 0.00219, RMSE = 0.032715 and MBE = 0.005351. The results showed that the yield of essential oil was inversely related to the moisture content present in the biomass, given that the yield of dried leaves after 192 h was 2.7 times higher than from the initial fresh ones. Therefore, it is recommended that leaves from *P. aduncum* L. are submitted to drying process prior to the extraction in order to have a greater yield efficiency in a larger scale production.

Acknowledgements: Embrapa Amazônia Ocidental, FAPEAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Nitrogen Sources on Growth, Production and Quality of Essential Oil in *Cymbopogon citratus* and *Cymbopogon flexuosus*

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Keywords: Lemongrass; compost; manure; β-myrcene; citral

Cymbopogon citratus (DC) Stapf and *Cymbopogon flexuosus* (Nees ex Steud.) Will. Watson, (Poaceae) are aromatic plants which produce essential oil rich in citral used in food, pharmaceutical and cosmetic industries. This study aimed to evaluate the effect of nitrogen sources on *C. citratus* and *C. flexuosus* development, essential oil production and the physiological efficiency of nitrogen at 160 and 220 days after planting (DAP). The experiment was carried out at field conditions in a randomized block design comparing the effect of four nitrogen sources (composting of bovine manure, goat manure, poultry manure and urea), with four replications, each one with ten plots and twenty five plants. The results showed that the nitrogen sources did not affect the biomass production and essential oil yield and composition of *C. citratus* for both crops, *C. flexuosus* fertilized with composting bovine manure resulted on decrease of fresh and dry biomass accumulation and essential oil yield at 220 DAP. The increase in essential oil production derived by N fertilization was due to an increase of leaf biomass. *C. flexuosus* produced higher biomass and essential oil yield than *C. citratus* with average between harvests of 3257.5 kg ha $^{-1}$ and 29.1 40 kg ha $^{-1}$ and 592.9 kg ha $^{-1}$ and 11.5 kg ha $^{-1}$ respectively. The essential oil content of *C. citratus* was of 17.7 g kg $^{-1}$ to 160 DAP and 21.3 g kg $^{-1}$ to 220 DAP, 52 % higher than to *C. flexuosus* that reached 10.0 g kg $^{-1}$ and 8.4 g kg $^{-1}$ respectively. The *C. flexuosus* was also more efficient in the use of physiological N in biomass yield than *C. citratus* in both crops. The nitrogen source did not affect the essential oil composition. The major components of the essential oil of *C. citratus* were geranial (39.7 to 42.2%), neral (29.6 to 31.1%), geraniol (4.4 to 5.7%) and β-myrcene (11.7 to 15.2%), while *C. flexuosus* presented geranial (43.6 to 50.7%), neral (29.3 to 34.4%), geraniol (1.6 to 7.5%) and absence of β-myrcene. The citral content in *C. flexuosus* was 10 % higher than *C. citratus*.

Acknowledgements: Embrapa

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Plant Density and Harvest Season Influence the Essential Oil yield and α-humulene Content in *Varronia curassavica* DC.

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Keywords: Chemical composition; essential oil; medicinal plant; cropping system.

Varronia curassavica is a native species from Brazil, known as "erva-baleeira" and traditionally used as anti-inflammatory. This activity is scientifically validated and assigned to the essential oil constituents stored in their leaves.[1] It is known that the yield of active compounds and therefore the therapeutic properties can be changed due to several factors, including the harvest season and cropping system.[2] This work aimed to evaluate the essential oil yield and α-humulene content in plants grown at different densities between plants and harvested in three seasons. *V. curassavica* seedlings were obtained from CPQBA-UNICAMP and transplanted in five densities between plants (0.4 X 1.0 m; 0.6 X 1.0 m; 0.8 X 1.0 m; 1.0 X 1.0 m; 1.0 X 1.6 m) at the Experimental Research Station of EPAMIG, Oratórios-MG, Brazil. They were harvest on three seasons: December 2013 (summer), April 2014 (autumn) and August 2014 (winter). The leaves were separated of stems, weighed and dried in an oven with forced air circulation (40 °C) to constant weight. Dried leaves (100 g) were subjected to hydrodistillation separately in a Clevenger-type apparatus for 3 h each. The oils were analyzed by GC/FID and GC/MS. The content of α-humulene were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Oil yields were significantly higher (1 and 0.8 %) in winter for 0.8 X 1.0 m and 1.0 X 1.0 m spacing between plants, respectively. The α-humulene content changed between seasons: summer (4.9 %), autumn (4.9 %) and winter (3.9 %).

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Acknowledgements: FAPEMIG, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Potential Use of *Baccharis dracunculifolia* Essential Oil to Control White Mold in Bean

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Keywords: Alternative control; *Phaseolus vulgaris*; plant diseases.

Common bean (*Phaseolus vulgaris* L.) is a susceptible plant to diseases caused by pathogens. *Sclerotinia sclerotiorum* (Lib.) de Bary is a causative agent of one of the most destructive diseases of bean called white mold. White mold control is accomplished mainly through the use of synthetic fungicides, which can lead to higher costs of production and environmental problems. The research of alternative methods to control pests and diseases through essential oils and plant extracts has increased considerably.[1,2] This study aimed to evaluate the antifungal activity of *Baccharis dracunculifolia* essential oil on *S. sclerotiorum* (Ss) of bean leaves. *Sclerotia* were disinfected with ethanol-70 % followed by 1 % sodium hypochlorite for 3 min. After that, they were washed in sterile water and transferred to Petri dishes (9 cm diameter) containing 15 mL of PDA (Potato Dextrose Agar) with chloramphenicol (100 mg L^{-1}), which were maintained at 23 °C in the dark. Bean plants were grown in 5 L pots with a commercial substrate. Thirty days after plant emergence, leaflets of the youngest trifoliate leaf of each plant were detached, brought to the laboratory and put into plastic boxes lined with moist paper towel. The *B. dracunculifolia* essential oil was sprayed on the leaflets using a Potter tower in the concentrations of 0, 0.5, 1, 2 and 4%. Ss mycelial discs (5 mm diameter) were transferred from the Petri dishes to the leaflets surface. Boxes were maintened at 23 °C in the dark. The colony diameter was measured after 24 and 48 h using a digital caliper. The experimental design was completely randomized with four replicates per treatment. At the concentration of 4 % of essential oil of *B.dracunculifolia* there was complete inhibition of the fungal growth. This result indicates that the essential oil of *B. dracunculifolia* field could be used for the management of Ss on common beans.

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Acknowledgements: FAPEMIG, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antifungal Activity of the Essential Oil and Semisynthetic Derivatives Dillapiole against Wood Degrading Fungi

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Keywords: *Pycnoporus sanguineus*; *Trametes villosa; Lenzite strabea*.

Fungi are biological agents that attack wood, as are conditions for use of wood constituents for its development. The control of wood decaying fungi is is usually done with the use of chemicals such as at the fungicide Dynasty. *Piper* species are sources of bioactive essential oils and are of great interest to the industry. Previous studies have demonstrated fungistatic activity of etheric derived dillapiol (methyldillapiol ether, ethyldillapiol ether and butyldillapiol ether) using the method of bioautographic front of *Pycnoporus sanguineus*, *Trametes villosa* and *Lenzite strabea* fungus.[1] In our study, from the essential oil (2.0 g) of *Piper aduncum* was isolated dillapiol as major substance (831.3 mg) by CC silica gel with hexane and ethyl acetate gradient, identified by spectroscopic methods. The dillapiol synthetic derivatives have been prepared and all isolated by flash CC and preparative chromatography, whose identities were confirmed by ^{1}H NMR and MS.[2] This study aimed to evaluate the antifungal activity of the essential oil , dillapiol and its derivatives (D1: diacetyldillapiol; D2: isopropyletherdillapiol; D3: hydroxymonobenzoyldillapiol; D4: propyletherdillapiol; D5: dihydroxydillapiol; D6: diolcarbonylateddillapiol; D7: 2,2-dimethylmethylenedioxydillapiol; D8: dillapioloctylether) against wood degrading fungi, as an alternative in the search for wood preservatives. These substances were tested for fungistatic activity using bioautography method front fungi *P. sanguineus*, *T. villosa* and *L. strabea*. One mg of each substance was diluted with hexane and applied in 10 and 40 μg at TLC plates on silica gel G60 Merck F_{254} (2 X 5cm) previously autoclaved at 120 °C for 15 min. After 6 h, the plates and the inoculum of the fungus were placed on malt agar culture medium and incubated for 72 h in an oven at 25-27 °C. After fifteen days were made evaluations observing that the essential oil showed activity, inhibiting the growth of fungus *T.villosa* in 40 μg. From the derivatives, only the D4 showed activity inhibiting the growth compared to the three fungi in both 10 and 40 μg. The D2 and D3 inhibited *P. sanguineus* and *T. villosa* in 40 μg. Derivatives D1, D6, D7 and D8 showed only front inhibition for s *L. trabea* in 10 and 40 μg. D5 showed no activity against the tested fungi.

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Acknowledgements: CNPq, FAPEAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of Morphological and Agronomic Characteristics and Essential Oil Production of Two Accesses of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) at Different Plant Ages

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Keywords: *Lippia alba*; seasonality; secondary metabolism.

Lippia alba (Mill.) NE Br. (Verbenaceae) is a plant species widely used in folk medicine, with recognized phytotherapeutic properties related to its essential oil.[1] Essential oil varies qualitatively and quantitatively, according to the genetic material and environmental conditions of growth changing continuously with local and season.[2] The present work aimed to evaluate the morphological and agronomic characteristics, and essential oil production of two *L. alba* accesses according to plant age. In this context, *L. alba* accesses were cultivated in the experimental field of the UFRB, at Cruz das Almas – Bahia, using randomized block in a 2x3 factorial design, with three replicates, A factor - *L. alba* accesses (L001 and L002) -, and B factor - plant age at 30, 60 and 90 days after transplanting-. The sampling periods presented the following climatic variations: (1) September 2014: pluviometric index (IP): 88.2 mm; maximum temperature (Tmax): 28 °C; minimum temperature (Tmin): 18 °C; (2) October 2014: IP: 28.2 mm; Tmax: 30 °C; Tmin: 19 °C; (3) November 2014: IP: 31.5 mm; Tmax: 31 °C; Tmin: 20 °C. Different quantitative and qualitative morphological characteristics, agronomic behavior and essential oil production were evaluated for each age and accesses. The L001 access demonstrated possesses prostrate stem with brown coloring, limbo adaxial surface bright green, rough leaves and purple petals clear, whereas the L002 access possess erect stem with color ranging from brown to purplish, limbo adaxial surface green, soft leaves and clear purple petals. For agronomic characterization, differences were also observed between accesses. L002 access stands out during all the evaluation period, for the parameters fresh (326.9 g plant^{-1}) and dry (109.3 g plant^{-1}) weigh of leaves; leaf area (318.3 cm^2 plant^{-1}), leaf dry matter yield (4373 kg ha^{-1}), leaf area ratio (1.153 cm^2 g^{-1}), specific leaf area (2.92 cm^2 g^{-1}), mass leaf ratio (0.40), with higher averages at 30 days. On the other hand, the L001 access stands out in the content and oil yield, throughout the studied period, being the best results obtained at 60 days (1.4 and 19.4 L ha^{-1}, respectively), while for the L002 access the greatest results for this parameters were obtained at 90 days (0.40 and 5.33 L ha^{-1}, respectively) values not statistically different between the evaluated periods.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Preliminary Tests of the Acaricide Activity of Essential Oil from *Ruta graveolens* L. and *Ocimum gratissimum* L.

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Keywords: Acaricide; *Ruta graveolens* L.; *Ocimum gratissimum* L.

Ruta graveolens L., Rutaceae, is an aromatic species characterized by pentamerous flowers, radial symmetry and rarely zygomorphic. The main components of its essential oil are methylheptylketone (90%), methylnonylcarbinol (10%), methylnonyl alcohols, esters, phenols and terpene compounds. Other aromatic species is *Ocimum gratissimum* L., that can reach one meter in height, with essential oil composed of eugenol, methyl eugenol, linalool, cineol and α-terpineol.[1] The acaricidal activity of many essential oils is already recognized and the house dust mite is the major trigger of allergies due to its waste and remains of dead mites which have a cysteine protease homologous to papain, named Der p1.[2] The goal of this work is to evaluate the acaricidal activity of the essential oils from *R. graveolens* and *O. gratissimum* (donated by the Center for Agroecology Paranaense (CPRA) acquired from a private plantation in Itapetininga (SP)). The methodology of steam distillation was used to obtain essential oil from dry leaves through the Clevenger apparatus. Dust mites and house dust mite of the genus *Dermatophagoide spteronyssinus* were collected with a vacuum cleaner and posteriorly cultured in Petri dish containing brewer's yeast. They were kept at room temperature and humidity controlled for two years. The acaricidal activity was made by direct test with the oil of both plants evaluated without dilution, the negative control (alcohol 70 %) and the positive control (commercial acaricide - ADF Solution Plus). Readings were made at 24, 36, 42 and 96 h. The results were subjected to evaluation of statistic variance (ANOVA) and *Tukey* post test ($p < 0.05$). From the results it can be concluded that the first 24 h the essential oil of *O. gratissimum* did not provide an acaricidal activity greater than the positive control, but between 48 and 72 h both species showed a higher activity than the positive control, which is highly significant. Thus, we can conclude that essential oils of *R. graveolens* and *O. gratissimum* are potential miticides, but other tests should be performed to confirm the acaricidal activity.

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Acknowledgements: CAPES, Fundação Araucária.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Influence of Essential Oils from *Lippia gracilis* and *L. sidoides* Genotypes, their Major Compounds and Nanoemulsions against *Lasiodiplodia theobromae*

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Keywords: *Lippia*; thymol; carvacrol; phytopathogens.

The aim of this work was to study the antifungal activity of essential oils, major compounds and formulations of *Lippia gracilis* and *L. sidoides* genotypes. The essential oils were extracted by hydrodistillation. Nanoemulsions were produced according to the spontaneous emulsification method of essential oils from the genotypes LGRA-106 (Nano-106) and LGRA-109 (Nano-109) of *L. gracilis*, LSID-102 (Nano-102) and LSID-104 (Nano-104) of *L. sidoides*, and the major compounds thymol (Nano-thymol) and carvacrol (Nano-carvacrol). The nanoemulsions obtained were characterized in terms of macroscopic, zeta potential, particle diameter, polydispersity and pH. The antifungal activity of the essential oils, thymol, carvacrol and nanoemulsions were tested against the fungus *L. theobromae* at concentrations 10.0, 5.0, 1.0, 0.5 and 0.1 mL L^{-1}. The percentage of inhibition of mycelial growth was calculated, in relation to the control, after 7 days of incubation at 25 ± 3 °C, with a photoperiod of 12 h. The characterization of the nanoemulsions identified Nano-106 and Nano-102 with the smallest particle sizes, 15 nm and 18.83 nm, respectively. The Nano-106 formulation presented as main components thymol (63.4 %) and β-caryophyllene (13.5 %). In the Nano-109 formulation, the major components were carvacrol (45.6 %) and *p*-cymene (12.9 %). The major components of Nano-102 were thymol (83.0 %) and methyl thymol (9.4 %). The Nano-104 formulation presented carvacrol (38.7 %) as the major compound. In tests against the fungus *L. theobromae* the essential oil from genotype LSID-104, and the monoterpene carvacrol showed better activity with percentage of inhibition of mycelial growth of 50 and 100 %, respectively, at the lowest tested concentration. The nanoemulsion that showed the best biological activity was Nano-carvacrol with percentage of inhibition of mycelial growth of 84.6 % at a concentration of 1.0 mL L^{-1}.

Acknowledgements: CNPq, FAPITEC/SE, CAPES, FINEP, RENORBIO.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Influence of Essential Oils from *Lippia alba* Genotypes and their Major Monoterpenes against Stored Grain Insects

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Keywords: Brazilian lemon balm; monoterpenes; insect pests.

The insects *Sitophilus zeamais* and *Tribolium castaneum* cause losses in stored grains and are considered pests of wide distribution and global significance. In the present study, we evaluated the toxicity and repellency of essential oils of different *Lippia alba* genotypes (carvone genotypes LA-13 and LA-57 and citral genotypes LA-10 and LA-44) and their major monoterpenes, carvone and citral, on *S. zeamais* and *T. castaneum.* Toxicity bioassays by exposure of the insects on treated filter paper were performed to determine the concentration and lethal time. Repellency tests were performed using the most toxic compounds according to the toxicity bioassays. The carvone genotypes were more toxic than the citral genotypes for both species: for *S. zeamais*, the LC_{50} values were 15.2 µL mL^{-1} (LA-13) and 16.7 µ LmL^{-1} (LA-57) and for *T. castaneum,* the LC_{50} values were 28.7 µL mL^{-1} (LA-13) and 19.7 µL mL^{-1}(LA-57). Isolated carvone (LC_{50} = 8.8 µL mL^{-1}) was more toxic than citral. For *S. zeamais*, the monoterpene citral had the lowest lethal time (LT_{50} = 6 h), whereas for *T castaneum*, the monoterpenes carvone and citral showed a more rapid toxicity (LT_{50} = 7.3 h). The compounds tested were highly repellent to *T. castaneum*; however, no repellency's effect was observed against *S. zeamais*, except for LA-13 genotype. The essential oils from the carvone genotype and the monoterpene carvone have potential for the development of natural insecticides against stored grain insects *S. zeamais* and *T. castaneum*.

Acknowledgements: CNPq, FAPITEC/SE, CAPES, FINEP, RENORBIO.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Yield and Composition of the Essential Oil from Mint (*Mentha x villosa*) in Monocrop and Intercrop with Vegetables

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Keywords: *Rumex acetosa*; *Lactuca sativa*; essential oil.

The intercrop between vegetables and medicinal plants can promote beneficial interactions among plants of results in diversification of products for different markets and ensure a more secure and continuous source of income for small farmers.[1] Therefore we have chosen lettuce (*Lactuca sativa* L.), azedinha (little bitter - *Rumex acetosa* L.) and mint (*Mentha* x *villosa*) to evaluate the efficiency of intercropping. Lettuce is the leafy vegetable economically most important in Brazil, being cultivated in almost all regions of the country. Mint is a medicinal plant from Europe and cultivated in Brazil because of its essential oil medicinal properties. Environmental stimulus may redirect the metabolic pathway promoting the biosynthesis of different compounds and influencing essential oil composition and yield. The intercrop is a usual agriculture practice in Brazil and may influence the oil composition and yield.[2] The influence of intercropped was verified between lettuce, azedinha and mint on the yield and on essential oils composition of mint. The experiment was carried out from August to December 2014 in Prudente de Morais, State of MG, Brazil. Treatments consisted in intercropping between lettuce, azedinha, mint and monocultures of each species in spacing 0.25 X 0.25 m. Lettuce was harvested on October, azedinha on November and mint on December. The mint essential oil was analyzed by GC/FID and GC/MS. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Oil yield were 0.19, 0.14 and 0.21 % for intercropping between mint and lettuce, mint and azedinha and mint monocrop, respectively. The intercropping between mint and lettuce increased piperitenone oxide content (79 %) of mint oil when compared with the mint monocrop (52 %). The intercrop between mint and lettuce is feasible, since the yield of both species is similar between cropping and intercropping systems, with no change in yield of the essential oil and increase the chemical constituent of interest in the mint oil.

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Acknowledgements: FAPEMIG, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Essential Oil Content of Parsley Submitted to the Hygienizing and Drying Process

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Keywords: *Petroselinum crispum*; processing; essential oil.

Parsley [*Petroselinum crispum* (Mill) Nym.], Apiaceae, is an aromatic herb that is cultivated worldwide.Their strong flavor is derivative from components apiol and myristicin of essential oil. The aim of this study was evaluate the effects of cleaning and drying parsley leaves on the content and quality of its essential oil. The herbs used were cultivated by farmers at the southern state of Rio de Janeiro. Two cases were examined: The A case, which leaves did not undergo by the cleaning process and the B case, which leaves were clean in water and sanitized with 200 ppm of sodium hypochlorite for 15 min. Parsley from the two processes were uniformly distributed on a thin layer and dried at different air temperatures (40, 50 and 60 °C) in an electric dryer tray. The drying process was completed when the product reached a water content of 0.11 d.b. The essential oil of the leaves was extracted using a Clevenger apparatus and quantified based on dry matter.The identification and quantification of chemical compounds were performed using the GC. The *data* were *s*ubmitted to the *variance analysis* at 5% probability (*Tukey* test), compared the treatments with the fresh herb from each case.The average drying time was 40 (40 °C), 24 (50 °C) and 14 h (60 °C). The essential oil content was 0.30 %, 0.36 %, 0.32 % and 0.34 %, for fresh leaves dried at 40, 50 and 60 °C, respectively at the A process and 0.18 %, 0.16 %, 0.14 % and 0.18 %, fresh leaves dried at 40, 50 and 60 °C, respectively at the B process. The lowest essential oil content of the samples was obtained at the B process. The difference is because the plants were taken at different times, in a 2-month interval, February (A) and April (B). On both cases there was a higher essential oil content compared to the fresh herb when they were subjected to drying at 40 °C, however, there was no statistically significant difference between the treatments. Apiol and myristicin were the major components of the essential oil, corresponding to 70 % of the total composition. There was no statistical difference for myristicin content at A process, as follows in percent: 23.05^{a} (fresh), 21.11^{a} (40°C), 23.01^{a} (50°C) and 20.64^{a} (60 °C). At B process, a statistical difference was observed between all the treatments: 31.97^{a} (fresh), 23.20^{b} (40°C), 28.90^{c} (50°C) and 26.14^{d} (60°C). For apiole content, after the A process, a significant loss on drying at 60 °C was observed for all treatments (in %): 40.42^{a} (fresh), 42.41^{a} (40°C), 41.48^{a} (50°C) and 34.45^{b} (60°C). Considering apiole content after process B, no significant difference between fresh and dried plant samples was recorded: 47.93^{a} (fresh), 49.56^{a} (40 °C) 46.56^{a} (50 °C) and 46.67^{a} (60 °C). The significant differences between the temperatures studied leads us to consider the temperature of 50 °C as the indicated for preservation of the main components at the two processes, but even with the statistical results and considering the time required to perform the drying process, that at 60 °C is reduced at cost and yield of essential oil equivalent to the other treatments, letting the best temperature option be chosen by the producer and/or company based on the marketing.

Acknowledgements: FAPERJ, UFF.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Volatiles Compounds of Three *Plumeria* Species

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Keywords: *Pseudosphinx tetrio; Plumeria*; HS-SPME.

The identification of volatiles from the flowers of three species (*rubra*, *alba* and *obtusa*) of the genus *Plumeria*, in white, red and yellow colors, as well as a comparison of emission of these compounds during the day with the night shift, and its possible relationship in attracting *Pseudosphinx tetrio* (Linnaeus) has not been reported yet. The fragrances issued by flowers are mean of orientation and attraction to their pollinators of interest serving as important aspects in choosing the pollination agent. Some volatile compounds emitted by plants act as a defense.[1] The flowers emit different compositions in their scents according to their growth stage, and they are subject to influences in the formation of aromas by climate conditions and or nutrient availability or attacks by predators.[1,2] The analysis was performed by GC/MS using HS-SPME with an SE 54 column (95 % methyl/ phenyl 5 %) with 30 meters length and internal diameter of 0.25 mm (Supelco). Twenty-two different compounds were identified, with majority occurrence of linalool (86.5 % daily and 75.0 % night) and 2-phenyl ethyl alcohol (15.4 % and 6.0 % nighttime and daytime), in white flowers. Major volatiles in red flowers were methyl benzoate (56.0 % and 62.4 %, daytime and evening) and 2-phenyl ethyl alcohol (21.7 % daily and 13.0 % night). The major compounds in yellow flowers were methyl salicylate (30.3 % daily and 20.6 % night) and methyl benzoate (14.6 % daily and 27.3 % night). The volatile compounds were identified by comparison with the data of NIST11 and Wiley7 libraries and with C7-C30 hydrocarbon standard retention times using Arithmetic Index (AI). Using a standard linalool an analytical curve was made, which provided the linearization straight from the equation y = 194321X + 1.108 with R^2 = 0.9923. Thus, it was possible to determine the concentration of linalool as 1660.7 ppm during the day and at night as 773.69 ppm. Food and pollination choice of *P. tetrio* for *Plumeria* with white flowers can be suggested because of linalool and 2-phenyl ethyl alcohol as major compounds in white flowers. The change in ratio of linalool and 2-phenyl ethyl alcohol in different periods may be due to the nocturnal *P. tetrio* with the plant promoting a change in your metabolic route to attract the moth. These studies help us understand the ecological relationship of species of *Plumeria*a nd *P. tetrio* and guide for new experiments to verify the effectiveness of the identified compounds.

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Acknowledgements: Fapemig, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Selected *Origanum dubium* Boiss Genotypes With High Essential Oil Yields and Carvacrol Rates

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Keywords: *Origanum dubium;* essential oil; carvacrol

Origanum dubium Boiss is one of the economically important wild oregano species in Turkey and it is collected from the natural flora of Antalya. This species is used mainly for essential oil production due to its high essential oil and carvacrol yield. In preliminary works, one hundred genotypes of *O. dubium* originated from the wild flora of Antalya were selected according to their agronomic and chemical features. Also, essential oil colours were observed in all genotypes since it could be important for marketing choices. This study was conducted in Antalya located in Mediterranean Region of Turkey and this location was characterized by Mediterranean climate. Essential oils of different genotypes were obtained by hydrodistillation of the aerial parts of plants and they were analysed by GC/MS. According to the results, ten genotypes were selected and propagated by stem cuttings. After that, each genotype (clone) was planted in a separate plot with three replications. Among the selected genotypes carvacrol was the major component and followed by p-cymene, γ-terpinene, α-thujene and myrcene. Essential oil yields varied between 8 to 11.5 %; carvacrol rates varied between 82.7 % to 88.2 %. These genotypes could be good candidates for developing new cultivars.

Acknowledgements: This research was supported by the Scientific, Technological Research Council of Turkey.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Seasonal and Circadian Study of the Essential Oil of *Ocimum gratissimum* L. (basil) and its Antifungal and Antioxidant Activities

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Keywords: *Ocimum gratissimum*; seasonal and circadian variation; antifungal and antioxidant activities.

The species *Ocimum gratissimum* belongs to the Lamiaceae family and its essential oil has many pharmacological activities.[1] Despite these characteristics, the species hybridizes too easily and, in addition, there are reports on the influence of external factors such as temperature, humidity, soil type, light incidence, age and development of the plant, among other factors, in the variation of the chemical composition of its essential oils.[2] Here, the essential oil of *Ocimum gratissimum* was analyzed by GC/MS and then evaluated for its antioxidant and antifungal activity, besides circadian and seasonal influences. It was observed that the weather conditions have direct influence on the yield and content of the chemical compounds present in essential oil. The yield of the essential oil varied significantly (4.75 - 7.06 %), apparently increasing in warm days with high incidence of sunlight and decreasing in inverse conditions. An average of 28 compounds was identified, around 98.9 % of the total composition of the oil obtained. All samples showed thymol (33.2 - 63.4 %) and γ-terpinene (21.0 - 45.1 %) as major components, as well as high levels of *p*-cymene (2.1 - 22.5 %). As there were significant variations in the content of the constituents, by combining the studies conducted it was possible to set the collection of the essential oil according to the chemical interest. It was also possible to confirm the known antifungal and antioxidant potentials of *Ocimum gratissimum* essential oil, with MIC of 0.7 μL mL^{-1} against the *Corynespora cassiicola* and 0.5 μL mL^{-1} against their sporulation. Related to the standard antioxidant Trolox in the DPPH radical scavenging capacity, values ranging from 1587.68 to 1674.05 mg TE g^{-1} were observed. Despite the variations in the chemical composition of the oils, no significant difference in biological activities was observed. This may be due to the action of other constituents of high content, such as *p*-cymene and γ-terpinene, which also varied significantly during the study and have properties similar to thymol (antioxidant activity) or may be acting synergistically with the compound (antifungal).

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[2]Gobbo-Neto, L.; Lopes, N.P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova* **2007**, *30,* 374. [CrossRef]

Acknowledgements: UFMA, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Perfumes From Amazonian Aromatic Plants

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Keywords: *Aniba rosaeodora; Cyperus articulates*; brazilian market.

The economy of the Amazon region is primarily focused on producing commodities such as meat, soy, energy and ore from mining operations. Non-timber forest products occupying a negligible share (0.1 % of total exports) in this economic context. To be preserved the Amazon must go through a Research & Development (R & D) process that would transform Biodiversity into bioproducts for society. The P&DBIO - Research & Development Laboratory on Natural Bioactive Products in Santarem, in the heart of the forest, uses biotechnology to standardize raw materials for the cosmetics and perfumery markets. The Brazilian cosmetic market is one of the most important in the world with US$ 15 billion in sales in 2012, and it is also the most important in perfume market with sales of US$ 6 billion. Nevertheless, almost all raw materials are imported and the Amazon practically does not participate in this market. An important item in the cosmetic industry is essential oils. A thousand aromatic plants have been recorded in the Amazon, however only the essential oil of rosewood (*Aniba rosaeodora*) – an endangered species – is exported to the main perfumery companies. Barata et al.[1] cultivated cassava intercropped with rosewood in the Amazon leading to a sustainable procedure of trimming trees (4 to 5 years of age) producing a fragrant essential oil now in use for fine perfumes. This expertise has been adapted by Magaldi, a small enterprise in Maués in the Amazon, which is now exporting rosewood oil from the leaves at the price of US$ 220 kg^{-1}. In another agro-industrial project, the P&DBIO developed a priprioca (*Cyperus articulatus*) plantation having two family farms as partners in Low Amazon. The essential oil extracted from roots has economic importance in perfumery for its strong and pleasant smell, therefore, it was introduced in 2003, in the national cosmetic and perfumery industry. Our research group introduced the priprioca in the Western Amazon in a project involving cultivation, management and sustainable production of essential oil in order to improve its quality to the international market. The experiments leading to the harvest of 1 kg of rhizomes in 2014, giving an essential oil that is already being used to create fine fragrances. The technology from the agro-industrial project with P&DBIO partnership is about to be transferred to small local producers bringing economic opportunities to Amazon communities and thereby offering an alternative to the devastation of the Amazon forest.

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Acknowledgments: UNICAMP, Banco da Amazônia, CAPES, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Antioxidant Activity of Essential Oil from *Ocimum campechianum* Mill and Methyleugenol Standard

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Keywords: *Ocimum campechianum*; methyleugenol; antioxidant.

Ocimum (Lamiaceae) is a genus with about 160 species and innumerable varieties distributed at least three main centers of diversity: tropical parts of Africa, South America and Asia.[1] It contains around 30 species native to the tropics and subtropics of the Old and New world[2]. In Pará State, *Ocimum* spp. are cultivated in gardens, in the countryside, on small farms and used in the treatment of many diseases, as well as culinary ingredient. It is sold in open markets from Belém city, mostly in the Ver-o-Peso market. *Ocimum campechianum* Mill (syn *Ocimum micranthum* Willd) is a native shrub of Brazil, popularly known as alfavaca and alfavaca-do-campo. The specimen was collected in the municipality of Abaetetuba, Pará state, Brazil. A voucher specimen (MG 213374) was deposited in the Herbarium of Museu Paraense Emilio Goeldi. The essential oil from leaves (EOL) and inflorescences (EOI) were obtained by hydrodistillation separately in a Clevenger-type apparatus for 3 h. The oils were analyzed by GC/FID and GC/MS in a Shimadzu QP 2010 and a Shimadzu QP 2010 plus systems, both with Rtx-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and Helium for GC/MS, both with a flow rate of 1.2 mL min^{-1}. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70 eV. Identification of the compounds were made by comparison of their mass spectrum and GC retention data with those in NIST-05 library and cited in the literature. Antioxidant activity of leaves and inflorescences essential oils and methyleugenol standard was determined by DPPH radical method. For each sample was prepared a solution test of 20 mg mL^{-1} and aliquots (50 µL) mixed with 1950 µL of DPPH 60 µM. The absorbance was measured at 517 nm after incubation for 120 min. Oil yields were 2.1 % and 2.3 % for leaves and inflorescences, respectively. The GC/MS analysis resulted in identification of 26 compounds and the main compound was the phenylpropanoid methyleugenol in leaves (85.2 %) and inflorescences (83.0 %). In minor proportions were identified sesquiterpene hydrocarbons such as β-elemene (EOL: 4.0 %, EOI: 4.6 %), α-humulene (EOL: 1.2 %, EOI: 1.5 %), β-selinene (EOL: 3.5 %, EOI: 4.0 %) and α-selinene (EOL: 2.8 %; EOI: 3.3 %). The DPPH radical scavenging for the essential oils from leaves, inflorescence and methyleugenol were 58.5 ± 0.4, 68.4 ± 2.1 and 54.0 ± 2.9 mg TE g^{-1}, respectively. The results suggest that essential oils have antioxidant activity equivalent to methyleugenol.

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[2]Paton, A. A synopsis of *Ocimum* L. (Labiatae) in Africa. *Kew Bull* **1992**, *47*, 403. [Link]

Acknowledgements: CNPq, UFPA, MPEG.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Dermal Acute Toxicity of *Cyperus articulatus* var. *nodosus* Essential Oil and Hydrolate from Santarém-Pará

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Keywords:*Cyperus articulatus* var*. nodosus*; essential oil; dermal alergenicity.

Priprioca (*Cyperus articulatus* var. *nodosus* Lin.; Cyperaceae) is a tuber that exhales an aroma traditionally used in baths and preparation of handmade perfumes in Amazon Rainforest. Essential oil (EO) has wet wood, green and spicy notes and it is used in the production of fine perfumes of Natura S/A. In our research group, (P&DBIO) *priprioca* has been target of multidisciplinary studies involving agronomical, chemical and biological areas. The plant was cultivated in a hectare in West region of Pará State (Santarém-Brazil), and the EO was extracted in laboratorial, pilot and industrial scales with yield approximately of 0.5 %, as well a hydrolate (HD) was obtained as a sub product. These studies aimed the formulation of cosmetics using these raw materials. EO and sub products could present side effects as contact allergic dermatitis, photosensitivity, neurotoxicity and carcinogenicity. That is the reason to study the dermic absorption and toxicity of *priprioca* EO and HD *in vivo*. In the primary cutaneous irritation assay were used twelve rabbits New Zealand breed, male or female, healthy and body weight higher than 2.0 kg for each treatment. Six rabbits were used for both treatment, EO and HD. Animals were kept in individual cages with constant temperature and were trichotomized in two areas in dorsal region 24 h prior the beginning of the assay. A volume of 0.5 ml of EO or HD was applied in one side of the trichotomized area, while the other one was used as control. After treatment each area was covered with gauze and both EO and HD remained in contact with the skin for a period of at least 4 h. After this period, the semi-occlusive patch was removed to collect the residue of the product. The readings were done between 24 and 72 h and the values were registered measuring the skin edema of the test area with a pachymeter and calculating with a formula. The grade of intensity of cutaneous reaction was based on Draize method. The arithmetic averages of the readings were calculated. The value found for Index of Primary Cutaneous Irritation (PCI) of *C. articulatus* var. *nodosus* EO classified it as moderated irritant, while PCI for HD was considered not irritant.

Acknowledgements: FIOCRUZ, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Composition of the Essential Oil of *Schinus terebinthifolius* Raddi (Anacardiaceae) Cultivated with Poultry Manure and Organosuper

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Keywords: Brazilian pepper; hydrodistillation; medicinal plant.

Schinus terebinthifolius Raddi (Anacardiaceae, Brazilian pepper) is native from Brazil and used in traditional medicine as anti-inflammatory, anti-hemorrhagic, for respiratory disorders, among others. Scientifically, its leaves and fruits showed anti-inflammatory, antioxidant, antimicrobial and antitumor potential. The species is used in cooking and there are studies that confirm its low oral toxicity. This study aimed to investigate the chemical composition of the essential oil of Brazilian pepper cultivated under different organic residues. The plants were grown at the *Garden of Medicinal Plants* from the Federal University of Grande Dourados (*UFGD*). A voucher specimen was deposited in the herbarium (DDMS 4602). The treatments resulted from the addition of semi-decomposed poultry manure or Organosuper® to the ground as coverage or incorporated at a dose 10 t ha^{-1}. Treatments were arranged as 2 (residues) x 2 (way of addition) factorial + 1 (control without residue), in a randomized block design with five replications. Samples of fresh fruits (50 g) from each treatment were collected in January 2015 and submitted to hydrodistillation, separately, in a Clevenger-type apparatus for 4 h each. The oils were analysed by GC/MS using capillary column DB-5 (30 m X 0.25 mm X 0.25 mm). The analysis conditions were: carrier gas helium (99.9 % and flow rate of 1.0 mL min^{-1}), injection volume 1 µL in split mode (1:20). Initially, the oven temperature was kept at 50 °C reaching 250 °C at a rate of 3 °C min^{-1}. The temperatures of the injector, detector and transfer line were kept at 250 °C. The parameters included scanning MS voltage electron impact ionization of 70 eV, a range of mass 45-600 *m/z* scan a range of 0.5 s. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature.[1] In the essential oil obtained from the fruits, 32 compounds were identified, in which 20 compounds were found in the treatment with incorporated poultry manure, 8 in poultry manure coverage, 13 in incorporated Organosuper®, 19 in Organosuper® coverage and 13 in the control sample. The components α-pinene, β-pinene, myrcene, α-phellandrene, δ-carene, limonene, terpinolene e γ-muurolene were identified in all samples. The major components were α-pinene in the control sample (80.3 %) and in the Organosuper® coverage (71.9 %), whereas in incorporated Organosuper®, δ-carene (51.8 %) was the major compound, followed by α-pinene (30.8 %). In both applications of poultry manure, δ-carene was the major compound.

[1]Adams, R. P.; *Identification of essential oil components by Gas Chromatography/Mass Spectroscopy*, 4a ed. Allured Publishing Corporation: Carol Stream, 2007.

Acknowledgements: FUNDECT, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Variability of Priprioca Essential Oil (*Cyperus articulatus* var. *nodosus*) in Different Soil Conditions in Amazon West

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Keywords: Essential oil; *Cyperus articulates;* soil

Priprioca (*Cyperus articulatus* var. *nodosus*, Cyperaceae) is an Amazonian plant used for aromatic baths. The essential oil (EO) is used in commercial scale by only one company, "Beraca" in Belém, to produce the "Natura" range of fine perfumes. This study aimed to define experimental parameters for commercial cultivation, EO production and determination of chemical composition of the different agronomical experiments in Santarém city (Tabocal I and II) and Belterra (Embrapa). The cultivated material was collected in November 2014 and a voucher specimen was deposited in Herbário Museu Paraense Emílio Goeldi under registration nº 207174. Soil samples were collected according to recommendations of Embrapa and analyzed in the laboratory of Embrapa Amazônia Oriental. The chemical profile was determined according by GC/MS in CPQBA-UNICAMP. Identification of chemical compounds was done through calculation of retention indices and comparison with library NIST11 and literature data.[1] Results from soil analysis revealed a sandy texture in Tabocal I (544 g kg^{-1}), and claylike in Belterra (700 g kg^{-1}) and Tabocal II (634 g kg^{-1}). Respectively, pH in water: 5.0; 4.6 and 5.5; M.O. (g kg^{-1}): 5.10; 6.39 and 34; N (%): 0.19 in Tabocal I and 0.41 in Belterra; P (mg $(dm^3)^{-1}$): 2; 7 and 14; K (mg $(dm^3)^{-1}$): 25, 67 and 60; Na (mg $(dm^3)^{-1}$): 17 in Tabocal I and 34 Belterra; Ca (mg$(dm^3)^{-1}$): 2.3; 2.0 and 4.6; Ca+Mg ($cmol_c(dm^3)^{-1}$): 3.0; 2.6 and 5.8; Al ($cmol_c(dm^3)^{-1}$): 0.3; 0.8 and 0.0; H+Al ($cmol_c(dm^3)^{-1}$): 3.80; 6.77 and 5.25; and effective CEC ($cmol_c(dm^3)^{-1}$): 3.54; 3.32 and 11.3 (mg$(dm^3)^{-1}$). Yield of EO was 0.58 % in Tabocal I, 0.62 % in Tabocal II and 0.49 % in Belterra. Chemical profile of priprioca showed that volatile compounds are in different concentrations in the samples. Sesquiterpenes are the major compounds in all samples including isocorimbolone, mustacone, aristolone, *trans*-pinocarveol and caryophyllene oxide. The same compounds were previously identified in priprioca.[3] Myrtenol, viridiflorene, verbenone, 1,3,8-*p*-menthatriene, β-humulene, *trans*-calamenene, α-selinene, with a further five unidentified compounds with concentrations > 2%. Clay soils presented minor concentration of compounds like α-pinene (0.84-0.98 %) than sandy soil (5.13 %), similarly to β-pinene in clay soil (0.58 %) versus sandy soil (2.82 %). Mustacone has higher concentration in clay soil (14.25 %) than sandy soil (11.86 %). The aromatic differentiation of EO's produced in different soils and growing conditions could define favorable factors of the fragrances appreciated by perfumers.

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[2]Potiguara, R. C. V.; Zoghbi, M. G. B. Priprioca: um recurso aromático do Pará. Museu Paraense Emílio Goeldi/Universidade do Estado do Pará: Belém, 2008.

Acknowledgements: IBEF-UFOPA, CPQBA-UNICAMP, FAPESPA, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Natural Oxidation of Monoterpenes in *Protium heptaphyllum* Oilresin

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Keywords: *Protium heptaphyllum;* essential oil; oxidation.

Protium heptaphyllum (Burseraceae) produce an oilresin rich in volatile and non-volatile terpeneswhich has cosmetic and medicinal application. The oilresin texture changes as it ages, ranging from a semi-solid to a solid texture. The aim of this present work was to evaluate whether this aging process affects the volatile composition of the oilresin. Oilresins were periodically collected in São João da Barra, State of Rio de Janeiro, during the years 2013 and 2014. They were characterized by their texture: the oilresins of recent exudation (ORE) had semi-solid texture, while the oilresins of late exudation (OLE) had solid texture. Mixtures (MO) of ORE and OLE have also been collected. The oilresins (n=20) were hydrodistillated with a Clevenger apparatus for 4 h. The essential oils (EOs) obtained were analyzed by GC/FID and GC/MS, using Agilent system 5973N, with a capillary column HP-5 (30 m X 0.25 mm X 0.25 µm). The temperature was programmed from 60 to 240 °C (3 °C min^{-1}). The identification was made by comparison of the mass spectra (Wiley database) and retention indices calculated from the injection a series of n-alkanes. The major constituents of EOs were α-pinene (3.6-19.4 %), *p*-cymene (4.3-43.0 %), limonene (5.8-11.6 %), terpinolene (8.8-69.7 %) and *p*-cymen-8-ol (2.7-31.8 %). Though there were differences in the content of EOs directly related with the collection time, we found that the percentage content of *p*-cymene, terpinolene and *p*-cymen-8-ol was highly depending on the aging stage of the oilresin. The EOs obtained from ORE (n=10) were mainly constituted of terpinolene (28.2-69.7 %), whereas the EOs obtained from OLE (n=8) were mainly constituted of *p*-cymene (18.7-43.0 %) and *p*-cymen-8-ol (8.2-31.8 %). The percentage contents of *p*-cymene and *p*-cymen-8-ol in the EOs obtained from ORE were 4.3-23.3 % and 2.7-9.8 % respectively, while the content of terpinolene in the EOs obtained from OLE was 8.8-20.9 %. Also, the MO (n=2) presented increased percentages of terpinolene (16.3-35.5 %) and *p*-cymene (19.8-29.9 %), and a content of *p*-cymen-8-ol (11.1-14.6 %) in-between the ranges found for these components in semi-solid and solid oilresins. These results bring strong evidence that terpinolene could be oxidated into *p*-cymene, and then *p*-cymene into *p*-cymen-8-ol as a natural aging process.

Acknowledgements: Embrapa, Faperj, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Cytotoxic and Genotoxic Effects of Essential Oil from Leaves of *Casearia sylvestris* Sw. (Salicaceae) on A549 Tumor CellLine

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Keywords: Antitumor activity; *Casearia;* germacrene D

Casearia sylvestris Sw. belongs to the family Salicaceae, and can be found in all extension of Brazilian territory. This plant is included in the public health system (SUS) of Brazil. According to literature, *C. sylvestris* showed cytotoxic and genotoxic effects in different tumor cell lines and the anticancer potential of extracts could be due to casearins (clerodane diterpenes). On the other hand, there are few studies related to the antitumor activity of the essential oils from this species.[1] This study aimed to evaluate the cytotoxicity and DNA strand break effects of the essential oil from leaves of *C. sylvestris* against A549 tumor cell line (human lung carcinoma). The botanical material was collected in Tijuca National Park and deposited under as RB 570651 (Botanical Garden of Rio de Janeiro). Fresh leaves (150 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 2 h. The essential oil was diluted in dichloromethane and then analyzed by GC/MS and GC/FID (Agilent 6890N coupled to Agilent 5973N), equipped with HP-5MS fused silica capillary column (30 m X 0.25 mm X 0.25 µm). Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Compounds were identified by comparison of mass spectra, linear retention indices and literature comparison. For the cytotoxic and genotoxic assays, A549 cell line was used. Different concentrations of the essential oil (0.5, 1.0, 2.0 and 4.0 µg mL^{-1}) were assayed to evaluate the cytotoxicity by wst-1 and clonogenic assay. DNA damage (genotoxicity) was evaluated by the comet assay.[2] Results were expressed as the half-maximal response (EC_{50}). In total, 63 compounds were identified in the oil (90 %). Germacrene D (9.9 %), viridiflorol (7.9 %) and (*E*)-caryophyllene (5.4 %) were identified as the main compounds. The essential oil showed cytotoxic activity against A549 tumor cells with EC_{50} at 4 µg mL^{-1} and showed dose dependent pattern (r = -0.79, p = 0.03). Genotoxicity assay showed DNA damages of types I – IV at 4 µg mL^{-1} according to the Damage Index (DI). So, the essential oil of *C. sylvestris*is is rich in sesquiterpenes and displays cytotoxic activity and promots DNA damages.

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Acknowledgements: FAPERJ, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In Vitro Antibacterial Activity of Individual and Blended Essential Oils on Pathogenic and Probiotic Gut Bacterial Microbiota of Poultry and Pigs

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Keywords: pathogenic bacteria; probiotic bacteria poultry; swine.

The aim of this research was to evaluate, in vitro, the antibacterial activity of different essential oils, individually and in binary blends, on pathogenic and probiotic bacteria of pig and poultry microbiota. A screening was performed with five essential oils (EO) obtained from *Eucalyptus globulus*, *E. exserta*, *Pimenta pseudocaryophylllus*, and two EO's which are by-products of orange juice production: Orange oil phase essence, and Citrus terpens. The EO's and blends were tested by disk diffusion method on five pathogenic bacteria: *Salmonella enteritidis, Escherichia coli, Staphylococcus aureus, Listeria innocua* and *Enterococcus faecalis*, and on three probiotic bacteria*: Lactobacillus plantarum, Lactobacillus rhamnosus* and *Bacillus subtilis*. The ANOVA (*Tukey* $p \leq 0.05$) showed a significant difference in the antibacterial activity between the evaluated products. Thus, orange oil phase essence, and the binary blend of essential oils (BEO), constituted of *E. globulus* and *P. pseudocaryophylllus*, were selected based on their highest activity on pathogenic bacteria and their lowest activity on probiotic bacteria, and also according to the availability of oils that were used in this study. These two oils were tested by microdilution method to determine the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) on the most resistant pathogenic bacterium, *E. faecalis*, and the least resistant probiotic bacterium, *L. rhamnosus*. Two-fold serial dilutions from 14.80 to 0.116 mg mL^{-1} were tested. MICs were determined by construction of growth curves and by the resazurin test. Hence, the MIC of orange oil phase essence was 14.80 mg mL^{-1} for both bacteria, *E. faecalis* and *L. rhamnosus,* by growth curves. By the resazurin test, the MIC was not observed in this oil for *E. faecalis,* because, at all concentrations, a change in resazurin color was observed. The MICs of BEO for *E. faecalis* and *L. rhamnosus* was 14.80 mg mL^{-1} and 7.40 mg mL^{-1}, respectively. The BEO was bactericidal at 14.80 mg mL^{-1}(MBC) for *L. rhmanosus*. Observed that by microdilution methods, it was not possible to observe a selective effect from the oils tested on probiotic bacteria, in contrast with disk diffusion method. Nevertheless, it is important to emphasize the potential antibacterial activity of the oils tested. Limonene was detected as a major compound in orange oil phase essence (87.2 %) by GC/MS. In the case of oils that made up the blend, Chavibetolin *P. pseudocaryophylllus* (29.2 %), and 1,8 cineole (Eucalyptol) in *E. globulus* (83.7 %).

Acknowledgements: PRONABEC for Carmen M. Sinche Ambrosio's scholarship.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Myrtaceae Species Growing in the Rio de Janeiro Botanical Garden: Preliminary Characterization of their Essential Oil Composition

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Keywords: Myrtaceae; essentialoil; Rio de Janeiro Botanical Garden.

The family Myrtaceae, with 120 genera and more than 3800 species, is very representative and important in all the Brazilian ecosystems. This study presents the first part of the overall analysis of the Myrtaceae species growing in the Research Institute Botanical Garden of Rio de Janeiro (IPJB/RJ) campus. Between July 2010 and June 2011, twenty-one species, native and exotics, were collected and their leaf essential oils were obtained by hydrodistillation from fresh material, using a Clevenger apparatus during 4 h. The species were comprised in the genera *Campomanesia* (3), *Eugenia* (10), *Gomidesia* (1), *Melaleuca* (2), *Myrcia* (1), *Myrciaria* (1), *Plinia* (1), *Tristania* (1) and *Ugni* (1). The oils were analyzed by GC/MS (Agilent 6890N) with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Helium was used as carrier gas with a flow rate of 1.0 mL min^{-1}. Oven temperaturewas 70 (held for 5 min) to 250°C at 3 °C min^{-1}. Detector at 70 eV and acquisition mass range 10-400 *m/z* (3.66 scan sec^{-1}). The injections were standardized to 1 µL from a 3 mg mL^{-1} CH_2Cl_2 solution. As a mean to obtain a qualitative indication of the oils' main composition, the chromatogram threshold was conveniently adjusted to allow registering up to 15 main signals in each analysis, and their relative areas were registered. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Considering all the samples, the oil yields ranged between 0.02 % (*Plinia edulis*) to 1 % (*Campomanesia phaea*). Content of α- and β-pinene, limonene, linalool were variable (30-58 %) in two *Campomanesia* species, but *C. guazumifolia* presented exclusively sesquiterpenes (β-caryophyllene, germacrenes and guaiol-type alcohols). Monoterpenes were relevant in 5 *Eugenia* species (pinenes, ocimenes, 1,8-cineole) but were absent in *E. feijoi*, *E. velloziana* and *E. luschnatiana*. Sesquiterpene alcohols were relevant in the former (50 % α-cadinol and 7-epi-α-eudesmol, 92 % total alcohols) and in the second (51 % guaiol-type, 72 % total alcohols), whilst the latter was characterized by the cadinol-type (34 %) along with 66 % hydrocarbons (40 % β-caryophyllene). E-β-ocimene (61 %) was predominant in the oil of *E. kiaerskovana*, nerolidol (80 %) in *Melaleuca armilaris*, and caryophylenols in *E. crenata* (64 %) and *E. sulcata* (58 %). *E. itaguahiensis* showed 50 % of phenolic compounds besides 30 % β-caryophyllene. *Myrciaria tenella* presented 43 % pinenes, 18 % β-elemene and 10 % aspidinol (phloroglucionol derivative). Sesquiterpenes were exclusive in *P. edulis* (42 % β-caryophyllene and its oxide, 29 % guaiol-type alcohols) and *Ugni molinae* (55 % β-elemene in 73 % hydrocarbons). Quantitative CG/FID analysis are presently ongoing.

Acknowledgements: Ricardo C. C. Reis, curator of the Living Collections of IPJB/RJ during 2010-11.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Composition of Cultivated *Campomanesia adamantium* L.(Myrtaceae) Leaves

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Keywords: guavira, hydrodestillation, medicinal plant.

The *Campomanesia* (Myrtaceae) species, known as guavira or gabiroba, are used in traditional medicine against hypertension, throat infections and intestinal infections. In the essential oil from the leaves of *C. adamantium* L., 82 compounds were identified in which limonene was the major component.[1] Based on the assessment of the morphological characteristics, it was perceived variability of a collection of *C. adamantium* grown in the Garden of Medicinal Plants-GMP, at UFGD. This work was carried out in order to evaluate if the composition of the essential oil of *C. adamantium* grown in the GMP is correlated with the morphological variability. A voucher specimen was deposited in the herbarium DDMS no. 2192. The plants evaluated had the following data for plant height (m), stem diameter (mm), average leaf length (cm) and leaf color, respectively: 1) 1.62; 23.64; 9 and dark green with yellow spots; 2) 1.80; 37.75; 7 and light green leaves with yellow spots; 3) 2.05; 53.87; 6 and grayish green leaves with brown spots; 4) 1.98; 52.68; 7, dark green leaves with brown spots on the edges; 5) 1.75; 52.31; 6.5 and light green. Samples of leaves (200 g) from each plant were collected in January 2015 and submitted to the extraction by hydrodistillation using a Clevenger-type apparatus for 4 h. The oils were analysed by GC/MS using capillary column DB-5 (30.0 m × 0.25 mm × 0.25 µm) and helium as carrier gas. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature.[2] Forty-six compounds were identified in the essential oil of *C. adamantium* and the same compounds were identified in all samples. The major compounds were α-pinene, globulol, limonene, β-pinene, trans-hydrate sabinene and bicyclogermacrene, confirming earlier results in literature, however, in a wild condition.[2] The morphological variability of plants evaluated through plant height, stem diameter, leaf size and leaf color showed parallel pharmacognostic variability in the levels of identified secondary metabolites.

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Acknowledgements: FUNDECT, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of Essential Oil from Leaves of *Spiranthera odoratissima* A. St.-Hil. (Rutaceae) in the Brazilian Cerrado

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Keywords: medicinal plants; manacá; GC/MS.

The Brazilian Cerrado is known for its biodiversity, with around 12,000 species found in this biome. The Rutaceae family presents a large variety of secondary metabolites that are flavored due the presence of essential oils. *Spiranthera odoratissima* is classified as a subshrub, and popularly known as manacá, and its leaves are frequently used like a medicinal tea to blood cleanser, to kidney and liver disorders[1,2]. The aim of this study was to compare the yield and composition of essential oil of the manacá in 4 different times of extraction. The samples were collected in Luziânia/GO city, in July 2014 and dried at room temperature. The samples were crushed and designed to hydrodistillation in Clevenger-type apparatus, modified by different times (3, 4, 5 and 6 h). Leaf essential oils obtained were analyzed using a GC-MS, Shimadzu QP5050A, using a fused silica capillary column CBP- 5 (30 m X 0.25 mm X 0.25 µm). The essential oil constituents were identified by comparing their mass spectra with those from the NIST, 1998 as well as by comparing the calculated linear retention indices (LRI) with values in the literature. The yields obtained in the extraction of 3, 4, 5 and 6 h were 2.1 %; 1.6 %; 1.1 % and 1.4 %; respectively. It was identified 23, 17, 18 and 19 different compounds in each extract, and majority components found in the samples were sesquiterpenes like β-caryophyllene (19.9 % - 3 h; 20.5 % - 4 h; 23.5 % - 5 h and 22.9 % - 6 h), γ-muurolene (34.6 % - 3 h; 36.3 % - 4 h and 30.1 % - 5 h), bicyclogermacrene (22.4 % - 3bh; 20.8 % - 4 h; 19.3 % - 5 h and 19.4 % - 6 h) and amorpha-4,7(11)-diene (29.2 % - just at 6 h). Pearson correlation was used to determine the real level of association among the components with the different extraction time. Therefore, considering the extraction time, β-caryophyllene presented a significant positive correlation (R=0.9; α=15 %), and bicyclogermacrene showed a strong negative correlation with the extraction time (R=-0.9; α=10 %).

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Acknowledgements: CNPq, FAPEG, IFG.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oils from the Leaves and Flowers of *Callistemon viminalis*: Chemical Composition and Insecticidal Activity

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Keywords: escova de garrafa; essential oil; insecticide activity; Myrtaceae.

Popularly known as "escova de garrafa", *Callistemon viminalis* (Myrtaceae) is an important source of chemical compounds with insecticidal, antifungal, and antimicrobial activities, among others.[1] Several studies have been conducted to find natural products that can be used as alternatives for insect control because the synthetic compounds used for this purpose are poorly selective and aggressive towards both man and the environment. The essential oils from the leaves and flowers of *C. viminalis* were characterized chemically, and the insecticidal activity was evaluated. The essential oils were extracted by hydrodistillation using a modified Clevenger[2] apparatus and characterized by GC-MS. The insecticidal activity was evaluated against the aphid *Myzus persicae* using the no preference method with and without choice.[2] The oils were diluted in a solution of water and Tween 80 at concentrations of 0.1 and 0.5 %. The design for the test with choice was a completely randomized block design (RBD) and that for the no choice test was completely randomized (DIC), using the statistical program SISVAR. The major constituents in the essential oils from *C. viminalis* leaves and flowers were 1,8-cineole (67 % and 69.1 %), α-pinene (16 % and 18.9 %), limonene (10 % and 5.9 %) and α-terpineol (2.2 % and 1.75 %); respectively. Regarding the insecticidal activity in the free choice test, the essential oil from the flowers, at 0.5 %, was able to influence the preference and reproduction of aphids. These effects did not change over time. The essential oil from the leaves caused a decrease in the mean number adults at 48 h. For the no choice test, the average number of adults was lower for both oils.

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Acknowledgements: Fapemig, CNPq and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation on Chemical Composition of Essential Oil from *Lippia sidoides* with Different Storage Conditions

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Keywords: *Lippia Sidoides*; essential oil; store conditions.

Lippia sidoides is a shrub that belongs to the Verbenaceae family, found in Northeastern hinterland in the States of Ceará and Rio Grande do Norte, Brazil.[1] It is an aromatic plant locally known as alecrim-pimenta' and the essential oil from its leaves is rich in thymol. Bactericidal and fungicide properties have been described for the essential oil, especially in the treatment of skin, mouth, throat, and vaginal infections.[2] The aim of this work was to evaluate the changes on the composition of the essential oil from *L. sidoides* essential oil according to the temperature storage. Dried leaves (200.0 g) were subjected to hydrodistillation in a Clevenger-type apparatus. The oil was splitted in three samples and stored at room temperature (25 ^{o}C), refrigerator (4 ^{o}C) and freezer (-10 ^{o}C). The oil was analyzed just after extraction and after six months of storage by GC/FID and GC/MS in an Agilent 7890N and an Agilent 5975C systems, both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, with flow rates of 1.5 and 1.0 mL min^{-1} respectively. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID signal. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. The samples of the oil stored in the refrigerator and at ambient temperature presented almost the same composition, with 29 and 27 compounds identified respectively. Major components were thymol (75.4 %), *p*-cymene (7.3 %) and (*E*)-caryophyllene (4.3 %). For the sample kept in the freezer 36 compounds were identified and the major compounds were thymol (56.8 %), *p*-cymene (12.2 %), (*E*)-caryophyllene (7,6 %).

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Acknowledgements: FAPERJ, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Fungicide Evaluation of the Essential Oils from the Leaves of *Cinnamomum verum* J. Presl (Lauracea) of Maranhão

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Keywords: *Cinnamomum verum*; benzil benzoate; eugenol.

Cinnamomum verum J. Presl is a tree of great importance in the world market because it's used in culinary and responsible for pharmacological activities. *C. verum*, known as "cinnamon", "Srilankan cinnamon", "cinnamon India" or "Ceylon cinnamon" is a tree that reaches up to 9 m tall, with leathery leaves, lanceolate and light green.[1] Two leaf samples were collected in February 2014, in different locations in the capital São Luís (SL) and inside Santa Inês (SI), Maranhão, Brazil. The herbarium samples were identified in João Murça Pires Herbarium of the MPEG under registration 165477. Dried leaves were subjected to hydrodistillation process separately on a Clevenger-type apparatus. Essential oils were analyzed by GC-FID and GC-MS, in a FOCUS equipment (Thermoelectron) equipped with a capillary column DB-5 (30 m X 0.25 mm X 0.25 µm), nitrogen as carrier gas (flow 1.2 mL min^{-1}). Essential oils were tested *in vitro* against *Colletotrichum musae*, characteristic of anthracnose and a very common disease in banana fruits. The methodology used was adding the essential oil amid flux BDA and the sulfactant agent DMSO. The concentrations tested were 0.0 µL mL^{-1}; 0.5 µL mL^{-1}; 1.0 µL mL^{-1}; 2.0 µl mL^{-1}; 3.0 µL mL^{-1} and 4.0 µL mL^{-1}. Standards of the major components of the oils were also tested in the same concentrations. The oil yields were 2.2 % (SI) and 2.4 % (SL). The essential oils from leaves showed two different chemotype. For the sample SI were identified benzyl benzoate (95.0 %), followed by linalool (1.2 %). For the sample SL was found eugenol (94.6 %), followed by (*E*)-caryophyllene (1.0 %). The other constituents of the essential oils in percentages lower than 1 %. Chemical composition variations may be related to the phases of growth, environmental conditions, weather effects and altitudes.[2] The essential oils of *C. verum* were able to inhibit the mycelial growth, sporulation and speed index of mycelial growth. The standard benzyl benzoate did not lead to good results to the test, different from the standard eugenol, which provided similar results to those of the essential oils of *C. verum* tested. Thus, the essential oils of cinnamon (*C. verum*) appear as an alternative to control the disease caused by the fungus. The study of the chemical composition is necessary, since different components have different biological activity against organisms.

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Acknowledgements: FAPERJ, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Secretory Structure, Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from *Lavandula dentata* L. (Lamiaceae)

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Keywords: Lavanda, lavender; labiatae; herbal drug.

Lavandula is one of the most important genera of Lamiaceae. This genus is originally found in Mediterranean region, Arabian Peninsula, India and Canary Islands and shows very popular plants of aromatic, ornamental, and medicinal properties.[1] The aim of this study was to analyze the secretory structures of *Lavandula dentata* as well as to extract the essential oil, to determine its chemical composition and to evaluate its antioxidant and antimicrobial activities in Brazil. Aerial parts of *L.dentata* were collected in Horto Medicinal of UEPG, Campus Uvaranas (25°5'23"S 50°6'23"W), Ponta Grossa – Brazil. Usual techniques of electron and light microscopy were used. The essential oil was extracted through hydrodistillation using a Clevenger apparatus during 6 h. Volatile composition of *L. dentata* essential oil was performed by GC-MS. Antioxidant potential was investigated by phosphomolybdenum, 2,2-diphenylpicrylhydrazyl (DPPH), and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods. Antimicrobial effect was determined by minimum inhibitory concentration using microdilution broth method. Microplates containing bacterial inoculum and serial dilutions of essential oil were incubated at 35 °C for 24 h. Minimum bactericidal concentration was then evaluated in Petri dish containing BHI agar for 24 h at 35 °C. Secretory structures were represented by glandular trichomes which comprise capitate and peltate ones. The major volatile component of *L. dentate* essential oil was 1,8-cineole (63.2 %). Regarding antioxidant activity, *L. dentata* essential oil at 200 µg mL^{-1} had a relative antioxidant activity of 28.0 % compared to ascorbic acid (100 %) by phosphomolybdenum assay. This volatile oil showed an antioxidant activity of 11.9 % at 20 mg mL^{-1} for DPPH scavenging assay comparing to rutin and gallic acid. By ABTS method, *L. dentata* essential oil achieved 22.0 % ofactivity after 30 min compared to the same standards. A minimum inhibitory concentration (MIC) of 54.7 µg mL^{-1} was observed for *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. *L. dentata* essential oil demonstrated a MIC value of 437.5 µg mL^{-1} for *Pseudomonas aeruginosa*. Minimum bactericidal concentration (MBC) of 54.7 µg mL^{-1} was achieved for *E.coli, C. albicans*, and *S. pyogenes*. For *S. aureus*, *L. dentata* essential oil presented a MBC value of 218.8 µg mL^{-1}, while no MBC value was detected for *P. aeruginosa*.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Secretory Structure, Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from *Eucalyptus alba* Reinwex. Blume

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Keywords: *Eucaliptus*; herbal drug; Myrtaceae.

Eucalyptus is represented by over 700 species worldwide.[1] Due to the main use in wood processing and wood-based materials, paper and cellulose production and vegetable coal, some particular taxa of *Eucalyptus* as *E. alba* play an important role in Brazilian economy.[2] Therefore, this study aimed to analyze the secretory structures of *E. alba* as well as to extract the essential oil, to determine its chemical composition and to evaluate its antioxidant and antimicrobial activities. Leaves of *E. alba* were collected in Ponta Grossa – Brazil. Usual techniques of electron and light microscopy were used. The essential oil was extracted through hydrodistillation using Clevenger apparatus during 6 h. Volatile composition of *E*. alba essential oil was performed by GC-MS. Antioxidant potential was investigated by 2,2-diphenylpicrylhydrazyl (DPPH), and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods. Antimicrobial effect was determined by minimum inhibitory concentration using microdillution broth method. Microplates containing bacterial inoculum and serial dilutions of essential oil were incubated at 35 °C for 24 h. Minimum bactericidal concentration was then evaluated in Petri dish containing BHI agar for 24 h at 35 °C. As secretory structure, leaves showed oil cavities in mesophyll. The major volatile component of *E. alba* essential oil was 1-8 cineole (55.2 %). This volatile oil showed antioxidant activity of 25.5 % at 20 mg mL^{-1} for DPPH scavenging assay comparing to rutin and gallic acid. By ABTS method, *E. alba* essential oil also achieved 25.5 % of activity after 30 minutes compared to the same standards. A minimum inhibitory concentration (MIC) of 416.5 µg mL^{-1} was observed for *Escherichia coli* and *Pseudomonasae ruginosa*. *E. alba* essential oil demonstrated a MIC value of 208.3 µg mL^{-1} for *Staphylococcus aureus*. A MIC value of 104.1 µg mL^{-1} was verified for *Candida albicans* and *Streptococcus pyogenes*. The same values were observed as minimum bactericidal concentration (MBC) for *E. coli, C. albicans*, *S. aureus*, and *S. pyogenes*, while no MBC value was detected for *P. aeruginosa*.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Study of Aroma Liberation in Air Fresheners of Lavender Essential Oil Using Gas Chromatography/Olfactometry

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Keywords: air fresheners; lavender; gas chromatography/olfactometry.

Air fresheners are conventionally used to provide a desired fragrance to ambient air, to mask, neutralize or counteract undesirable odors in the air, or to achieve a combination of these functions. However, a conventional liquid air freshener often present certain disadvantages and limitation as, for example, limited or even unacceptable fragrance performance, product longevity, esthetical appearance or temperature product stability over time. The oil nanoemulsions can be an alternative because nanoemulsions are fine oil-in-water dispersions, kinetically stable and also long-term physically stable.[1]The objective of this study was to evaluate and compare the liberation of aroma volatile compounds present in lavender essential oil in samples of air fresheners produced by two different methods, using gas chromatography/olfactometry (GC/O). The tests were done blindly. One sample was produced by traditional method mixing alcohol, propylene glycol, water and the essential oil. The other, a nanoemulsion of lavender essential oil, was produced by phase inversion temperature method.[2] Five mL of air freshener samples were added in a closed environment at constant temperature. Solid-phase microextraction (SPME) was applied to extract the volatile compounds, using the fiber DVB/CAR/PDMS. SPME was performed at 40 °C, 15 min of equilibrium and adsorption time, and 2 min of the desorption time. Analyzes were performed on the 7th, on the 14th and on the 21st days preparation time. To perform the GC/O analysis, a GC/FID was used and a sniffing port with HP-5MS fused silica capillary column (30 m X 0.25 mm X 0.25 µm) and nitrogen was used as carrier gas. Trained evaluators performed the olfactometry analysis. Volatile compounds of lavender essential oil present in air fresheners samples were identified by comparison of spectra and linear retention indices of pure essential oil, using GC/MS with the same column and conditions. Comparing two samples, the aroma intensity of the sample with traditional preparation differs from the 7th day, as well as this sample presented a faster volatilization process. The nanoemulsion sample had better fragrance performance during the time of the experiments and the aromas lasted longer time with a little variation of the intensity over time.

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Acknowledgements: FK Biotec, Fapergs, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Acute Toxicity of the Essential Oil of *Copaifera reticulata* (Ducke) from Seasonal Collections in the Tapajós National Forest, Belterra, Para, Brazil

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Keywords: copaiba; essential oil; seasonality.

The *Copaifera* genus consists of economic and ecological high value species widely distributed in Africa and tropical and subtropical regions of South America. In Brazil they are mainly found in the states of Para and Amazonas.[1] *Copaifera reticulata* Ducke is one of the most important specie economically exploited in the Amazon region with potential anti-inflammatory, antitumor and healing. The oleoresinis composed primarily of a volatile fraction consisting of sesquiterpenes and other corresponding to diterpenes, seasonality directly influences the production and oleoresin composition of copaiba. Studies of seasonal variations in chemical composition of oleoresin and its biological effects are scarce, so the objective was to evaluate chemically the volatile fraction of the oleoresin of *C. reticulata* and its toxic effect against nauplii of *Artemia salina* Leach. The oleoresin was collected in October 2014, dry period, in the Tapajos National Forest, Para. Aliquots of 30 mL were fractionated with technique of fractional distillation in triplicate to determine the yield of essential oil. Chemical analysis of the volatile fraction was performed by GC-MS. The toxicity was tested via an adapted bioassay with *Artemia salina* in triplicate.[2] Ten different concentrations of the essential oils were evaluated which were dissolved in Tween 80. Statistical analysis was carried out through the PRISM3.0 software and found to be of low toxic when the 50 % lethal dose (LD_{50}) was > 500 µg mL^{-1}; LD_{50} for moderate between 100 and 500 µg mL^{-1} and too high when the LD_{50} was < 100 µg mL^{-1}. The yield of the essential oil was 41 % (12.3 mL). Twenty-five compounds were identified in the volatile fraction of the oleoresin, and the major compounds were caryophyllene (29 %), α-bergamotene (24 %), β-bisabolene (14.3 %) and β-eudesmene (13 %). The volatile fraction was found to be highly toxic (LD_{50}= 7 µg mL^{-1}) to microcrustacean tested. In animal tests, the oleoresin showed relatively low toxicity. Although widely studied, *C. reticulate* oil still has differences in their composition and variations of the biological effects caused by different levels of substances that make up the oil. The result of acute toxicity showed that the volatile fraction has high toxicity and the dry period presents high essential oil incomes.

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Acknowledgements: UFOPA, Unicamp, LBV, P&DBio.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Investigation of the Essential Oil of *Tanacetum albi pannosum* Hub.-Mor. & Grierson for Cholinesterase Inhibitory and Antioxidant Activities

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Keywords: *Tanacetum albi pannosum;* antioxidant; anticholinesterase.

The genus *Tanacetum* L. (Emend.Briq.)(Asteraceae) is represented in the world by 160 species and in the Flora of Turkey by 46 species[1]. A number phytochemicals and biological properties have been reported for *Tanacetum* species[2,3]. *T. albi pannosum* Hub.-Mor. & Grierson has earlier been reported for its sesquiterpenic lactones and flavonoids. Cholinesterase inhibitory potential and partenolide content in the extract of *T. albi pannosum* has been evaluated[5]. *Tanacetum albi pannosum* was collected in Sivas province of Turkey and subjected to hydrodistillation using a Clevenger-type apparatus. Composition of the essential oil was investigated using GC-FID and GC/MS techniques. The oil was subjected to *in vitro* biological activity tests. Cupric reducing antioxidant capacity and free radical scavenging properties of the oil were investigated with CUPRAC and DPPH tests. Cholinesterase inhibitory (anti-AChE) potential of the essential oil was evaluated in Ellman's colorimetric assay. The main compounds of the essential oil were found as bisabolone oxide A (36.0 %), 1,8-cineole (18.3 %), α-bisabolol oxide A (7.2 %), α-bisabolol oxide B (4.5 %) and α-pinene (4.2 %). Anti-AChE activity was compared to galantamine as a standard while antioxidant activity was tested against to butylated hydroxytoluene and gallic acid. The essential oil of *T. albi pannosum* demonstrated weak anticholinesterase (> 30 mg mL^{-1}) and antioxidant potential (> 34 mg mL^{-1}) when compared to the standards.

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Acknowledgements: Authors thanks Anadolu University Scientific Research Foundation (BAP project № 1505S379) for supporting this research and to Biol. SüleymanYur, Biol. Bilge Kara and Biol. YeşimHaliloğlu for assistance with the biological activity assays.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Rectification Process of Essential Oils: Modeling and Simulation

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Keywords: Batch distillation; thermodynamic correlation; COSMO-SAC.

The fractionation process of essential oils on industrial scale receives little attention from the scientific community because of its complexity and application quite limited. Since the essential oil is a mixture of thermolabile compounds and has little known physical properties, studies on the subject are scarce. Thus, this work seeks to define thermodynamic correlations that describe the properties necessary for the calculation of chemical equilibrium, the definition of a dynamic model to describe the essential oil batch distillation process. The determination of physical properties is the crucial step when it comes to essential oils rectification processes, due to the reduced experimental data available, which leads to the use of predictive models. A consistent model that suits this requirement is the combination of group contribution methods with the corresponding states theory (CSGC), which uses only the groups within the molecule and its normal boiling point for the calculation of the vapor pressure of the substance. For the activity coefficient, COSMO-SAC technique is used. On the other hand, the batch distillation process is widely studied and numerous models have been developed, but all these were employed for the separation of well-known mixtures, as petroleum fractions. This work also aims to validate a dynamic model proposed by Domench e Enjalbert[1] in order to describe the separation of essential oil constituents. The model also has a few fundamental considerations like the constant plate holdup and negligible vapor holdup, which generate constant internal flow and eliminate the need to use hydrodynamic correlations. The vaporization rate is determined in relation to the enthalpy of vaporization and the feed composition in the reboiler. The temperature profile is calculated as a result of thermodynamic equilibrium. Simulations were performed in the equation-oriented simulator EMSO in order to demonstrate the feasibility of the method. *Eucalyptus citriodora* Hook and *Eucalyptus globulus* Labill essential oils were chosen due to their large production, economical potential of their fractions and both are produced by steam distillation. *E. globulus* oil has an initial composition of eucalyptol (83 %) and α-pinene (4 %), among others. The simulation provided four fractions: the first main-cut has a purity of 99.5 % of α-pinene, the second one has 99 % of eucalyptol. Each batch has a 35 % recovery of the initial feed and the remainder is intended for a recycle batch with the same starting oil composition. *E. citriodora* simulation provided an 80 % recovery of the initial feed with 95 % of citronellal and a minor cut with 99.6 % of (-)-isopulegol. The simulations were carried on a 20 stages column, with constant pressure of 133 Pa, leading to a temperature range from 345 K to 373 K for the *E. globulus* oil and 330 K - 420 K for the *E. citriodora* oil.

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Acknowledgements: CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Yield and Composition of the Essential Oil from *Varronia curassavica* Jacq. in Response to Suppression of Irrigation

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Keywords: *Varronia curassavica*; erva baleeira; essential oil; α-humulene; deficit water.

Varronia curassavica Jacq, popularly known as "erva baleeira", is a medicinal plant native of Brazil that has aroused the interest of the pharmaceutical industry because it contains in its essential oil α-humulene, a substance with anti-inflammatory activity. Due to the economic importance of the essential oil, this study evaluated the effects of withholding irrigation on yield and chemical composition of the essential oil. The experiment was conducted in a green house and it were used micro propagated seedlings growing in plastic pots with sub-irrigation system. The treatments were represented by the control (irrigated daily), and three levels of water stress. These levels were expressed by the leaf water potential l (Ψ_w): T1 - control (Ψ_w ~ -0.3 MPa), T2 –Ψ_w ~ - 1.0 MPa; T3 – Ψ_w ~ -1.7 MPa and T4 –Ψ_w ~ -2.5 MPa. The Ψ_w was measured daily at 4:30 a.m. with a pressure camera. The plants were harvested eight, nine and ten days after the suppression irrigation, and they were dried in oven with air flow until reaching constant weight. The essential oil was extracted by hydrodistillation in a Clevenger-type apparatus for 2 h 30 min. The analysis of the chemical composition of the essential oils were performed through gas chromatograph equipped with a flame ionization detector (GC-FID) and gas chromatograph coupled to mass spectrometer (GC-MS). The smallest Ψ_w (-2.5 MPa) differed significantly from the other treatments and showed the highest oil yield (0.2 %), without causing significant losses in the production of biomass. The suppression irrigation was effective in increasing the productivity of the essential oil from *V. curassavica*. Twenty-five substances were identified in the essential oil from *V. curassavica.* The same substances were found in all the treatments. The major compounds were α-pinene (40.0 %), (*E*)-caryophyllene (22.9 %), α-humulene (4.0 %), and β-bisabolene (2.5 %). There was no statistical difference among treatments in the relative proportion for the main classes of compounds. The relative proportions of the active principals, (*E*)-caryophyllene and α-humulene, also did not differ significantly among the treatments.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Crem (*Tropaeolum pentaphyllum* Lam.): Extraction and Quantification of Volatile Oils tubers

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Keywords: Tropaeolaceae; spice; taste.

The crem, native species of southern Brazil, is an unconventional food plant that has several versatility of uses, and its leaves and flowers eaten in salads and the 'crem potato' as it is called, is traditionally grated and stored in a red vinegar, used as a condiment for fatty meats and soups, in European colonization regions. On the other hand, crem is used in popular therapies for preventing hypercholesterolemia and detoxification of the body. However, it is the use of the condiment that has stood out for its characteristic flavor and aroma, providing a new economic option for family agribusiness. Other species of the family are also Tropaeolaceae food and the potential to store essential oils, both in the shoot as the booking agencies. This study aimed to evaluate the production of essential oils from crem tubers, through the extraction and quantification of volatile compounds. The study was conducted at the Laboratory of Horticulture of the Faculty of Agronomy and the Laboratory of Pharmacognosy of the Faculty of Pharmacy of Federal University of Rio Grande do Sul. The material used was a sample of tubers taken from a population anthropogenic crem cultivated in the didactic garden of the Agronomy School. The tubers were washed, cut into pieces of 2x2 cm, placed in volumetric flasks 6L and supplemented with distilled water. The essential oil extractions were performed in triplicate graduated by hydrodistillation in Clevenger apparatus, under a recirculating cooling system, for three hours. In the extraction process, the essential oil became miscible with the distilled water being recovered with the aid of a dropping funnel, adding to the emulsion the organic solvent hexane. The oil obtained was analyzed in a GC/MS equipped with a fused silica capillary column (25 m X 0.25 mm X 0.25 µm film thickness 5 % phenyl-polydimethylsiloxane phase). The essential oil components were identified by comparing their retention index, and the mass spectrum data obtained in the scientific literature. The results showed an average yield of 0.02 % (w/w) essential oil from crem tubers and confirmed the presence of a major component (69.6 %) of terpenic core with nitrogen and sulfur in its molecule, identified as fluorescein isothiocyanate benzyl. The presence of the major component essentially crem oil can be related to the effects observed empirically by the traditional use of this species as cholesterol reducer.[1]

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Acknowledgements: CAPES, UFRGS.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Effect of Sub-chronic Treatment of the Essential oil of *Lippia origanoides* Kunth. (Verbenaceae) on the Behavior of Rats

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Keywords: *Lippia origanoides*; memory; salva-do-marajó.

Lippia origanoides Kunth, known in northern Brazil as "salva-do-marajó" and "alecrim-d'Angola", is a shrubby specie with a perennial life cycle, whose aerial parts are used in cooking as a flavoring of regional dishes, in the treatment of gastrointestinal disorders, respiratory diseases, and as an antiseptic for mouth and throat irritation.[1] *L. origanoides* already has many ethno-pharmacological applications. This species possess several chemotypes.[2] It's essential oil, extracted from its leaves, has been identified as producer of bioactive compounds important in the antibacterial, antiviral and antioxidant protection.[1]The present work evaluated the behavior effect of the essential oil extracted from *L. origanoides* leaves on the motor coordination, learning and memory in rats. The essential oil of *L. origanoides* (EOLo) (collected in Santarém, Pará State, Brazil) was obtained by hydrodistillation and analyzed by gas chromatography mass spectrometry and its major compounds were carvacrol (46.1 %) and thymol (11.8 %) as the main components. Sub-chronic toxic effects of orally administered oil were investigated in male Wistar rats (n=6) using the standard methods for Morris water maze (MWM), Rota rod (RR) and passive avoidance (PA) tests. Doses of 30, 60 and 120 mg kg^{-1} of the EOLo were administered for 21 days, and in the 22^{nd} day the experiments were performed. All the doses of EOLo did not induce significant changes in the rats spatial memory for the MWM test. In the RR and PA tests, the doses of EOLo didn't present significant difference compared to the control, suggesting that EOLo doesn't affect spatial, recent and later memory and locomotion in rats after oral administration in the experimental model used in this study.

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Acknowledgements: LabBBEX; UFOPA; CNPq; Projeto CSF-PVE's, Processo 88881.030.467/2013-01.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Study on Essential Oils of *Alpinia zerumbet* Varieties

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Keywords: Zingiberaceae; *Alpinia zerumbet*; essential oil; hydrodistillation; GC-MS.

Zingiberaceae family, the largest of Zingiberales order, consists of 53 genera and over 1.200 species native to tropical regions, especially Southern and Southeast Asia. Its leaves are long, wide, bright and have a characteristic aroma. It's essential oil is popularly used for anxiety problems, pain and hypertension.[1,2] In this context, the aim of this study was to identify and compare the essential oil composition of the leaves of *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. and its variety *Alpinia zerumbet* var. *variegata*. *A. zerumbet* leaves were collected in 2012 and 2013 and *A. zerumbet* var. *variegata* leaves were collected in 2013, both from cultivated area in Rio de Janeiro. The essential oils were obtained by hydrodistillation with 4 L of water and 200 g of fresh leaves of both species in Clevenger apparatus. The extractions were performed in triplicate and the technique was considered satisfactory for obtaining the respective oils. All extracts were analyzed by gas chromatography coupled to a quadrupole mass spectrometer with ionization by electronic impact (70 eV), fitted with a DB-5MS column and helium as carrier gas. The GC oven program was as follows: 50 ^{o}C to 290 ^{o}C at 5 ^{o}C min^{-1}. Interpretation and identification of the fragmentation mass spectrum was carried out by comparison with the Wiley NBS mass spectrum database. The comparison of the three essential oils showed similar qualitative profile. The major identified substances of both *A. zerumbet* essential oil were 4-terpineol (22 %), 1,8-cineole (18 %) and γ-terpinene (14 %). The major constituents of *A. zerumbet* var. *variegata* essential oil were 1,8-cineole (39 %), β-pinene (11 %) and β-caryophyllene (10 %).These results indicated that the species *A. zerumbet* and its botanical variety were considered chemically similar to those cultivated in other countries.

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Acknowledgements: PIBIC/CNPq, Farmanguinhos-FIOCRUZ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Seasonality Effect on Essential Oil Chemical Composition of Fresh Leaves of *Syzygium cumini* Collected in the Amazon Region

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Keywords: Seasonality, *Syzygium cumini*, α-pinene.

Essential oils with medicinal importance can be found in the families Apiaceae, Asteraceae, Geraniaceae, Lamiaceae, Lauraceae, Liliaceae, Myrtaceae, Piperaceae, Pinaceae, Poaceae, Rutaceae, Rosaceae, Santalaceae and Zingiberaceae. The main chemical constituents present in the essential oil are hydrocarbons (pinene, limonene, bisabolene), alcohols (linalool, santalol), acids (benzoic and geranic acid), aldehydes (citral), cyclic aldehydes (cuminal), ketones (camphor), lactones (bergapten), phenol (eugenol), phenolic ethers (anethole), oxide (1,8-cineole) and esters (geranyl acetate. The chemical composition of essential oils can vary according to geoclimatic location, culture conditions (type of soil, climate, altitude and water availability), plant development stage (e.g., before or after flowering), harvest time, etc.. The medicinal properties of essential oils include antimicrobial, analgesic, sedative, anti-inflammatory, anesthetic, etc. *Syzygium cumini* (L.) Skeels belongs to the Myrtaceae family, is original from India and Java. In Brazil, it is found in various states of the Southeast, Northeast and North.. The essential oil from leaves was obtained by hydrodistillation of fresh material (1000 g) in Clevenger apparatus (6 h), analyzed by GC-MS Shimadzu QP2010 mass spectrometer in a DB-5MS column, 30 m x 0.25 mm, inner film thickness of 0.25 µm. Identification of the constituents was made by interpretation of their mass spectra with the aid of the library database NIST, the Kovat's index calculation and by comparison with literature. Essential oil yield was obtained in a minimum of 0.072% in Feb/2013 and up to 0.130% in Dec/2014 (volume/mass). The chemical composition varied in all periods analyzed. In October/2013, 37 substances were identified and in June/ 2014, 22 substances, corresponding to a composition of 93.6 % and 97.9 %, respectively. The major constituents were α-pinene (56.1 %), camphene (1.2 %), β-pinene (13.9 %), β-myrcene (3.9 %), *p*-cymene (1.0 %), limonene (7.7 %), (*Z*)-β-ocimene (23.9 %), (*E*)-β-ocimene (8.1 %), α-terpineol (7.0 %), bornyl acetate (2.4 %), *trans*-caryophyllene (12.4 %), α-humulene (5.6 %) and caryophyllene oxide (5.9 %). The *α*-pinene was the substance with the highest percentage in area in the whole period.

Acknowledgements: FAPEAM, UFAM and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antibacterial Activity of Essential Oil from Fresh Leaves of *Conobea scoparioides* (Cham. & Schltdl.) Benth. from Santarém, Pará, Brazil

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Keywords: Pataqueira; essential oil; antibacterial activity.

Essential oils are used for centuries for medicinal purposes, which has stimulated the search for biologically active substances, especially for microorganisms. The bacteriostatic activity and/or bactericidal activity in essential oils are mainly exerted by terpenoids. *Conobea scoparioides* (Cham. &Schltdl.) Benth., popularly known as "pataqueira" is a species from the Amazon with very high interest to the pharmaceutical and cosmetic Brazilian industries, and of the main sources of thymol from aromatic plants.[1] The objective of this study was to evaluate the antibacterial activity of "pataqueira" essential oil on strains of microorganisms: *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteritidis* by disk diffusion technique. The essential oil was obtained by standard methodology hydrodistillation using a Clevenger-type apparatus. Clinical isolates of bacteria kept in the microbiology laboratory of the Federal University of Western Pará – UFOPA were tested. The essential oil obtained was tested by diffusion method in paper disc into Petri plates containing Mueller-Hinton medium, seeded with bacterial suspension previously adjusted. Discs containing 10 µL of the essential oil without any dilution were added to the plates at 36 °C for 24 h. Ciprofloxacin discs (CIPRO) of 50 mg, were used as positive control and Tween 80 discs as negative control. The test was performed in triplicate. For the comparative analysis between treatments, we used the statistical software PRISM (version 3.0), by ANOVA followed by Tukey test, statistical differences were considered significant in situations where $p < 0.05$, demonstrating the potential difference antibacterial tested oil. It was found that the essential oil of "pataqueira" presented antibacterial activity to *S. aureus*, *S. enteritidis* and *E. coli*; with halos of 14.67 mm, 16.33 mm and 12.33 mm, respectively, where $p < 0.001$ compared to the negative control group, allowing to conclude that the essential oil of "pataqueira" (*C.scoparioides*) showed antibacterial activity to bacteria tested.

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Acknowledgements: Laboratory of Plant Biotechnology UFOPA Microbiology Laboratory of UFOPA, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Antioxidant Activity of Essential Oil from Lippia thymoides Mart. & Schauer (Verbenaceae)

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Keywords: *Lippia thymoides*; essential oil composition; antioxidant activity.

Lippia thymoides Mart. & Schauer (Verbenaceae) is a shrub growing up to 2 m, erect, branched, with little and aromatic leaves. The species is native and endemic from Brazil and popularly known as ''alecrim-do-mato'' or ''alecrim-do-campo''. In folk medicine, is used to treat injuries, fever, bronchitis, rheumatism, headaches, and weakness.[1,2] In the present study, the specimen was collected in Abaetetuba (Pará State, Brazil) in February and September 2014. Aerial parts of *L. thymoides* were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were analyzed by GC/MS in instrument Thermo DSQII systems. The operating conditions were as follows: DB-5MS fused-silica capillary column (30 m X 0.25 mm X 0.25 µm film thickness); programmed temperature: 60-250 °C (3°C min^{-1}); injector temperature: 250 °C; carrier gas: helium, adjusted to a linear velocity of 32 cm s^{-1}; Mass detector was operated in electronic ionization mode at 70 eV. The retention index was calculated for all the volatiles constituents using an *n*-alkane homologous series. Individual components were identified by comparison of both mass spectrum and retention index data with authentic compounds cited in the literature.[3] The oil yields were 0.9 % (February) and 0.4 % (September). The main constituents were the oxygenated monoterpene thymol in February (75.7 %) and September (60.0 %), followed by thymol acetate (5.2 %, 5.9 %) and monoterpene hydrocarbons: *p*-cymene (6.4 %, 8.5 %) and γ-terpinene (4.9 %, 7.9 %), and sesquiterpene β-caryophyllene (2.9 %, 4.6 %), respectively. The antioxidant activity of the essential oils was evaluated by DPPH radical scavenging method. The oils did not showed statistical differences to Trolox Equivalent Antioxidant Capacity (TEAC) with average value of 150.2 ± 3.9 mg ET g^{-1}. These results suggest that antioxidant activity do not have direct correlation with thymol concentration.

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Acknowledgements: UFPA, SEDUC-PARÁ, MPEG.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Biotransformation of Geraniol by *In Vitro* Cultures of *Aloysia triphylla* (L'Herit) Britton: Effect on Growth and Volatile Components

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Keywords: *Aloysiacitriodora*; verbenaceae; micropropagation; biotransformation.

Aloysia triphylla (L'Herit) Britton (= *A. triodora* Palau), is an aromatic and medicinal plant. Late spring cuttings are the current propagation method of this species, but there can be losses during winter. Micropropagation is a promising technology for plant multiplication without disruption as well as for production of diverse plant metabolites. The biotransformation capacity of *in vitro* cultures of *A. triphylla* shoots after the addition of the oxygen-containing monoterpene geraniol, by assessing the cultures growth and volatiles composition, was studied. Explants were aseptically inoculated in 15 mL WPM medium [1] with and without (control cultures) geraniol addition at 25 mg L^{-1}. Cultures were maintained at 24 °C in a photoperiod of 16 h light. Growth and volatiles production were evaluated at the inoculation time (0), 8, 24 and 48 h after inoculation and weekly during four weeks. Shoots growth was evaluated by measuring fresh weight and by using the dissimilation method as in [2]. Volatiles were isolated by hydrodistillation and analyzed by GC and GC–MS. Substrate addition had no negative influence on shoots growth. Geranial, neral, limonene and 1,8-cineole were the main constitutive volatiles components of *A. triphylla* shoots, although in variable amounts during the growth period. No new volatiles were detected after geraniol addition. Given the possibility of geraniol integration into non-volatile components, as it was found in other *in vitro* plant culture systems[2], running experiments will evaluate the possibility of geraniol glycosylation.

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Acknowledgements: This study was partially funded by CNPq, CAPES, FAPEMIG and Fundação para a Ciência e a Tecnologia (FCT) under Pest-OE/EQB/LA0023/2011, UID/AMB/50017/2013and research contract PEOPLE MC-IRSES, FP7-PEOPLE-2011-IRSES, PIRSES-GA-2011-295251.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development of an Analytical Method for the Analysis of Monoterpenes in *Alpinia zerumbet* Essential Oil by Gas Chromatography Using Central Composite Design Optimization Combined with Different Column Types

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Keywords: *Alpinia zerumbet*; essential oil; central composite design.

Alpinia zerumbet is native of West Asia and has a large distribution in South America. In Brazil, it is popularly named as "colônia". The essential oil of *A. zerumbet* (EOAZ) has been reported by pre-clinical studies to exhibit antihypertensive activity, a pharmacological effect related to the presence of monoterpenes.[1] A selective GC analytical method for the analysis of monoterpenes in EOAZ has not been reported. Therefore, the aim of this study was to develop a selective GC analytical method for analysis of monoterpenes in EOAZ using central composite design (CCD) combined with different column types. Fresh leaves of *A. zerumbet* were obtained from Fundação Oswaldo Cruz, in the city of Rio de Janeiro, Brazil, in August 2014. The essential oil was extracted by hydrodistillation, using Clevenger apparatus. The CCD experiments were carried out, starting with a full factorial design (with a replicate at center point) with axial points. Three GC parameters were chosen as factors, oven initial temperature, heating rate and flow rate with a total of 16 runs and one replicate. The columns chosen were DB-5, DB-1, DB-17ht, DB-35, DB-1301 and DB-1701. The method was developed in a CG/FID and the substances were identified by CG/MS. Terpinen-4-ol, 1,8-cineole, sabinene and γ-terpinene corresponded to 22 %, 21 %, 20 % and 11 % of the crude oil, respectively. DB-17ht and DB-1 resulted in asymmetric and tailed peaks with poor separations. Intermediate polarity columns showed better results. Nevertheless, the best conditions were observed in DB-5 and DB-35 columns, all the peaks corresponding to the monoterpenes in DB-5 analysis had baseline resolution with exception of the separation between 1,8-cineole and limonene. However, a resolution of 1.56 was possible in DB-35, after optimization by CCD. So, the final optimized method had an initial oven temperature of 70 °C, heating rate of 4 °C min^{-1} and flow rate of 2 mL min^{-1} with a DB-35 column. This is the optimized method ideally suited for the separation and identification of monoterpenes in EOAZ by gas chromatography.

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Acknowledgements: Antonio Carlos Siani for lending the Clevenger apparatus.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Secretory Structures of *Vernonia polyanthes* Less Sheets and Production of Essential Oils in Different Seasons

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Keywords: *Vernonia polyanthes*; essential oil; histochemistry.

Vernonia is one of the most complex genres from a taxonomic point of view due to the high diversity of its biological forms. *Vernonia polyanthes* Less., popularly known as assa-peixe or assa-peixe branco, is a common wild species in the cerrado of Minas Gerais, São Paulo, Mato Grosso and Goiás. The leaves have disposal alternate, with short petioles, lanceolate, serrated edges, attenuated base and acute apex. They are rough on the abaxial surface and hairy on the adaxial and have about 13 cm long and 3 cm wide. The essential oils extracted from its leaves are important in the treatment of colds, flu, bronchitis, bruises, hemorrhoids and infections of the uterus.[1] Through the phytochemical characterization of its aerial parts, it was reported the presence of fixed acids, alkaloids, amino compounds, coumarins, steroids, triterpenes, anthraquinone glycosides, flavonoid glycosides, saponins and hydrolysed glycosides.[2] However, despite the medical importance of this species, detailed studies of the producer sites such as biologically active substances are scarce. The objective of this study was to identify the main essential oils production sites in the leaf blade of *V. polyanthes*, seeking to assess whether the production of these substances occur in different seasons. Expanded leaves were collected from adults at the vegetative stage in the didactic orchard of the Department of Horticulture, FCA, UNESP, Botucatu, São Paulo, in three different periods - November 2014, February and May 2015. Samples of fresh material were freehand sectioned, processed according to conventional light microscopy techniques and treated with Nadi reagent. Essential oils were detected in epithelial cells and in the lumen of secretory cavities in glandular trichomes and glandular located on both sides of the leaf blade and leaf common mesophyll parenchyma cells, in the three periods. Thus, it can be concluded that the synthesis and storage of essential oils occur in different secretory structures during all times analyzed, requiring quantitative and qualitative assessments of the secreted content to determine possible differences in the yield and chemical composition of substances produced.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of *Lippia origanoides* Kunth Essential Oil According to the Harvest Moment

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Keywords: Composition; carvacrol; Verbenaceae; semiarid.

Lippia origanoides (Verbenaceae) is a specie native from Brazil, commonly known as "alecrim-do-tabuleiro". Traditionally the leaves and flowers are used in the popular medicine as infusion to stomach pain, indigestion and flatulence. The medicinal potential found in this species is mainly related to the chemical components present in the essential oil. The aim of this work was to evaluate the effect of the interval between harvests in chemical compounds from *L. origanoides* essential oil. The study was held in the experimental field of the State University of Feira de Santana. *L. origanoides* plants were propagated by cuttings from grown matrix plants in the collection of aromatic plants of that University. The experiment was installed in the Horto Florestal of the State University of Feira de Santana - Bahia, using the experimental design of randomized blocks with four replications and four harvest intervals (90, 180, 270 and 360 days after the first regrowth). Then leaves and inflorescences were packed in paper bag and dried at 40 ^{o}C until constant weight. Briefly, 40 g of dried and milled plant material were extracted with sufficient water amount during 3 h in a Clevenger-type apparatus. The determination of the chemical composition of essential oils of *L. origanoides* was analyzed by GC/FID and by GC/MS. From the obtained essential oil were identified 28 components, with carvacrol as the main compound ranging (39.2-46.5 %), following γ-terpinene (10.5-15.5 %), *p*-cymene (11.0-13.0 %), (*E*)-caryophyllene (3.6-4.5 %) and linalool (1.0-4.1 %).The remaining components were considered as minor components, presenting values below 4 %. From the main components only linalool was influenced over time of harvest regrowth, with low concentrations at 90 days after the first regrowth. For carvacrol, γ-terpinene, *p*-cymene, (*E*)-caryophyllene and linalool no statistical differences were observed between harvest moments. According to the conditions at which the experiment was conducted, it was concluded that the harvest moment influence quantitatively the composition of essential oils. Carvacrol a compound identified in great quantity, could decisively contribute for this species important bioactive characteristics, which can be applied in the pharmaceutical industries, food and cosmetic sectors.

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Acknowledgements: FAPESB, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In vitro Antioxidant Activity of Essential Oil of *Mentha piperita* L. Leaves Grown Under Hydric Stress

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Keywords: *Mentha*; moisture levels; total antioxidant activity; chelating power.

Water deficit is a strategy to increase phytochemicals in plants, however to avoid the negative effects of excessive ROS production, such as cell damage and death, this must be carefully managed.[1] The aim of this study was to compare the influence of different levels of soil moisture in antioxidant activity of the essential oil of *Mentha piperita* L. Plants were grown in 5 L pots filled with 4 kg of soil and cattle manure mixture 3:1. In the first 30 days plants were irrigated by drip at 100% of field capacity (FC) every 72 h. FC was determined by gravimetric method after 72 h draining. Irrigation system design consisted of four levels of moisture: (T1) 100 % FC; (T2) 80 % FC; (T3) 60 % FC; (T4) 40 % FC. Experimental design was laid out in randomized block with 4 treatments in 5 blocks with 5 pots containing 1 plant/pot. After 70 days, leaves were harvest and oven dried at 40±2 ºC. The essential oil obtained by the leaves hydrodistillation was analyzed by GC and GC/MS and evaluated its antioxidant capacity. Total antioxidant capacity was measured based on the reduction of ammonium molybdate method. Degree of chelating ferrous ions by samples was evaluated and DPPH free radical scavenging activity was accomplished. Data were analyzed by one-way analysis of variation (ANOVA), and differences among treatments were determined by a comparison of means using Tukey's test ($p < 0.05$). There was statistical difference in the menthol content between soil moisture levels. The lowest value was observed with 40 % of field capacity (25.98 %), the other treatments showed an average of 29.11 %. Menthone and menthofuran did not differ statistically among treatments. However, highest limonene content was observed in plants grown in 40 % of FC. Plants grown in 60 % and 40 % of the FC had higher total antioxidant activity. Total antioxidant activity in this treatment increased about 55 % compared to control (100 % FC). DPPH free radical scavenging activity had no significant differences between treatments, but low values of IC_{50} (3.32 to 4.73 mg mL^{-1}) denoted high activity. The essential oil extracted from plants grown in 80 % of FC showed the highest power of chelating iron ions (IC_{50} = 0.18 mg mL^{-1}). The results of this study suggest that hydric stress has effect on menthol and limonene content in the peppermint essential oil, which influenced total antioxidant activity and chelating power.

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Acknowledgements: FAPEMIG, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antifungal Activity of Essential Oils, Its Major Constituents and Emulsions of *Lippia gracilis* Genotypes against *Colletotrichum acutatum*

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Keywords: *Lippia gracilis*; volatile oil; antimicrobial activity.

The aim of this study was to evaluate the antifungal activity of emulsions and essential oils of *Lippia gracilis* genotypes against the plant pathogenic fungus *Colletotrichum acutatum* in vitro. The essential oils were obtained by hydrodistillation using a Clevenger apparatus. The emulsions were obtained from the mixture of essential oils from two genotypes of *L. gracilis* with surfactants and water. Samples of essential oils and emulsions were analyzed by GC-MS/FID. For testing the inhibition of mycelial growth of the fungus, according to the methodology described by Costa e Carvalho[1], we used Potato Dextrose Agar (PDA) medium and different concentrations of the essential oils, thymol, carvacrol and emulsions to determine the lethal and inhibition concentrations. Discs of 0.7 cm diameter containing mycelia were transferred to the center of plates containing the solid medium. The major compound identified for genotype LGRA106 was thymol (63 %) and for LGRA109 was carvacrol (46 %). For LGRA106 we observed a minimum inhibitory concentration (MIC) of 0.2 % and a minimum lethal concentration (MLC) of 0.2 %. For LGRA109 we observed a MIC of 0.2% and a MLC of 0.2 %. For thymol and carvacrol we observed a MIC of 0.2 % and a MLC of 0.4 %. For the emulsions we observed a MIC of 0.6 %. Essential oils, emulsions and the major compounds showed fungicidal profile, being the lowest concentration tested 0.1 % capable to inhibit the mycelial growth by over 50 % for all tested substances against fungal plant pathogen *C. acutatum* in vitro.

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Acknowledgements: FAPITEC/SE, CNPq, CAPES,FINEP, Embrapa Tabuleiros Costeiros, EMDAGRO.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

A Study of the Composition of the Essential Oil from Aroeira (*Myracrodruon urundeuva)* in Different Stages of Development

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Keywords: *Myracrodruon urundeuva*; Aroeira; essential oil.

The northeast region of the state of Minas Gerais, specifically the Salinas region, is inserted into the transition between the Cerrado and Caatinga areas. The vegetation of this ecosystem is exposed to water stress (dryland vegetation) and adapted to a harsh climate with low annual rainfall distributed in a small time window. The type of vegetation that predominates in this region is called the Mata Seca (Dry Forest), which has a very diverse flora and vegetation characterized by a high degree of leaf deciduousness during the dry season.[1] One of the most significant plant species in this biome is the Aroeira (*Myracrodruon urundeuva*) of the Anacardiaceae family. The composition of the essential oil from Aroeira at different stages of development was determined. Five individuals were selected from plants with two diameters of trunk. The leaves were collected, the plant material in the same diameter class homogenized, and the essential oil extracted by hydrodistillation in a Clevenger-type apparatus for 4 h. The essential oils were analyzed by GC-MS on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass detector. An HP-5 capillary column (30 m X 0.25 mm X0.25 µm) was used. The injector temperature was 250 °C, the initial column temperature, 60 °C (1 min), the heating rate was 4 °C min^{-1} to 250 °C, where the temperature was maintained for 1 min, and the split ratio was 1:10. The yield of oil was 0.5 %. The essential oil from younger individuals contained high concentrations of 3-carene (70.2 %) and low concentrations of α-pinene (2.7 %) and limonene (2.6 %). The presence of terpinolene (3.1 %), β-myrcene (3.3 %), eucalyptol (0.1 %), caryophyllene (1.6 %) benzaldehyde (1.9 %) and linalol (0.1 %) was also observed. Older individuals contained higher concentrations of α-pinene (24.9 %) and limonene (24.9 %), whereas the concentration of 3-carene (35.1 %) was lower than that observed in younger plants. The concentrations of terpinolene (1.8 %), benzaldehyde (0.5 %) and linalol (0.4 %) were higher, but in a lower degree. β-Myrcene, eucalyptol and caryophyllene were not detected in the older individuals.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Evaluation of 16 Populations of *Rosmarinus officinalis*

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Keywords: Rosemary; chemical variability; essential oil.

Rosmarinus officinalis, commonly known as Rosemary, is an aromatic perennial shrub native to the Mediterranean region, with white, pink or blue flowers, which can reach the height of 1.5 meter. It is a member of the family Lamiaceae. The plant flowers in spring and summer in temperate climates. It is one of the most well-known plants since ancient times because of its fragrant, edible and medicinal properties. Rosemary oil can be a colorless or a yellow liquid with camphorated odor. It can be useful in diverse situations, such as pest control effects, food flavor, pharmaceutical and in cosmetics.[1] The purpose of the research was to identify the chemical variability of 16 populations of *Rosmarinus officinalis*. Samples were cultivated at Universidade de Caxias do Sul, Caxias do Sul, RS, and harvested in April 2014. Nine samples of fresh leaves (50 g) and 7 samples of dry leaves (20 g) were collected. The leaves were subjected to hydrodistillation in a Clevenger-type apparatus for approximately 2 h. The essential oils were analyzed GC/MS in an Agilent 5973N system, equipped with HP Chemstation software and Wiley 275 mass spectral library. HP-5MS fused silica capillary column (30 m X 250 µm) 0.50 µm of film thickness was used. Column temperature was raised from 60 to 180 °C at 3 °C min^{-1} and 20 °C min^{-1} to 230 °C. Helium was used as carrier gas for GC/MS with a flow rate of 1.0 mL $minute^{-1}$ and mass detector was operated in electronic ionization mode at 70 eV. Thirty three compounds and 4 chemotypes were identified in the oil samples. As expected, the major components were terpenes followed by 1,8-cineole, camphor and borneol. The first chemotype high in terpenes and oxide contains α-pinene (16.6 %) 1,8-cineole (20.6 %) and verbenone (7.4 %). The second chemotype identified high in oxide and ketone has 1,8-cineole (16 %) and camphor (36.7 %). The third one contains α-pinene (18.9 %), 1,8-cineole (7.2 %) and camphor (12.4 %). The forth chemotype high in ketone and terpene has camphor (20 %) and borneol (13.5 %).

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Acknowledgements: UCS, SDECT.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Schinus weinmannifolius* and *Schinus polygamus* Collected in Rio Grande do Sul

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Keywords: *Schinus weinmannifolius*; *Schinus polygamus*; essential oil.

The genus *Schinus* belongs to the family Anacardiaceae and comprises about 600 species found in several typical plants of tropical and subtropical regions[1], with a prevalence of about 27 species in South America. The plants belonging to this genus are commonly known as mastic and can be identified by the production of essential oils, among other constituents. One of the most abundant characteristics, mainly present in its fruits is the strong and concentrated smell.[2] Therefore, this study aimed the chemical characterization of the volatile oil from leaves and fruits of *Schinus weinmannifolius* and leaves of *Schinus polygamus*, native of Rio Grande do Sul. The aerial parts of both species were collected in Lombas, Santo Antônio da Patrulha. A voucher specimen was deposited in the Herbarium of the UFRGS (ICN).The oil from the leaves and fruit of the species was obtained by hydrodistillation, using the Clevenger-type apparatus for 4 h. The chemical analysis was carried out by GC/MS.The identification of compounds was based on comparison of both retention indices and mass spectra with authentic samples and data from literature.[3] The yield of volatile oil from leaves and fruits of *S. weinmannifolius* was 0.5 % and 2.0 %, respectively, and from leaves of *S. polygamus* was 0.4 %. The major compounds identified in the leaves of *S. weinmannifolius* were the sesquiterpenes α-cadinol (21.2 %), spathulenol (11.0 %) and cubenol (9.8 %). For the fruits of this species, the major compounds were the sesquiterpenes α-cadinol (20.5 %) and spathulenol (9.9 %) and the monoterpene limonene (9.8 %). For the leaves of *S. polygamus*n-nonane (33.5 %), an aliphatic compound and α-cadinol (15.4 %) were the main compounds. These results demonstrate that the cadinane cyclization pathway is one of the biosynthetic pathways that are present, especially by the α-cadinol, a common compound for both species.

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Acknowledgements: Capes, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

The Role of HSCCC to Separate Volatile Metabolites from Brazilian Native Species

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Keywords: Separation process; *Piper*; *Eugenia*; terpenes; HSCCC.

Essential oils (EOs) are hydrophobic liquids, usually extracted by hydrodistillation, containing volatile aromatic compounds. The preparative separation of volatile compounds from these complex mixtures is always a challenge due to their structural similarity, strongly hydrophobic properties and poor stability. High Speed Counter Current Chromatography (HSCCC), a support-free liquid–liquid partition chromatographic technique, is the chromatographic method that permits a wide range polarity phase operation, and a fast running time separation process. It is as saving solvent process useful in the isolation of compounds, with a very limited thermal and chemical stability. In this work, we present the successful separation process strategy of major volatile compounds from native Brazilian plant species in preparative scale using HSCCC. The method uses a solvent system composed by hexane/acetonitrile (1:1) to isolate many terpenes in isocratic elution (normal and reversed modes) in run time average of 3 h recovering up to 94 % of the mass applied. In this process, we separate the major constituents of four Brazilian aromatic species: *Piper claussenianum*, *Manekia obtusa*, *Pectis brevipedunculata* and *Eugenia uniflora*. *P. claussenianum* showed to produce a very active leaf EO against *Leishmania* species. The HSCCC separation process provided the isolation of the major metabolites nerolidol (75 mg, 93.0 %) and linalool (230 mg, 98.2 %) as well as camphor (46 mg, 97.4 %), bornyl acetate (7 mg, 87.8 %) and camphene (35 mg, 81.8 %). Safrole represents roughly 8-10 % of the crude EO of *M. obtusa*. This compound has an important economic value since it is used as derivative in many chemical synthesis processes. Through HSCCC, we obtained 200 mg of safrole in 95 % of purity in 2 h of running separation process. The EO from the native grass *P. brevipedunculata* has a citric fragrancy due to the presence of citral as its major component. In 2 h running time it was possible to separate the isomers pair neral/geranial (780 mg, 98.7 %), as well as geraniol (8 mg, 86.0 %), geranial (12 mg, 91.0 %) and neral (7 mg, 81.0 %) with high percentage mass recovery and at high purity level. *Eugenia uniflora*, a popular Myrtaceae species, is known as Brazilian cherry due to its edible fruits. Using HSCCC it was also possible to isolate in high percentage their major constituents:(-)-oxidoseline-1,3,7-(11)-trien-8-one (375 mg, 97 %) and (364 mg, 92 %) of(-)-seline-1,3,7(11)-trien-8-one in just one separation step. HSCCC is a useful tool to separate terpenes and bioactive non-polar compounds in the essential oils from different Brazilian native plant species.

Acknowledgements: We thank to CAPES and CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Yield from Three Genotypes of *Mikania laevigata* Grown in Organic System

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Keywords: *Mikania laevigata,* essential oil, organic system.

In Brazil the species *Mikania laevigata* is widely used due to antiallergic, antispasmodic, anti-inflammatory, anti-ulcer, antimicrobial, bronchodilator and relaxing smooth muscles properties.[1] The influence of genetic and environmental variations in the secondary metabolites of plants may have implications on their biological effects.[2,3] The secondary metabolites identified in *M. laevigata* are: essential oils, stigmasterol, hydrolysable tannins, flavonoids, saponins and coumarin with pharmacological properties described in the literature.[4] The essential oil essential extracted from leaves of *Mikania* genus presents antimicrobial activity.[5] This work aimed to evaluate the essential oil yield in *M. laevigata* genotypes cultivated at organic system. Herbarium specimens of three genotypes (Embrapa, Cpqba and Unaerp) were incorporated into the Herbarium PAMG under the numbers 57032, 57033 and 57031 respectively. The cuttings were rooted and transplanted in a 2 x 1 m spacing at the Experimental Research Station of EPAMIG, Oratórios-MG, Brazil. The leaves were separated of stems, weighed and dried in an oven with forced air circulation (40 °C) to constant weight. Dried leaves (100 g) were subjected to hydrodistillation separately in a Clevenger-type apparatus for six hours each. Embrapa (0.3%) and CPQBA (0.28%) genotypes presents significantly higher essential oil yield than Unaerp (0.19%). The essential oil yield depends on the genotype and environment interaction.

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Acknowledgements: FAPEMIG, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

The Essential Oil Content of *Vernonia polyanthes* Less Evaluation at Different Times of Collection

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Keywords: assa-peixe; essential oil; seasonality.

The assa-peixe (*Vernonia polyanthes* Less.) is widely used in popular medicine in cases of bruises, hemorrhoids, uterine infections, bronchitis and as diuretic. The species has in its composition fixed acids, alkaloids, amino groups, coumarins, anthraquinone glycosides, flavonoid glycosides, saponins glycosides and essential oils. It is one of the plants found in the SUS interest list (RENISUS). Considering the value of medicinal plants not only as a therapeutic resource, but also as a source of income for family farms, it is important to establish lines of action aimed at developing management techniques, targeting the use of this species, together with the maintenance the balance of tropical ecosystems. Therefore, this study aimed to evaluate the essential oil content of *V. polyanthes* in three seasons collection in a native area located in the didactic orchard of the Department of Horticulture and deposited in the Herbarium Irina DelanovaGemtchujnicov, Universidade Estadual Paulista, Botucatu-SP under BOTU record 25797-assa-peixe. Completely randomized design was used with three treatments in 60 days gathering intervals, and the collection in November 2014, February and May 2015. Plant material of 10 plants was collected and made up the sample comprised a total of five repetitions. Fifty grams of dry biomass (dried at 38 °C in an oven with forced circulation) were used for each essential oil extraction. Essential oil from leaves was extracted by hydrodistillation in a Clevenger type apparatus 2 h. The results for the essential oil content were subjected to analysis of variance, followed by comparisons of means by Tukey test at the level of 5% probability. The content was determined based on 100 grams of dry biomass leaves. There was no statistical difference in the essential oil content presented in different times of collection, being found in November (0.10 %), February (0.15 %) and May (0.18 %). Based on the results, it is concluded that the collected plant material can be in any of the measured times, giving preference to those that have larger amount of plant leaves.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of the Coagulating Potential of the Essential Oils from *Hyptis carpinifolia* Benth. and *Lippia origanoides* Kunth.

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Keywords: essential oils; coagulation; snake venom.

New inhibitors of snake venom toxins, especially those compounds that neutralize the local effects of accidental poisoning, are essential to complement or even replace traditional serum therapy. Plants provide great choices for scientific research and represent a growing market. Brazil has an exceptional variety of flora; and the functional properties of the species that comprise this flora must be studied. *Hyptis carpinifolia* Benth., popularly known as rosemary, is a plant widely used in folk medicine for the treatment of diseases such as influenza, colds and rheumatism.[1] *Lippi aoriganoides* Kunth. (Verbenaceae), an aromatic bush native to Central America and northern South America, is used in traditional medicine for the treatment of gastrointestinal and respiratory diseases. This study sought to assess the potential of the essential oils from *H. carpinifolia* Benth. and *L. origanoides* Kunth. for inhibiting the coagulation induced by the *Lachesis muta* (bushmaster) venom. The plant material was collected in the Garden of Medicinal Plants of the Federal University of Lavras (*L. origanoides*) and the municipality of Itumirim-MG, Brazil (*H. carpinifolia*). The essential oils were obtained by hydrodistillation of the leaves using a modified Clevenger apparatus in the Laboratory of Organic Chemistry - Essential Oils of the same University. The inhibitory action of the essential oils against the clotting activity induced by snake venom was evaluated according to the method described by Valentin and Lambeau using human citrated plasma (200 µL) provided by a clinical laboratory of the city of Lavras-MG. *Lachesismuta* (8 µg) venom was used for inducing the clotting of plasma. The essential oils used in the bioassay (0.6; 1.2 and 1.8 µL) were pre-incubated with plasma for 10 minutes at 37 °C prior to adding the venom. The percentage reduction in clotting time with both essential oils varied in a dose-dependent manner. For the oil from *H. carpinifolia* the reduction was 32% for the lowest volume and 51% for the 1.8 µL volume. For the oil from *L. origanoides* the reductions were 40% and 56%, respectively. One can conclude that the essential oils had a pro-coagulant effect on human plasma. When added to plasma, these oils were probably capable of interacting with plasma lipids, whereas the proteins remained available for the coagulation cascade caused by the action of venom proteases.

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Acknowledgements: CNPq, FAPEMIG, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Characterization of the Essential Oil of *Vernonia polyanthes* Less.

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Keywords:essential oil; medicinal plant; cerrado.

Vernonia polyanthes Less.is a shrubby plant native to common Asteraceae family in the Cerrado of São Paulo, Minas Gerais and Goiás. Popularly known as assa-peixe, assa-peixe branco, estaca-sangue, tramanhém, mata-pasto, cambará-guassu, cambará-do-branco, erva-preá and enxuga.[1] Their leaf are used to treat disorders of the respiratory tract, kidney problems, fractures, indicated also as tonic, diuretic and emenagoga and is currently present in RENISUS list.[2] In a study conducted in Minas Gerais using the assa-peixe oil in controlling anthracnose, the major compounds were found: Germacrene D (4.18%) and biclyclogermacrene (17.23%).[3] Therefore, this study aimed to analyze the chemical composition of the essential oil of *V. polyanthes*. The plant material was collected in native area located in the didactic orchard of the Department of Horticulture and deposited in the Herbarium Irina Delanova Gemtchujnicov, Universidade Estadual Paulista, Botucatu, and registered as BOTU 2579-roast-fish. It was used 50 g of dry biomass for leaf essential oil extraction. The essential oil was extracted by hydrodistillation in Clevenger apparatus, for 120 min. The chemical composition of the essential oils was performed CG/MS equipped with an DB-5 column. The GC oven temperature was programmed at 60 °C to 230 °C, 3 °C min^{-1}, injector at 240°C, detector at 230 °C, helium as the carrier gas (1.0 mL min^{-1}). Fourteen components, which accounted for 98.2 % of the total oil composition, were identified: germacrene D (25.0 %), *trans*-caryophyllene (19.5 %), α-humulene (16.4 %), biclyclogermacrene (9.0 %), *n*-tricosane (8.7 %), α-copaene (5.8 %), caryophyllene oxide (3.1 %), germacrene A (2.6 %), β-elemene (2.5 %), *allo*-aromadendrene (2.5 %), spathulenol (1.8 %), δ-cadinene (1.5 %), 14-hydroxy-caryophyllene (0.8 %), β-bourbonene (0.7 %). Given the results, it is concluded that despite the majority compound is the same in both locations, there are differences in the levels of each substance, demonstrating the influence of the environment on the chemical composition of the essential oil.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Baccharis (Asteraceae): a Source of Odorants Compounds Diversity from South Brazilian Highlands

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Keywords: *Baccharis sp*.; GC-O; multivariate analysis.

Asteraceae is the biggest flowering plant family worldwide. Into such family, *Baccharis* genus is represented by more than 360 species exclusively in the American continent. In particular, South Brazil is a very rich region for this genus, mainly in highlands, an area of transition between different biomes and which is supposed to be a center of biodiversity; a reason by which this region might be preserved. Odorant chemistry diversity of the *Baccharis* genus is a characteristic fact and a reason to develop new aromatic products. In the frame of our study about the biologic impact of volatile composition in the *Baccharis* genus, we collected eight species (*B. anomala*, *B. articulata*, *B. milleflora, B. megapotamica*, *B. tridentata,B. trimera*, *B. uncinella* and *B. vulneraria*) from the highlands at the ´*Estação de Conservação e PesquisaPró Mata*´ (S.F. de Paula, Rio Grande do Sul). Aerial parts were dried and the volatile compounds extracted by simultaneous distillation extraction. Chemical analyses were performed by GC/MS using two capillary columns of different polarity and calculating the linear retention index for identification purposes.For the most promising species (*B. anomala*, *B. articulata* and *B. uncinella*) key odorants compounds were evaluated by GC-O. In all cases, a very complex volatile profile was found, with almost 200 compounds identified and many co-elutions detected. Mono and sesquiterpenes were the predominant components in all species with minor amounts of phenylpropanoids and aliphatic compounds.[1] Composition (identity and percentage proportion of each component) of species collected at the same time (*B. milleflora*, *B. tridentata*, *B. trimera* and *B. uncinella*) were used as raw data for statistical multivariate analyses. The results showed two separated groups: the first brings together *B. milleflora*, *B trimera* and *B. uncinella* volatile extracts, characterized by the presence of higher proportions of sesquiterpene compounds especially spathulenol. The second cluster was formed by *B. tridentata* containing α-pinene, β-pinene, limonene and (*E*)-β-ocimene as main components. *B. vulneraria* showed in its volatile profile the diterpene abienol as a major compound. Key odorants of *B. anomala* give to its essential oil an olfactive character sweet and woody while the aromatic notes of *B. articulata* and *B. uncinella* were mainly green, burnt and woody.

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Acknowledgements: ANII, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of Essential Oils from *Varronia curassavica* Jacq. Germplasm

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Keywords: *Varronia curassavica;* germplasm; essential oil.

Varronia curassavica Jacq., ex *Cordia verbenacea* DC., is a native medicinal species from Brazil. This species has a wide distribution in the Brazilian territory and is popularly used in the treatment of inflammation, rheumatism and ulcers. The anti-inflammatory property was assigned to α-humulene and *trans*-caryophyllene compounds present in the essential oil. Due to its anti-inflammatory property, it was developed a phytotherapeutic medicament in Brazil for topical use, obtained from the essential oil, indicated for the treatment of inflammation. The aim of this work was to characterize the chemical diversity of the essential oil from leaves of five native populations of *V. curassavica*Jacq. collected in Northeast Brazil. Leaf samples were collected of 59 *V. curassavica* plants, from five localities in the Sergipe State, Northeast of Brazil. The localities were Graccho Cardoso municipality, São Cristovão municipality, Japaratuba municipality, Graccho Cardoso municipality and Laranjeiras municipality. The essential oils were obtained by hydrodistillation and analyzed by GC/MS-FID. Sixty-three compounds were detected in the essential oil of *V. curassavica*, of which 53 were identified. High chemical diversity was observed among the plants, which are distributed within the chemical groups, regardless of the collection site. By cluster analysis, there was formation of five groups. The cluster 1, consisting of 14 plants, was characterized by presenting turmerone and *trans*-caryophyllene as the major compounds. Cluster 2, consisting of 4 plants, presented as major compounds tricyclene and camphene. Cluster 3, consisting of 5 plants, presented α-zingiberene and β-sesquiphellandrene as the major compounds. Cluster 4, consisting of 13 plants, was characterized by presenting *trans*-caryophyllene and/or 7-cyclodecen-1-one, 7-methyl-3-methylene-10-(1-propyl) as the major compounds. Cluster 5, consisting of 23 plants, presented 7-cyclodecen-1-one, 7-methyl-3-methylene-10-(1-propyl) as the major compound. High correlation was observed between the compounds α-humulene and *trans*-caryophyllene. In this study, besides the majority compounds characteristic of chemical groups, other compounds as sabinene (38.9 %), β-phellandrene (21.4 %), δ-elemene (12.5 %), α-gurjunene (11.7 %), bicyclogermacrene (12.1 %), shyobunone II (14.7 %) shyobunone IV (13.9 %), viridiflorol (15.2 %), ar-turmerone (15.7 %), 1-*epi*-cubenol (13.3 %), and shyobunol (14.3 %) were among the major constituents identified in at least one plant of *V. curassavica*.

Acknowledgements: CNPq, FAPITEC/SE, CAPES, FINEP, RENORBIO.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Chemical Composition of Essential Oils Obtained from Three *Piper* Genus Species

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Keywords: *Piper*; essential oil; antioxidant activity.

Plants of the *Piper* genus are the most representative and abundant plant species of the Piperaceae family, which is distributed in the pan-tropical zone, located in the subtropical forests in America, Africa and the South Pacific.[1,2] They have many applications, as antiparasitic, antifungal, anti-inflammatory, and food-flavoring properties.[1,2] The species under study, *Piper medium* (COL 582360), *P. holtonii*(COL 582357) and *P. cf. subflavum* (COL 582361), were collected in Palmira and Dagua, Valle del Cauca, and their essential oils were obtained by steam distillation. Essential oils analyses were carried out by gas chromatography (*Agilent Technologies 6890N)* with mass spectrometric detection (AT 5975 Inert XL, EI, 70 eV), using two capillary columns: non-polar [5%-phenyl-poly(methylsiloxane) (DB-5MS) 60 m X 0,25mm X 0,26 µm]; and polar [poly(ethyleneglycol) (DB-WAX) 60 m X 0,25 mm X 0,25 µm] stationary phases. Helium was used as the carrier gas at 1 mL min^{-1}. The identification of compounds was performed by comparing linear retention indices and their mass spectra with a built-in library database. Oils from *P. medium* and *P. holtonii* species, both collected in Palmira, showed a very similar chemical composition, with germacrene D (11% and 10%, respectively), dillapiole (14% and 55%, respectively) as main components. β-phellandrene (21%) appeared in *P. medium*, and (*E*)-caryophyllene (9%) in *P. holtoni*. For *Piper cf. subflavum*, collected in Dagua, the essential oil chemical composition was very different, featuring 1,8-cineole (21%), piperitone (17%) and apiole (14%) as major components.Research on the chemical composition and antioxidant activity of this*Piper* species essential oils is relatively new, however, the chemical constituents of *P. medium*, *P. holtonii* and *P. cf. subflavum* have not been described. The antioxidant activity (expressed as µmolTrolox /g substance) of obtained essential oils were: 1410 ± 20 (*P. holtonii*), 1610 ± 50 (*P. cf. subflavum*) and 1820 ± 50 (*P. medium*). The results obtained by the ORAC method showed that all essentials oils studied presented antioxidant activity higher than that of BHT (459 ± 10) and α-tocopherol (550 ± 10), typical standard antioxidants which were used as reference compounds.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antioxidant Activity and Chemical Composition of *Cordia curassavica* Essential Oil Collected in Santander, Colombia

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Keywords: *Cordia curassavica*; antioxidant capacity; essential oil; gas chromatography.

Cordia curassavica is a shrub of the Boraginaceae family, native of tropical and subtropical America. It is geographically distributed from Southern Mexico to Northern South America, although it behaves as an invasive weed in Malaysia and Christmas Island[1]. *C. curassavica* has ovoid leaves with short, thin hair, white or greenish flowers, with red fleshy fruits and is commonly used in folk medicine for its anti-inflammatory properties. Plant materials were collected in the surroundings of Girón, Santander department. Taxonomical identification of the botanical sample was performed at the Colombian National Herbarium, where voucher specimens were deposited (COL 559446). Leaves and stems of the shrub were subjected to microwave-assisted hydrodistillation to afford the essential oil, 0.2 % by weight. Essential oil characterization was carried out by gas chromatography coupled to mass spectrometry. Two columns with different stationary phases were used: polar and non-polar. Individual components were tentatively identified by matching their mass spectra (EI, 70 eV) with those of spectral libraries (ADAMS, NIST, Wiley) and by comparing their linear retention indices with those reported in the scientific literature. The major components found in *C. curassavica* essential oil were: α-copaene (16.5 %), *trans*-β-caryophyllene (21 %), and germacrene D (18 %). The components found in essential oil, but *trans*-caryophyllene, differ from those found in essential oil obtained from Brazil-cultivated plants, where α-pinene appears as the main compound (22.7 %)[2]. The antioxidant activity, determined by the ORAC method, showed that *C. curassavica* essential oil antioxidant capacity exceeded those of reference antioxidants, BHT and α-tocopherol.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Evaluation of the Antioxidant Activity of *Tagetes lucida* Essential Oil

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Keywords: *Tagetes lucida*; MWHD; ORAC.

Tagetes lucida is an aromatic plant, popularly known as pericón, anisillo, hierbanis, and tarragon. This species belongs to the Asteraceae family and is native to Guatemala, Honduras and Mexico. It is distributed mainly in Central and South America, where it is used for flavoring food, for medicinal and ornamental purposes. Pericón possesses biological activities such as anti-inflammatory, insecticidal, bactericidal and anesthetic.[1] The aim of this study was to analyze the essential oil (EO) and to evaluate its antioxidant activity. The EO was obtained by microwave radiation-assisted hydrodistillation (MWHD) in a Clevenger-type apparatus with a Dean Stark distillation receiver. The chromatographic analysis was performed using Agilent Technologies 6890 GC equipment with 5973 mass selective detector (EI, 70 eV). Chromatographic columns DB-5 and DB-WAX (60 m X 0.25 mm ID X 0.25 µm) were used. The injection port temperature was 250°C, split injection mode (30:1). The oven temperature was programmed from 45-150 °C, at 4 °C min^{-1}, was increased to 250 °C (5 min) at 5 °C min^{-1} and 275 °C (15 min), at 10 °C min^{-1}. The identification of compounds was performed by comparing their mass spectra with those of the databases ADAMS, NIST and Wiley and their linear retention indices with those found in the literature. The antioxidant activity was determined by oxygen radical absorbance capacity (ORAC) for hydrophilic antioxidants, with some modifications. The predominant essential oil component was estragole (99.1 %), accompanied by β-myrcene (0.6 %) and *trans*-ocimene (0.1 %). Guzmán and Manjarrez reported methyl eugenol (80 %) and estragole (12 %) as the major components of *Tagetes lucida* EO; and Cicció found 95-97 % of estragole.[2,3] The EO antioxidant capacity (234 ± 8 µmol Trolox® / g EO) was lower than that off commonly used substances, such as BHT (457 ± 9 µmol Trolox® / g substance) and α-tocopherol (550 ± 13 µmol Trolox®/ g substance).

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Acknowledgements: Colciencias-Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of Secondary Metabolites of the *Ambrosia peruviana* (Asteraceae) Essential Oil by Gas Chromatography Coupled to Mass Spectrometry

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Keywords: *Ambrosia peruviana*; essential oil; GC-MS.

The genus *Ambrosia* belongs to the Asteraceae family and it has 40 species; it is known for its ethnobotanical uses. The antioxidant, antifungal and cytotoxic effects of its essential oils have been studied.[1,2] *A. peruviana* is one of the species of this genre. It is native to Central and South America and is commonly known as altamisa, artemisa, altamiz, alcanfor, ambrosia silvestre and maki. This work aimed to study the chemical composition of the essential oil (EO) of *A. peruviana*. The vegetal material used was collected in the municipality of Zapatoca, Santander, Colombia. The taxonomic identification of the botanical samples was done at the Colombian National Herbarium (Bogota) and a voucher specimen was kept as Nº COL 579246. EO extraction was performed by hydro-distillation assisted by microwave radiation (MWHD). The total extraction time was 45 minutes, divided by three stages (each one of 15 min). The analysis of secondary metabolites was carried out by gas chromatography coupled to mass spectrometry (GC/MS), by using two capillary columns: a polar stationary phase of poly (ethylene glycol) (DB-WAX, J & W Scientific, Folsom, CA, USA) of 60 m X 0.25 mm X 0.25 µm, and a non-polar stationary phase of 5% phenyl-poly (methylsiloxane) (DB-5MS, J&W Scientific, Folsom, CA, USA) 60 m X 0.25 mm X 0.25 µm. The GC oven was programmed from 45 °C (5 min), to 150 °C (2 min) at 4 °C min^{-1}, then to 250 °C (5 min) at 5 °C min^{-1} and finally to 290 °C (60 min) at 10 °C min^{-1}; the injection mode was split (30:1*)*. *ar*-Curcumene (24 %) was identified as the major component of *A. peruvian* *α*EO, followed by *β*-bisabolene (17 %), *γ*-curcumene (13 %), phytol (5 %) and spathulenol (4 %). Studies of antimicrobial activity of *A. peruviana* EO have shown a minimum inhibitory concentration (MIC) between 350-500 µg ml^{-1}.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101 for access to genetic resources and derivatives for scientific research with bioprospecting aims, between MinisteriodelMedioAmbiente y DesarrolloSostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Rectification of Essential Oil of *Lippia alba* (Mill.)

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Keywords: *Lippia alba* essential oil, fractional distillation.

Lippia alba (Mill.) N.E. Brown, also known as *L. geniculata* HBK or *Lantana alba* (Mill.) is a shrub about 0.8 m high belonging to the family Verbenaceae.[1] *L. alba* is used in folk medicine for gastric and intestinal diseases.[2] The essential oil of *L. alba* citral chemotype, cultivated in the municipality of Barbosa, Santander, Colombia, was obtained by steam distillation. The oil was subjected to fractional distillation using a spin column tower B / R Instrument 800 (B/R Instrument Corporation, Easton, MD, USA) of 15 theoretical plates, at pressures of 6, 9 and 14 Torr. We performed experiments on reproducibility and the tests for each pressure were done in triplicate. In all tests, four fractions were obtained: three in the top of the tower and one in the background. This research was carried out to find operating conditions favoring the rectification of the essential oil and obtaining volatile fractions and of background of higher added value. The fractions Identification were by GC/MS (Agilent) with quadrupole analyzer. Nonpolar capillary fused-silica DB-5MS column of 60 m X 0.25 mm X 0.25 µm with stationary phase 5 % phenyl-poly (methyl siloxane) was used and, a polar fused-silica DB-WAX column of 60 m X 0.25 mm X 0.25 µm with stationary phase of poliethyleneglycol, were used temperature program from 50 to 280 °C and from 50 to 200 °C respectively; the mode of injection was split (30:1). The chromatographic analysis of essential oil of departure revealed the presence of 92 compounds of which were identified 46 (50 %). In the top fractions there was an increase in the composition of monoterpenes oxygenated mainly citral (neral and geranial) and the bottom fraction of sesquiterpene hydrocarbons, however in this fraction is highlighted the increased content of geraniol. The mass of the fractions obtained at the pressure of 9 Torr accounted for 11.9, 16.4, 16.1, and 43.2 % of the initial mass of essential oil. The composition of citral in the volatile fractions was of 41.9, 40.8 and 40.5 %, in bottom fraction the citral represented 29.2 % and geraniol 20.7 %. There have been studies on the rectification under reduced pressure in essential oils such as *Cymbopogon martinii* (Palmarosa), but we have not found this type of study for the essential oil of *L. alba* (citral).[3]

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0343-2013.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antioxidant and Antifungal Activity of Essential Oil of *Lippia gracilis* Schauer

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Keywords: *Lippia gracilis*; antioxidante; antifungal.

The essential oils are found in various parts of plants and are composed of volatile substances responsible for flavor and aroma characteristic of the plants. Besides their use as flavoring material, the essential oils stand out as raw material to pharmaceutical and agricultural industries due to their several biological properties. *Lippia gracilis* Schauer is an aromatic plant, belonged to Verbenaceae family and widespread in northeastern Brazil. The essential oil from its leaves is rich in monoterpene compounds highlighting thymol, which has been evaluated for its pharmacological activities. This work aims to analysis the chemical composition of the essential oil from *L. gracilis* leaves and evaluate its antioxidant and antifungal activities. The species was collected in Chapada das Mesas National Park, in the Municipality of Carolina-MA, Brazil in January 2013. The essential oil was extracted by hydrodistillation in a Clevenger-type apparatus. The identification of the compounds were performed by GC/MS and GCQFID, using a FOCUS equipment (Thermoelectron). The chromatography conditions were the same in both analyses, using a DB-5 capillary column (30m X 0.25 mm X 0.25 mm); nitrogen as carrier gas (1 ml min^{-1}); injection splitless (split flow 20:1); injector and detector temperatures of 250 °C and heating column program from 60 to 240 °C. The fungicidal activity was evaluated by *in vitro* technique against endophytic fungus *Corynespora cassicola*, using the PDA medium at 1, 1.5 and 2.0 µl mL^{-1} concentrations. The antioxidant activity was analyzed using DPPH radical scavenging assay and the result was expressed as milligrams of trolox equivalent (TE) per gram of oil (mg TE/g oil). The yield of essential oil was 8.0 % (w/w), in relation to the weight of dry leaves. Twenty-three components were identified corresponding to 99.5 % of the oil. Among them, thymol (77.0 %) was the major constituent, followed by *p*-cymene (10.7 %), *γ*-terpinene (8.0 %), and carvacrol (4.5 %). The oil showed a significant antifungal activity, inhibiting the fungus growth at all concentrations tested with an average inhibition of 64 %. The antioxidant assay revealed that the essential oil has 1,616.49 ± 20.32 mg of trolox equivalent per gram of oil, exhibiting a scavenging capacity on DPPH radical about 161 % higher than this synthetic antioxidant. Hence, these results show the potential of *L. garacilis* essential oil to be used on the development of antioxidant and antifungal formulations.

Acknowledgements: UFMA, CAPES, Bionorte.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Influence of Seasonality on the Content, Chemical Composition and Antibacterial Activity of the Essential Oil of *Lippia alba* (Mill.) N.E.Brown Grown in Santarém-PA

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Keywords: *Lippia alba*; essential oil; seasonality.

Lippia alba (Mill.) N. E. Brown (Verbenaceae), is an aromatic specie of a real pharmacological importance and is used in phytotherapy programs for several countries, including Brazil, where it is widely used because of its calming properties, spasmolytic, analgesic, sedative, anxiolytic and expectorant.[1] In order to contribute to the study of this species grown in Santarém and used in the city phytotherapy programs, we evaluated the influence of seasonality on the content, chemical composition and antibacterial activity of *L. alba* collected in dry and rainy seasons at Santarém-PA in 2014. Samples (aerial parts) were collected in the planting of Ponta de Pedras community at 8 h (voucher at Herbarium of the Federal University of Juiz de Fora, CESJ 65,276). The material was dried at 40 °C followed by hydrodistillation in a Clevenger type apparatus for 3 h. The content of the EO was calculated based on fresh biomass and the chemical components were analysed and identified by GC/MS. The antibacterial activity was evaluated against Gram-positive bacterium *Staphylococcus aureus* - ATCC 25923 and tested in triplicate using the disk diffusion method in agar and broth micro dilution. The EO content obtained was 3.1 ± 0.3 % in the dry season and 4.3 ± 0.2 % in the rainy season. Quite all EO constituents were identified during the rainy season, with the major compounds geranial (25.8 %), neral (17.0 %), *p*-cymene (8.1 %), carvone (8.0 %), geraniol (7,0 %) and hedicariol (10.2 %). Major compounds in the dry season were geranial (41.3 %), neral (27.7 %), limonene (7.6 %), elemol (3.0 %), *p*-cymene (2.7 %) and carvone (2.5 %). Average inhibition zones for *S. aureaus* growth were higher than 40 mm for 10 µL EO, in both seasonal periods, superior to standard gentamicin (19.3 ± 0.1 mm) highlighting strong activity, while minimal inhibitory concentration (MIC) was 1.25 mg mL^{-1} in the rainy season and 2.50 mg mL^{-1} in the dry season. These results show that the collection period influenced the chemical composition and yield of *L. alba* EO, and significantly interfere with the antibacterial activity. Thus, the yield of the EO from *L. alba* grown in Santarém-PA is above 2 %, and considerable antibacterial activity for *S. aureus*, may become a promising source of bioactive compounds against this micro-organism.

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Acknowledgements: UFOPA, APL-FITO, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antioxidative Activities of the Essential Oils of Two *Lippia sidoides* Cham.(Verbenaceae) Chemotypes

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Keywords: *Lippia sidoides*; carvacrol; thymol.

The medicinal plant *Lippia sidoides* (Verbenaceae) is known as “alecrim-pimenta” in Northeastern Brazil, and is used in the production of antiseptics. In traditional medicine, *L. sidoides* has been used to treat acne, infected scabies, skin mycosis, dandruff, bad odor on feet and armpits, sores, and mouth and throat inflammations.[1] These activities are accredited to its essential oil which is rich in thymol, compound that has proven microbiological activities. Despite being considered native from the Brazilian Caatinga, *L. sidoides* has been found in the south of Minas Gerais state. In this region, the essential oil of *L. sidoides* is mostly composed by carvacrol. In this context, the aim of this study was to characterize and evaluate the antioxidative activity of the essential oils of *L. sidoides* plants from the state of Ceará (Caatinga) and from the state of as Gerais that were cultivated in the same place. Leave samples of the two chemotypes o*f L. sidoides* cultivated in Itumirim-MG under the same conditions were collected in April 2015 (at the herbarium of the Federal University of Lavras no. ESAL 01943). Fresh leaves were subjected to hydrodistillation separately in a Clevenger-type apparatus for 2 h each. The oils were analyzed by GC/FID and GC/MS. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. The antioxidative activities of the essential oils and their major compounds, namely thymol and carvacrol, were determined through assays that evaluate free radical-scavenging of DPPH (g of essential oil g^{-1} of DPPH), oxidation of the β-carotene/linoleic acid system (g of essential oil g-1 of trolox) and through ferric reducing antioxidative power (FRAP) assay (μM of ferrous sulphate g-1 of essential oil).[2]Carvacrol was the major compound (47.7 %) in the essential oil of plants from Minas Gerais while thymol was the major compound (75.9 %) in the essential oil of plants from Ceará. The carvacrol-rich essential oil showed greater antioxidative activities (DPPH method: 202.06 ± 15.92; β-carotene bleaching assay: 25.23 ± 1.51; FRAP assay: 3629.1 ± 60.86) than the thymol-rich essential oil (DPPH method: 269.71 ± 46.21; β-carotene bleaching assay: 58.51 ± 2.30; FRAP assay: 5479.86 ± 165.38). Thymol and carvacrol activities were: 322.58 ± 14.54 and 288.30 ± 30.24 at the DPPH assay, 55.10 ± 0.41 and 26.44 ± 0.23 at the β-carotene bleaching assay and 10161.09 ± 173.63 and 9381.57 ± 224.8 at the FRAP assay, respectively.

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Acknowledgements: Fapemig, CNPq,Redemineira de química.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Pimenta dioica* (L.) Merrill from Guatemala

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Keywords: *Pimenta dioica*; essential oil; Guatemala.

Piment adioica (L.)Merrill, a tree to 20 m high, grows and is cultivated in wet forest, usually on limestone at 350 m or less in the Northern provinces of Petén, Izabal and Alta Verapaz, Guatemala. In Guatemala, the fruit is used as a condiment for flavoring food and sold in the markets. It is also employed in domestic medicine. In an ancient practice, some Maya people often apply the powdered seeds to the corpses of children, believing that thus they are preserved indefinitely. In Jamaica, the production of allspice from its fruits is an important industry.[1] As part of a project on screening the economic potential of the Guatemalan aromatic resources, the composition of the essential oil of leaves and fruits of *P. dioica* was determined as a first stage to evaluate its biological and anti-inflammatory activities. Leaves and flowers were collected in August 2014, at a population located at the Northern Province of Izabal. The fruits and leaves were separately air dried, milled and extracted by 2 h in a Clevenger-type apparatus. Average yields of 1.4 % and 0.6 % (w/w) were obtained for fruits and leaves, respectively. The GC/MS analyses were performed using a Shimadzu 2010 Plus system coupled with a Shimadzu QP-2010 Plus selective detector (MSD), equipped with a DB5-MS capillary fused silica column (60 m X 0.25 mm I.D. X 0.25 µm). The oven temperature program initiated at 60 °C, then rose at 3 °C min^{-1} to 246 °C, held for 20 min. He was used as carrier gas with a flow rate of 1.03 mL min^{-1}; split ratio of 1:50. Mass spectra were taken at 70 eV. The *m/z* values were recorded in the range of 40–700 Da. GC/FID analyses were carried out using a Shimadzu 2010 GC apparatus with DB5 fused-silica capillary column (60 m X 0.22 mm X 0.25 µm, Restek, France). The oven temperature was programmed from 60 to 246 °C at 3 °C min^{-1} and then held isothermally at 246 °C for 20 min. Nitrogen was used as carrier gas (1.44 $mLmin^{-1}$). The oil components were identified by their mass spectra and retention indices. Relative amounts of components were calculated based on GC peak areas without using correction factors. The major components of the fruit oil were eugenol (71.7 %), β-myrcene (11.2 %) and (*E*)-caryophyllene (7.7 %) and of the leaf oil were eugenol (76.2 %), β-myrcene (10.7 %) and (*E*)-caryophyllene (4. 2%). Due to the important presence of eugenol as major component and the reported antioxidant, anticancer and antifungal activities, the oil of both fruit and leaves will be tested for their activity.[2]

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Acknowledgements: CAPES, CNPq, IPPN/UFRJ, CONCYT/Guatemala (Proyecto FODECYT 12-2013).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Toxicity Evaluation of Essential Oils of Leaves and Fruit Peels of *Citrus aurantium* L. from Curuá-PA, Brazil

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Keywords: *Citrus aurantium* L.; essential oil; brine shrimp assay.

Citrus aurantium L. is an orange popularly known as "laranja da terra" and belongs to Rutaceae family. Leaves and fruit peels are largely used in folk medicine to treat respiratory and gastrointestinal disorders.[1] This work aimed to analyze the chemical composition of essential oils of leaves and fruit peels of *C. aurantium* L. and their toxicities by brine shrimp assay (*Artemia salina*). Samples were collected at Curuá city, Pará State, Brazil. Fruit peels and leaves were separately submitted to hydrodistillation in Clevenger apparatus both during 4 h, yielding 2.5 % and 0.4 % of essential oils, respectively. The essential oils were analyzed by GC/MS with following conditions: GC/MS Agilent Model HP-6890 coupled to a mass selective detector, column HP-5MS (30m X 0.25mm X 0.25 µm), temperatures: injector 220 °C, detector 250 °C, column 60 °C, 3 °C min^{-1}, 240 °C (20 min), carrier gas He 1.0 mL min^{-1}. The compounds were identified by comparison with NIST library-05, retention index calculation and compassion with literature. Chemical composition of essential oil from leaves presented 10 compounds, with linalool (49.5 %) being the major one, followed by linalool acetate (27.6 %), p-menth-1-en-8-ol or α-terpineol (9.5 %), geraniol acetate (5.1 %) and nerol acetate (2.6 %). On the other hand, essential oil of fruit peels presented nine compounds: limonene (92.3 %), *cis*-limonene oxide (1.9 %), β-myrcene (1.4 %), *cis*-carveol (1.0 %) and *trans*-limonene oxide, (0.9 %). For brine shrimp assay, *A. salina*eggs were incubated in artificial saline during 24 h under a lamp till the hatching of nauplii. Ten nauplii were transferred to tubes containing saline solution and the samples in the following concentrations: 1, 10, 100 and 1000 µL mL^{-1}. Bioassay was done in triplicate. The survival percentage of nauplii was calculated after 24 h and the results showed that essential oils of leaves and fruit peels of *C. aurantium* presented 100% mortality at concentrations of 100 and 1000 µL mL^{-1}.

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Acknowledgements: UFOPA, CNPq, CAPES, Fapespa, UNICAMP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In vitro antifungal activity of *Eucalyptus staigeriana* and *Eucalyptus globulus* against *Colletotrichum gloeosporioides* (PENZ),causer of grape black rot

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Keywords: alternative control; phytopathogen; fungicidal.

In Brazil, wine grape cultivation for winemaking is concentrated in the South region, mainly in the Serra Gaúcha - RS, which is responsible for 90% of the domestic wine production. However, climatic conditions of this region can be itself unfavorable to the cultivation of the vine due to high rainfall, which usually boosts the development of fungal diseases. The grape black rot caused by *Colletotrichum gloeosporioides*, generates great losses in quality and productivity, resulting in high economic losses. The use of chemicals to combat plant disease represents several environmental hazards and alternative control with low impact is required. This study aimed to evaluate the *in vitro* antifungal activity of the essential oils (EO) from *Eucalyptus staigeriana* and *Eucalyptus globulus* against the phytopathogen *C. gloeosporioides.* Leaves of *E. staigeriana* and *E. globulus* were collected in Caxias do Sul and the EO were extracted by steam distillation method from dried leaves for 1 h and analysed by GC/MS for chemical identification. The fungus *C. gloeosporioides* was isolated from grapes grown in Caxias do Sul. The major constituents from OE of *E. staigeriana* were citral (48.3 %) and limonene (17.3 %) and *E.globulus* were 1,8-cineol (64.6 %) and α-pinene (14.5 %). The EO was emulsified with Tween 20 (1:1) and added to the PDA medium autoclaved and fondant (40 °C) in the concentrations ranging from 0.05 to 0.6 µL mL^{-1}. The culture medium with different concentrations of EOwaspoured into Petri dishes of 9 cm (Ø), where it was inoculated at the center of each plate a disk of 5 mm (Ø) of a colony of *C. gloeosporioide*s. It was inoculated 3 Petri dishes per concentration and also a control without OE. The experiment was carried out at a temperature of 25 °C and photoperiod of 12h for 14 days. The radial mycelial growth of the colonies was measured at the 3rd, 5th, 7th, 10th e 14th day after inoculation. The EO of *E. staigeriana*showed significant inhibition compared to control from the concentration 0.05 µL $mL^{-1}$and the EO of *E. globulus* showed significant inhibition compared to control from the concentration 0.6 µL mL^{-1}. Transfer experiments were performed in order to make a distinction between the fungistatic and fungicidal effects of the essential oils on the phytopathogen. For this purpose, plug that did not grow were transferred to fresh PDA dishes and their viability and growth were assessed at the 5th day. It was not verified mycelial growth, demonstrating that both essential oils presented fungicidal action. These preliminary results suggest that the EO of *E. staigeriana* and *E. globulus* may be used as an alternative control of *C. gloeosporioides*.

Acknowledgements: Universidade de Caxias do Sul.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of the Volatile Fraction of *Sansevieria guineensis* (Agavaceae) and *Vanilla planifolia* (Orchidaceae) Flowers by HS-SPME/GC-MS

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Keywords: *Sansevieria guineensis*, *Vanilla planifolia,* HS-SPME.

Sansevieria guineensis (Agavaceaeis a plant native to South Africa. The flowers of this species are characterized by their intense white color and floral fragrance.[1]*Vanilla planifolia* (Orchidaceae family) is a climbing plant of food and perfumery uses, distributed in Mexico and Malasia.[2] The purpose of this study was to identify the components of the volatile fractions sampled with solid-phase microextraction (SPME) from *S. guineensis* and*V. planifoli a*flowers. Tests were run with various fiber coatings of different polarities. The largest chromatographic area was obtained when the CAR/PDMS fiber was used. The SPME fiber was exposed to the compounds emitted by flowers (ca. 2 g) of *S. guineensis* or *V. planifolia* for 30 min at 60 °C. The sampling was carried out at different times of day (6:00 am, 12:00 pm, 6:00 pm). The chromatographic analysis was performed on an Agilent Technologies 6890 gas chromatograph coupled to an AT MSD 5973 mass selective detector (EI, 70 eV). A DB-WAX column (60 m X 0.25 mm X 0.25 µm) was used, in splitless injection mode. The oven temperature was programmed from 50 °C for 5 min, then to 150 °C (2 min) at 5°C min^{-1}; finally to 230 °C (35 min) at 5 °C min^{-1}. The major components of the *S. guineensis* flower volatile fraction were *p*-cresol (27%), insect attractive compound,[3] and ethyl alcohol (14%). Methyl salicylate (31 %)was the major component of the *V. planifolia* flower scent, followed by benzyl acetate (16 %) and methyl benzoate (11 %). These compounds were also detected in aroma of cured vanilla beans by GC–MS and GC–olfactometry analysis.[2]

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Acknowledgements: Colciencias-Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0572-2012. Convocatoria 617 Doctorados Nacionales: Colciencias-UIS. Contract 101 for access to genetic resources and derived products for scientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In Vitro Evaluation of Inhibitory Activity of Some Species of *Croton* and *Piper* Essential Oils in Secreted Proteases of *Pseudallescheria boydii*

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Keywords: *Croton*; *Piper*; *Pseudallescheria boydii*.

*Pseudallescheria boydii*is a fungal organism known as a soil and water natural inhabitant and decaying vegetation, which has become an increasingly recognized pathogen among immunocompromised individuals, including HIV infected patients. It is related to cause madura foot and in severe cases, invasive infection of various organs. One potential drug target for the pharmaceutical industry is proteases. The aim of this study was to evaluate the inhibitory activity of some species of *Croton* and *Piper* essential oils (EOs) in *P. boydii* secreted proteases. The EOs of 3 species of *Croton* plants: *C. tricolor* (sacatinga), *C.pulegioides* (velandinho) and *C.blanchetianus* (marmeleiro); and 4 species of *Piper* plants: *P.marginatum* (capeba-cheirosa), *P.tuberculatum* (pimentadarta), *P.hispidum* (matico-falso)and *Piper* sp. were obtained by hydrodistillation using a Clevenger-type apparatus for 4 h. The identification of EO was performed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70 eV. The minimum inhibitory concentration (MIC) was evaluated in triplicate according standard method from the Clinical and Laboratory Standards Institute (CLSi). To evaluate the activity of *Croton* and *Piper* EOs in secreted proteases of *P. boydii* it was performed assays of proteolytic activity inhibition according to Buroker-Kilgore and Wang. Cell-free supernatants of *P. boydii*grown in RPMI broth, were incubated with bovine serum albumin (0.1 mg mL^{-1}), as proteic substrate, and some pH buffers.[1] The calculated MIC were >1250 µg mL^{-1}. The preliminary results showed that the extracellular peptidases were able to hydrolizate the proteic substrate on pH 6 in RPMI broth. The proteolytic inhibition obtained ranging of 29 % for *P. tuberculatum* and 100 % for *C. pulegioides* and *C. tricolor* (48 µL mL^{-1}) in RPMI supernatant of *P. boydii*. These results showed promising activity and suggests a possible target that justifies the antifungal activity of *Croton* and *Piper*EOs.

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Acknowledgements: FAPERJ, CAPES, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Characterization of volatile compounds in *Anemia tomentosa var. anthriscifolia* (Schrad.) Mickel *ex-vitro*

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Keywords: *Anemia*; SDE; tissue culture.

Anemia tomentosa var. *anthriscifolia* (Schrad.) Mickel is an aromatic fern growing on islands of vegetation associated with rocky substrates. Its woody aroma is derived from a triquinane sesquiterpene-rich essential oil,[1] which is also endowed with antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 100 μg ml^{-1}).[2] Our group indulged into the investigation of the chemical composition of this essential oil upon *in vitro* multiplication of the plant, and *in vitro* conditions imposed on the plant favored the production of monoterpenes instead of triquinane sesquiterpenes.[1] Here, we evaluated the contents of mono and sesquiterpenes present in the volatile fraction of germinated *in vitro* individuals in culture medium MS plus kinetin (0.5 μmol, 5 μmol) which were later acclimatized to the external environment (*ex vitro*). Plant's sporophytes were subjected to SDE extraction method (Simultaneous Distillation and Extraction), where small plant weights (± 5g) are extracted in solvent dichloromethane. The samples were analyzed by GC/FID and GC/MS (Shimadzu) with DB-5MS column (30 m X 0.25 mm X 0.25 μm). Oven temperature from 60 to 290°C at 3 °C min^{-1}. The percentage composition was obtained by normalization from FID (triplicate). Volatile components were identified by mass spectra and LRI. Monitored monoterpenes were *trans*-pinocarveol, *trans*-verbenol and pinocarvone, and in plants grown on MS medium without accretion of kinetin they showed respectively 1.4479 ± SD; 0.3029 ± SD and 1.2346 ± SD. In plants grown in MS + 0.5 μmol KIN, the levels were 1.5226 ± SD; 0.3012 ± SD; and 1.1352 ± SD. In plants grown in MS +5 μmol KIN, their levels were 1.4110 ± SD; 0.2779 ± SD and 0.9916 ± SD. The sesquiterpenes presilphiperfol-7-ene, silphiperfol-6-ene, α-guaiene and (-)-*epi*-presilphiperfolan-1-ol were monitored and showed in MS0 plants: 4.8473 ± SD; 19.8043 ± SD; 6.4682 ± SD; 27.7704 ± SD. In plants grown in MS + 0.5 μmol KIN levels were respectively 5.1114 ± SD; 21.1305 ± SD; 10.2357 ± SD; 25.8907 ± SD. For plants grown in MS + 5 μmol KIN, their levels were 4.6180±SD; 19.6240±SD; 9.2128±SD; 25.2812±SD. These results show that the in vitro protocol led to *ex-vitro* plants where the sesquiterpenes are the major volatiles.

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Acknowledgements: CNPq, CAPES e FAPERJ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Hematologic Parameters of Balb/c Mice Infected with *Plasmodium berghei* and Treated with Essential Oil of *Cyperus articulatus*

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Keywords: hematologic; *Plasmodium berghei*; *Cyperus articulatus*.

Malaria is a parasitic tropical disease that brings serious problems for public health and with huge economic impacts. Nearly 3.2 billion people live in areas considered under risk of malaria transmission. *Cyperus articulatus*L., popularly known as "priprioca" in Brazil mainly in the Amazon region, is used as antithermic, anticonvulsant and for the treatment of malaria by traditional medicine.[1,2] The hematological alterations most frequently associated with malaria infection are anemia, leukopenia, thrombocytopenia, neutropenia and eosinopenia. In order to determine hematological parameters, we used female mice of BALB/c weighing roughly 20 g, divided in 4 groups: Malaria (not treated); *C. articulatus* 200/mg kg/24h/V.O; *C. articulatus* 100 mg/kg/24h/V.O; *C. articulatus* 10 mg/kg/24h/V.O. Each group was inoculated with i.p. approximately 10^6 parasitized erythrocytes of *Plasmodium berghei.* They began the treatments on the fourth day after inoculation *plasmodium*, and were treated for 7 consecutive days. On the 11th day after the inoculation, the animals were submitted to a cardiac puncture for blood sample collection, which was used in the determination of hematological parameters. The results for red blood cells within the malaria group was 2.862 ± 0.488 x 10^6 μL^{-1}; in the *C. articulatus* the 200 mg group was observed a number red blood cells 3.668 ± 0.082 x 10^6 μL^{-1}; in group *C. articulatus* 100 mg, the number was 7.223 ± 0.422 x 10^6 μL^{-1}. Finally, for *C. articulatus* 10 mg, we estimated 3.566 ± 0.444 x 10^6 μL^{-1}. As for the analyses by hemogram, we found that the hemoglobin levels within the malaria group was 5.96 ± 0.44 gdL^{-1}; in group *C. articulatus* 200 mg, the estimate was 6.78 ± 0.38 gdL^{-1}; in group *C. articulatus* 100 mg, 11.94 ± 0.86 gdL^{-1} and for the 10 mg group *C. articulatus*, the estimate was 6.94 ± 0.44 g dL^{-1}. Concluding, the blood count analysis demonstrated that *C. articulatus* 100 mg group showed a significant increase ($p < 0.001$) in the number of red blood cells while compared to malaria group. Additionally, the analysis of hemoglobin levels showed a significant increase ($p < 0.001$) in this parameter *C. articulatus* 100 mg group, while compared to the group malaria.

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Acknowledgements: Foundation for Research Support of Pará, Brazil - FAPESPA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Vision of the Citrus Industry in Brazil

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Keywords: citrus peel oils, chromatographic analysis, by-products.

The citrus juice processing generates large amounts of by-products, almost 50 %, in the form of peel, segment membranes, rags, and seeds. Besides that, during juice concentration, volatile constituents contained on the vapour phase are condensed by the aroma recovery systems. The loss of these flavoring constituents results in a decrease of juice quality, unless these compounds are being reincorporated on it. The aroma recovery systems get two separated phases: essence oil or oil phase (OP) and aqueous essence or water phase (WP). The average yield of these products is very low; it is around 0.3 % for the peel oils (CPO), 0.05 % for the W P and 0.013 % for the OP, always in comparison to the fresh fruit. These products are rich in aldehydes, esters and other volatile compounds. As the storage and transportation of these products are expensive, the evaluation of concentration processes of these essences is of high interest. One of the objectives of this work is to explain the probable production of orange juice (FCOJ), as well the citrus oils production in the last ten years in Brazil. We will compare the yield of the orange products and also the variation costs for the different crops. From 2005 to 2015 the variation of the production quantities for these ten years was: FCOJ - 851,000 to 1,500,000 tons; CPOO - 24,104 to 40,863 tons; WP - 2,165 to 10,179 tons; OP - 2,980 to 1,560. By the commercial point of view, the total of citrus peel oils exported on these 10 years, generated in million of USD: orange - 908, lemon/lime - 60 and tangerines – 58.[1,2] The citrus single oils and also the folded ones, as well some special light fractions, were evaluated and performed by chromatographic and sensorial analysis. The study of the special light fractions of these flavoring volatile compounds from the oil phase, obtained by fractional distillation, is another point of this work. By fractional distillation of the orange oil phase, high contents of fragrant compounds such as ethyl butyrate, from 460 to 92,000 mg L^{-1} and valencene, from 1,300 to 12,600 mg L^{-1} were obtained by GC evaluation. For lemon and lime oils, we found citral since 4.23 to 40.15 (area % by GC); for tangerines we found methyl anthranilate from 0.04 to 2.84 (area % by GC).

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of Antinociceptive Effect of *Aristolochia trilobata* Essential Oil and its Major Component

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Keywords: *Aristolochia trilobata*; pain; antinociception.

Aristolochia trilobata (AT) is a Central American plant and its extracts (leaves and barks) showed topical anti-inflammatory and anti-bacterial activity. Our aim is to evaluate the antinociceptive activity of the essential oil from AT and its major component (sulcatyl acetate, AS). AT was collected in October/2011 at Estância, Sergipe/ Brazil. A voucher specimen is deposited at the herbarium of the Federal University of Sergipe (# ASE 23,161). The essential oil was obtained through steam distillation. Female Swiss *Webster* mice (22-25g, n=4-6) were pre-treated orally with AT or AS (1, 10 or 100 mg kg^{-1}) or vehicle 1h before 20 µL formalin injection (2.5 %) into the hind-paw. Formalin-induced licking time was evaluated 0-5 min and 15-30 min. In the hot plate model, animals were placed on a hot plate (Insight Equipment, Brazil) set at 55 ± 1 °C. At successive intervals of 30 min after oral administration of AT or AS (same doses as above), vehicle or morphine (2.5 mg kg^{-1}), the reaction time was observed when the animals licked their fore and hind-paws and jumped. The antinociceptive effect was quantified as area under the curve (AUC) of responses measured between 30 and 180 min. The results are mean ± SD. The AUC was calculated by the Software 5.0 (GraphPad Software, La Jolla, CA, USA). Statistical analysis was performed by ANOVA and Bonferroni's post-test (*$p<0.05$). Protocols for animal use a received number of #DFBCICB015-04/16. Two doses of AT and three doses of AS showed effect in the 1st phase (nociceptive) of formalin-induced licking, but only the higher doses inhibited the 2nd phase (inflammatory). 1st phase: AT: 1 mg kg^{-1}= 36.9 ± 6.3 sec; 10 mg kg^{-1}= 28.4 ± 8.7* sec; 100 mg kg^{-1}= 14.3 ± 6.4*sec and AS: 1 mg kg^{-1}= 27.5 ± 4.8* sec; 10 mg kg^{-1}= 19.1 ± 12.1* sec; 100 mg kg^{-1}= 19.1 ± 8.4* sec when compared with vehicle= 45.2 ± 6.8 sec. In the 2nd phase: AT: 100 mg kg^{-1}= 142.5 ± 23.3*sec and AS: 100 mg kg^{-1}= 120.8 ± 33.8* sec, when compared with vehicle= 213.0 ± 33.2 sec. In the hot plate test, the pre-treatment of mice with 1, 10 and 10 µL kg^{-1} of AT and AS were able to increase the AUC when compared with the vehicle group (AUC: vehicle= 1,833.5 ± 1,479.7; morphine= 13,054.2 ± 2,530.9; AT: 1 mg kg^{-1}= 8,378.2 ± 1,521.2*; 10 mg kg^{-1}= 6,313.5 ± 1,699.7*; 100 mg kg^{-1}= 6,544.8 ± 2,126.5* and AS: 1 mg kg^{-1}= 11,554.2 ± 2,852.5*; 10 mg kg^{-1}= 9,872.5 ± 2,092.8*; 100 mg kg^{-1}= 15,803.3 ± 3,226.7*. Our results suggest that the essential oil from *A. trilobata* and it major component, sulcatyl acetate, showed significant peripheral and central antinociceptive activities.

Acknowledgements: CAPES, CNPq and FAPERJ (financial support), Instituto Vital Brazil (for animal donation) and Alan Minho (technical support).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparison of Volatile Secondary Metabolites of the *Gardenia augusta* Flowers at Different Times of Day through Solid-Phase Microextraction and Gas Chromatography Coupled to Mass Spectrometry

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Keywords: *Gardenia augusta;* HS-SPME; GC-MS.

The *Gardenia* genus belongs to the Rubiaceae family and consists of 23 species. One species is *Gardenia augusta* native of China, also known as "*Jazmín del Cabo*" and its use is ornamental; it is identified by its white flower with a pleasant aroma.[1] The aim of this study was to compare the volatile profile of *G. augusta* flower at different times of day (6:00, 12:00 and 18:00h), sampled with headspace solid-phase microextraction (HS-SPME). Preliminary experiments using different fiber polarities (PDMS, Carboxen-PDMS and Carboxen-PDMS-DBV) showed that Carboxen-PDMS fiber and 30 min exposure afforded the largest chromatographic areas. Identification of secondary metabolites was based on mass spectra (electron ionization EI, 70eV) obtained under *splitless* injection with a gas chromatograph (GC, *Agilent Technologies* 7980) equipped with a mass selective detector (MSD*A.T.* 5975C), and a DB-WAX capillary column coated with polyethyleneglycol (60 m X 0.25 mm X0.25 µm, J&W Scientific, Folsom, CA., USA). The GC oven temperature was programmed from 50 °C (5 min) to 150 °C (2 min) at 5 °C min^{-1}, and then to 230 °C (35 min) at 5 °C min^{-1}. Mass spectra and reconstructed chromatograms were obtained by automatic scanning in the mass range *m/z* 40–350 at 3.5 scan/s. The major compounds present in *G. augusta* flower at 6:00h were: *trans*-β-caryophyllene (22 %), hex-3-en-ol (20 %), hexan-1-ol (10 %), α-humulene (4 %), α-cadinol (1 %); at 12:00h the main components found were *trans*-β-caryophyllene (36 %), hex-3-en-ol (9 %), α-humulene (8 %), hexan-1-ol (4 %), *trans*-β-elemene (2 %), α-cadinol (1 %), α-muurolene (1 %), and *p*-cadinene (1 %). At 18:00h the following metabolites were identified: *trans*-β-caryophylene (14 %), hexan-1-ol (11 %), linalool (11 %), α-humulene (2 %), and α-cadinol (1 %). The main component at all times was *trans*-β-caryophyllene. This compound has antidepressant and anti-inflammatory properties, and is used as an additive in the food industry.[2]

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Acknowledgements: Financial support from Colciencias-National Autonomous Equity Financing Fund for Science, Technology and Innovation, de Caldas, under grants RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Repellent Activity and Encapsulation of *Lippia origanoides* HBK Essential Oil and Fractions

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Keywords: *Lippia origanoides*; encapsulation; PEG.

Lippia origanoides (Verbenaceae) is an aromatic shrub 1 to 3.5 m tall, endemic to Central and South America. Its essential oils (EO) are rich in three main components: thymol, carvacrol and γ-terpinene.[1] The repellent activity of *L. origanoides* EO (obtained by steam distillation) and two fractions (LF and FF) obtained by fractional distillation at reduced pressure (BR-Instruments Equipment 800) was evaluated against *T. castaneum*, one of the main pests in grain storage. Since the effectiveness of essential oils as repellents decreases due to the high volatility of its components, they were encapsulated in individual particles or droplets of active material, loaded into a polymer melt (PEG-6000) and ground to produce capsules with sizes in the range of nanometers to millimeters. The area preference method was used to evaluate repellent activity.[2] The kinetic release of the compounds in the microcapsules was studied (20 mL vial, with ca. 0.2 g of the encapsulated material at 70 °C), using a headspace autosampler Agilent Technologies 7694E, coupled to GC-FID (Hewlett Packard 5890 Series II, column DB-WAX polyethyleneglycol of 60 m x 0.25 mm x 0.25 um. The EO, light (LF) and bottom fractions (FF) of *L. origanoides* showed a repellency percentage higher than the control compound (IR3535) at 2 and 4 h of exposure. The highest repellency values were observed for fractions with intermediate contents (5-15 %) of γ-terpinene, thymol and caryophyllene. Concentrations above 0.002 mL/cm^2 of carvacrol decreased the percentage of repellency against *T. castaneum*, at 2 and 4 h of exposure. Encapsulation of *L. origanoides* EO in PEG-6000 presented a slower release of volatiles, compared with light fractions (LF) and found fractions (FF). Percent encapsulation of *L. origanoides* EO in PEG-6000 was 60%, with liberation of the components for up to 20 days at 40 °C.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of Chemical Composition and Antioxidant Activity of *Aristolochia anguicida* and *Hyptis colombiana* Essential Oils Collected in Santander (Colombia)

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Keywords: *Aristolochia anguicida*; *Hyptis colombiana*; essential oil; GC-MS.

*Aristolochia anguicida*is an herbaceous species of the Aristolochiaceae family. It is an herbaceous liana, with triangular leaves, solitary flowers, axillary, purple, green and yellow. It is used in the traditional medicine as an antidote to snake venom.[1] *Hyptis colombiana* belongs to the Lamiaceae family. The plants of *Hyptis* genus are from South America and comprise about 400 species.[2] Plants were collected in the municipalities of Los Santos and Zapatoca, Santander, Colombia. These were identified at the National Herbarium of Colombia, with voucher numbers COL558352 and COL578972. The essential oils (EO) were obtained by microwave-assisted hydrodistillation (MWHD). Characterization of the essential oils constituents was performed on an Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 Plus Network mass selective detector (EI, 70 eV). Polar (DB-WAX, 60 m X 0.25 mm X 0.25 µm, with stationary phase of polyethylene glycol) and nonpolar (DB-5MS, 60 m X 0.25 mm X 0.25 µm, with stationary phase of 5% phenyl-polymethylsiloxane) capillary columns were employed. Oven temperature was programmed from 45 to 150 °C at 4 °C min^{-1}, maintained for 7 min, then 150 to 230 °C at 4 °Cmin^{-1} and held for 40 min. The ORAC method was performed in a Turner Biosystems multiplate reader. Several dilutions per sample were tested to establish the time required to quench the fluorescence to 5% of its initial value. Compounds identification was based on their mass spectra and retention indices. The major components found in *A. anguicida* oil were *trans*-β-caryophyllene (27 %), α-ylangene (10 %) and caryophyllene oxide (%). The major components for *H. colombiana* oil were *trans*-β-caryophyllene (34 %), germacrene D (22 %) and caryophyllene oxide (14 %). The antioxidant activity values by the ORAC method for essential oils were higher than those of reference substances, α-tocopherol and BHT. The main compound in those essential oils was β-caryophyllene, a sesquiterpene widely distributed in essential oils of various plants. Several biological activities are attributed to β-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Chemical Composition of the Essential Oils of Five Species of *Lepechinia* from Colombia

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Keywords: *Lepechinia*; essential oil; GC-MS.

The genus *Lepechinia* (Lamiaceae) grows from Northern Mexico to Chile. Ten species of this genus are used in traditional Colombian medicine to treat urinary, gastrointestinal infections and diabetes. *L. schiedeana* collected in Colombia was rich in ledol (37 %) and Δ-3-carene (22 %).[1] Five species of the *Lepechinia* genus were collected in different regions of Colombia, and identified in the Colombian National Herbarium: *L. vulcanicola* (Bogotá, COL 521090), *L. betonicifolia* (Bogotá, COL 521102), *L. bullata* (Guaca, COL 517763), *L. conferta* (Toca, COL 521068), *L. salviifolia* (Sogamoso, COL 521027,Samacá, COL 521070 and Bogotá, COL 521061). The essential oils were obtained using microwave-assisted hydrodistillation (MWHD) and characterized by gas chromatography-mass spectrometry (GC, Agilent Technologies 6890; MSD 5973 and 5975). Fused-silica capillary columns DB-5MS,coated with 5% phenyl polydimethylsiloxane (60 m X 0.25 mm X0.25 µm, J&W Scientific, Folsom, CA, USA) and DB-WAX, coated with polyethyleneglycol (60 m X 0.25 mm X 0.25 µm, J&W Scientific, Folsom, CA, USA) were used. Helium was used as carrier gas (1 mL min^{-1}, at constant flow). Compounds identification was based on chromatographic (retention times and linear retention indices, use of standards) and spectrometric (mass spectra interpretation, comparison with databases NIST, Adams, Wiley, and standards) criteria.[2] The EO chemical composition was compared by means of principal component analysis (PCA, statistics, version 6.0, StatSoft Inc.). The results showed that these *Lepechinia* species have high level of monoterpenes (40-60 %), mainly limonene, α and β-pinene and Δ-3-carene. Sesquiterpenoids represented 25-35 % of the composition, mainly with *trans*-β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, γ-curcumene and palustrol. The PCA applied to the composition of the EOs showed three principal groups: 1. ledol + palustrol (*L. vulcanicola*y *L. betonicifolia*); 2. camphor + borneol (*L. salviifolia*); 3. *p*-cymene + β-pinene (*L. conferta* and *L. bullata*). The extraction yields for the five species ranged between 0.7 and 1.1% w/w. For the first time the chemical composition of the essential oils of *L. vulcanicola, L. betonicifolia and L. conferta* was reported.

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Acknowledgements: Colciencias - PatrimonioAutónomoFondoNacional de Financiamientopara la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oils from *Nectandra cuspidata* Nees & Mart. (Lauraceae)

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Keywords: Lauraceae, *Nectandra cuspidata*, essential oil.

The Lauraceae is constituted by *circa* 50 genera and approximately 500 species, most of them trees. Its main distribution centers are South America and Southeastern Asia and the plants of this family are known for the production of a wide diversity of secondary metabolites. The genus *Nectandra* is well represented in the Brazilian flora, with several species presenting many benefits to man. The aim of this study was to evaluate the chemical composition of the volatile constituents from *Nectandra cuspidate* leaves and fruits were carried out on four samplings obtained from fresh leaves (FL), dried leaves (DL), fresh fruits (FF) and dried fruits (DF). Leaves and fruits from *N. cuspidata* were collected at Campus of Museu Paraense Emílio Goeldi, Belém, Pará State, Brazil in September 2014. The essential oil was obtained by hydrodistillation for 3 h. The essential-oil analyses were performed on a Shimadzu GC/MS Model QP 2010 Plus, equipped with a Rtx-5MS (30 m X 0.25 mm X 0.25 µm) fused silica capillary column. Helium was used as the carrier gas adjusted to 1.2 mL min^{-1}; with splitless injection of 1µL of a hexane solution; injector and interface temperature were 250 °C; oven temperature programmed was 60-240 °C at 3 °C min^{-1}. EIMS: electron energy, 70 eV; ion source temperature was 200 °C. The identification of the individual components was based on the matching of their mass spectra and retention index with those recorded in the libraries of the NIST-11 system and literature data.[1] The yield of essential oil from fresh and dried leaves contained 47.1 % and 9.3 % of the residual humidity, respectively, were 0.1 % and 0.2 %. The fruits oil (yield calculated at dried basis) were 1.9 % (fresh sample) and 1.1 % (dried sample). Quantitatively, the most abundant class of compounds identified was the terpenoids. Among them bicyclogermacrene (FL, 20.2 %; DL 19.2 %; FF, 5.3 %; FD, 4.2 %), (*E*)-caryophyllene (FL, 20.0 %; DL, 23.1 %; FF, 12,0 %; FD, 7.1 %), γ-elemene (FL, 12.5 %; DL, 11.2 %; FF, 7.2 %; FD 2.7 %), germacrene B (FL, 11.5 %; DL, 10.4 %; FF, 5.0 %; FD, 2.4 %) and germacrene D (FL, 9.0 %; DL, 8.3 %; FF, 21.5 %; FD, 2.3%), δ-elemene (FL, 3.7 %; DL, 4.0 %; FF, 6.3 %; FD, 5.3 %) , β-elemene (FL, 5.6 %; DL, 6.0 %; FF, 3.8 %; FD, 2.2 %), (*E*)-nerolidol (FL, 0.3 %; DL, -; FF, 2.3 %; FD, 8.4 %), spathulenol (FL, 0.5 %; DL, 1.8 %; FF, 0.4 %; FD, 6.4 %) and caryophyllene oxide (FL, 0.4 %; DL, 1.4 %; FF, 0.3 %; FD, 7.4 %).

[1]Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry.*4th ed. Allured Publishing Corporation. Carol Stream, Illinois, 2007.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Multivariate Analysis of Essential Oils from the Leaves of *Osteophloeum platyspermum*

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Keywords: *Osteophloeum platyspermum*; terpenes; antibacterial.

The present study evaluated variations in terpene composition of essential oils obtained from the leaves of a single adult individual of *Osteophloeum platyspermum*. The leaves were collected 13 separate times over a 2-year period. Correlations between terpenes and environmental changes as seasonal variations, the presence of El Niño and La Niña global phenomena, and climate variables as max temperature, relative humidity, total daily irradiation, and total daily precipitation were assessed. GC/MS let to the identification of 50 terpenes that were quali and quantitatively heterogeneously distributed in the 13 collections, with predominance of hydrocarbon monoterpenes and oxygenated monoterpenes. Set theory let to the identification of the constant presence of 17 terpenes as β-pinene (≈29-37 %), α-pinene (≈5-11 %), limonene (≈14-23 %), α-terpineol (≈2-8 %), terpinen-4-ol (≈0.4-1.3 %), myrcene (≈5-8 %), linalool (≈0.5-1.8 %), elemol (≈0.1-0.5 %), β-elemene (≈0.3-1.5 %), γ-elemene (≈0.2-1.3 %), neo-intermedeol (≈0.3-1.3 %), α-cadinol (≈0.6-2.2 %), 1-epi-cubenol (≈0.4-1.2 %), spathulenol (≈1-5.5 %), isospathulenol (≈0.6-2.4 %), viridiflorol (≈0.9-1.5 %), and ledol (≈0.3-1.5 %) in all oils, and these findings indicate that whatever the external conditions are, the species needs to sustain the production of the 17 terpenes. Cluster analysis in association to non-metric multidimensional scaling (NMDS) done with the percentage of each terpene in the oils resulted in the separation of the 13 essential oils in two distinct group: a dry season (DS) group and a rainy season (RS) group. The quantitative composition of the essential oils that were analyzed was significantly different among the samples that were obtained in the RS and the samples that were obtained in the DS according to the global analysis of similarities (ANOSIM) test (R = 0.242, p = 0.019). Thus, samples that were obtained in the same season (RS or DS) had a tendency toward showing similar amounts of the 17 terpenes, supporting the notion that terpene production may be influenced by climatic conditions. So, a canonic correspondence analysis (CCA) was performed as a direct analysis of gradients. In this analysis, terpinen-4-ol and α-terpineol (minor compounds) and myrcene and limonene (major compounds) may be associated with a warm environment and an environment that is exposed to more intense solar irradiation. Spathulenol, neo-intermedeol, and elemol (minor compounds) may be associated with environments that are exposed to higher humidity and higher precipitation. So, the constant presence of the 17 terpenes suggests they are required for protection against microorganisms and as an expression of its relationship to the environment.

Acknowledgements: The authors would like to thank FAPESP for financial support (grant no. 2009/58706-8) and Guilherme Lima Semencio for technical assistance.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Rectification for Two *Lippia origanoides* Chemotypes (Thymol and Carvacrol): Obtaining Reproducible Fractions from Different Initial Concentrations

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Keywords: *Lippia origanoides*; rectification; fractional distillation.

Widely known natural sources of thymol and carvacrol are thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*), both of Eurasian origin. The thymol and carvacrol contents in these essentials oils (EO) are: 37-55 % and 0.5-5.5 %, respectively, for thyme (ISO 14715:2010) and around 22% and 18%, respectively, for oregano.[1] The EO of some native American species have an important content of thymol and carvacrol. This is the case of *Lippia origanoides* (LO), a species for which several chemotypes have been found. Two of them afford essential oils rich in either thymol or carvacrol. Different agricultural conditions and post-harvest treatments cause variations in EO composition. The relative amount of carvacrol in the EO of the LO carvacrol-rich chemotype varies between 44.8 and 52 %. Similarly, thymol content EO chemotype is 53-61 %.[2] The present work studies LO EO rectification to obtain reproducible and enriched fractions of thymol and carvacrol. The EOs were obtained by hydrodistillation and rectification was carried out in a B/R Instrument 800, a reduced-pressure fractional distillation equipment that uses arotating B/R instruments, Easton, MD, USA). Reproducibility experiments were performed to standardize the rectification method. The effect of pressure on fraction composition was studied. The EO and fractions were analyzed by GC/MS and GC/FID(Agilent). Pressure and initial composition of EO had a significant effect on the rectification of thymol and carvacrol enriched fractions. Reproducible compositions (mass fraction) of fractions enriched in thymol (0.62-0.81), or carvacrol (0.52-0.62) was achieved depending on the initial composition and pressure. Two models were fitted to the experimental data for each EO. Cross validation was used as a warranty from fitting the model. The results of this work are the beginning of the EO rectification process escalation of promising natural compounds from Colombian native plants.

[1]D'Antuono L.F.;Glletti, G.C.; Bocchini P. Variability of Essential Oil Content and Composition of *Origanum vulgare* L. Populations from a North Mediterranean Area (Liguria Region, Northern Italy). *Annals of Botany* **2000**, *86*, 471. [CrossRef]
[2]Stashenko, E.E.; Martínez, J.R.; Ruíz, C.A.; Arias G.; Durán C.; Salgar, W.; Cala, M. *Lippia origanoides* chemotype differentiation based on essential oil GC-MS and principal component analysis. *Journal of Separation Science* **2010**, *33*, 93. [CrossRef] [PubMed]

Acknowledgements: Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antifungal Activity from *Nectandra* Species Essential Oils against Fungic Clinical Isolates

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Keywords: *Nectandra;* antifungal activity; dermatophytes.

Several studies evidence the antimicrobial activity of essential oils, due to synergistic effect between its compounds or to an isolated constituent, in order to provide therapeutic alternatives to conventional therapy.[1] Here, the chemical composition of the essential oils from *Nectandra megapotamica* and *N. lanceolata*, collected at different stages of plant development, were evaluated for their antifungal activity against yeast and filamentous fungi of clinical interest. Leaves of both species were harvested in Barracão – RS, in September (harvest 1 - H1) and November 2013 (H2) and April 2014 (H3). The essential oils were obtained by hydrodistillation in Clevenger apparatus for 4 h, and the chemical composition analyzed by GC-MS. The percentage composition was obtained by normalization and the compounds identified by IRL and mass spectra comparison. For the antifungal activity was determined the Minimum Inhibitory Concentration (MIC) against filamentous fungi and yeast, through of the broth microdilution method standardized by CLSI (M38-A and M27-A3). Relative to yield, there were no significant variations among the harvest of *N. megapotamica*, with 0.34 % for inflorescence (H1), 0.36 % for sterile phase (H2) and 0.37 % to the end of fruiting phase (H3). The chemical composition showed only quantitative variation among harvest, with a predominance of the sesquiterpene fraction. Bicyclogermacrene was the main compound identified in both samples (22.7, 22.9 and 36.7 % for H1, H2 and H3, respectively). β-Pinene (15.5 %) and germacrene D (15.0 %) were the major compounds for H1, germacrene D (10.9 %) and limonene (8.7 %) for H2 and germacrene D (19.2 %) and spathulenol (9.1 %) for H3. For *N. lanceolata*, H1 and H2 (sterile phase) oils showed average yield of 0.15 and 0.2 % for H3 (final stage of fruiting). Although in different stages of plant development, there was no significant variation in the chemical composition. β-caryophyllene (32.5 %), bicyclogermacrene (27.8 %), spathulenol (11.8 %) and germacrene D (5.1 %) were identified as compounds major. Both samples exhibited activity against filamentous fungi, but no effect against *Candida* species. *N. megapotamica* oils showed activity against *Trichophytonrubrum*, *T. mentagrophytes*, *Microsporumcanis* and *M. gypseum* (MIC in the range of 250 - 500 µg mL^{-1} for H1, 125 - 500 µgmL^{-1} for H2 and > 500 µgmL^{-1} for H3(similar for *N. lanceolata*). The essential oils of both species demonstrate selective antifungal activity, characterized as antidermatophytic.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Topical Anti-Inflammatory Activity of *Lantana radula* sw. (Verbenaceae) Essential Oil

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Keywords: *Lantana radula*; ear edema; croton oil.

Many species of the genus *Lantana* have been used in traditional medicine for the treatment of inflammatory diseases.[1] Here, the chemical composition of *L. radula* essential oil was investigated for its topical anti-inflammatory activity. *L. radula* aerial parts were collected at 7:00 am in the APA Aldeia Beberibe, Camaragibe, PE (voucher at IPA Herbarium no. 94,459). Fresh flowering aerial parts (200 g) were extracted by hydrodistillation in a Clevenger apparatus for 2h. Chemical analyses by GC/MS were performed using a gas chromatograph Agilent Technologies equipped with a FID and MS and a HP-5MS column (30 m X 0.25 mm X 0,25 µm). The oven temperature was programmed at 70 °C, 4 °C min^{-1} until 280°C (15 min), carrier gas was helium, (1.4 mL min^{-1}), ionization source at 280°C, ionization energy at 70 eV, and ionization current at 0.7 kV. Mass spectra were recorded from 30 to 450 *m/z*. Identification was made by mass spectra comparison with libraries and LRI. Topical anti-inflammatory activity was evaluated by croton oil-induced ear edema in mice. Briefly, mice were anesthetized with halothane and received 20 µL of croton oil 2% in acetone at the right ear. After drying, animals received 3 µL of essential oil diluted in acetone. Left ear received acetone and was used as control. The animals were euthanized after 6 h and samples of 6 mm of diameter were taken and weighted for edema measurement. The results were expressed as mean ± SEM and analyzed by ANOVA with posttest of Bonferroni, $p < 0.05$. Essential oil extraction yielded 0.03 %. Chemical analyses identified 87.1 % of the compounds, all of them sesquiterpenes. Among them, 26.6 % were oxygenated sesquiterpenes. The major compounds found were β-caryophyllene (24.4 %), β-cubenene (23.3 %), elemol (14.7 %), β-elemene (6.8 %), and eudesm-7(11)-em-4-ol (5.3 %). Caryophyllenes are reported as chemical markers of *Lantana* genus. Topical administration of *L. radula* essential oil decreased the croton oil-induced ear edema by 44.4 %, while dexamethasone (0.1 mg ear^{-1}) inhibited the ear edema by 81.3 %. This data points to a possible topical anti-inflammatory effect of the essential oil of the flowering aerial parts of *L. radula*.

[1]Barros-Júnior, M.R.; Santos M.R. R.; dos Santos E. A.; Sena-Filho J. G.; Fernandes, R.P.M.;Thomazzi, S.M. Anti-inflammatory and antioxidant activities of *Lantana radula Swartz* and its phenylethanoid glycosides. *Journal of Medicinal Plants Research* **2014**, *8*, 1354. [CrossRef][Link]

Acknowledgements: UFPE, FACEPE, CNPq and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Bioproducts from Different Brazilian Plants with Antimicrobial Activities against Sulfate-Reducing Bacteria (SRB)

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Keywords: Bioproducts; antimicrobial activity; sulfate-reducing bacteria (SRB).

The sulfate-reducing bacteria (SRB) represent a problem for the oil industry. The presence of SRB in pipelines, reservoirs and oil wells is associated with the formation of biofilms, biocorrosion and acidification (production of H_2S), and may result in the closing of the wells. Strategies for the control of SRB make use of high concentrations of biocides, which may induce bacterial resistance and, indirectly, lead to environmental impacts. Different bioproducts produced by plants show the ability to inhibit the growth of different microorganisms, being a possible alternative for the control of SRB. Therefore, the aim of this study was to evaluate the antimicrobial activity of bioproducts from Brazilian flora against the strain NCIMB 13491 of *Desulfovibrio alaskensis*. For this purpose, essential oils (EO) from the plant species *Croton cajucara* Benth (white and red "sacaca"), *Croton sacaquinha* ("sacaquinha"), *Aristolochia cymbifera* ("milhomem") and its major EO component (sulcatyl acetate) were tested against the SRB strain. In addition, the plant extracts (PE) from the species*Platycyamus regnelii* ("paupereira") and *Quassia amara* (bitter-wood) were also used in the tests.To evaluate the antimicrobial activity, a volume of 100 µL of each bioproduct was tested in 9 mL of Postgate C medium inoculated with 1 mL of the SRB strain. The use of EO from *C. sacaquinha* and the sulcatyl acetate resulted in the complete growth inhibition of the NCIMB 13491 strain when compared to the control without the EO. The same results were observed when PE from *P. regnelii* and *Q. amara* were tested in the same conditions. An amount of 1 mL, taken from the experiment described above, was re-inoculated in a new Postgate C without the addition of bioproducts. No growth was observed considering the addition of EO from *C. sacaquinha* and sulcatyl acetate and PE from *P. regnelii* and *Q. amara*, suggesting a bactericidal activity. Furthermore, the agar diffusion method was performed with the bioproducts from sulcatyl acetate, *P. regnelii* and *Q. amara* in Postgate C plates inoculated with the SRB strain. The OE of *A. cymbifera* showed a total inhibition of the SRB strain growth in agar plates, probably due to the OE volatility. The PE of *P. regnelii* and *Q. amara* showed an inhibition halo against the SRB strain of 1 cm. When serial dilutions of all bioproducts were done to determine the Minimal Inhibitory Concentration (MIC) against the SRB strain, the PE showed the best results when compared to the OE. The PE of *P. regnelii* and *Q. amara* inhibited the SRB strains in concentrations of 31.25 µg mL^{-1} for MIC, and 78 µg mL^{-1} for Minimal Bactericidal Concentration (MBC).

Acknowledgements: CNPq, FAPERJ, Capes and Petrobras.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Piper regnellii*, *Piper umbellatum* and *Piper xylosteoides* from Rio Grande do Sul

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Keywords: *Piper regnellii*; *Piper umbellatum*; *Piper xylosteoides.*

Plants are rich natural sources of compounds capable of performing the various pharmacological actions. This feature, combined with its great chemical diversity makes vegetable products excellent raw materials for the synthesis of new drugs. Morphological variations can occur within the same species depending on their adaptation to different locations and climates. Knowledge of the chemical composition can also assist in its botanical classification. Among the plant secondary metabolic products most promising and easier access to the research of active compounds are the volatile or essential oils, which have been regarded as the largest group of natural products and potential source of biologically active substances. Piperaceae family belongs to Piperales order and is one of the earliest families of Angiosperms. *Piper* and *Peperomia* are the largest genera, respectively with 265 and 166 species. Among the species of Piperaceae are included several varieties of peppers, with economic and medicinal importance.[1] *Piper* genus comprises about 2000 species and only 12 % were studied from the chemical point of view.[2] Thus, the objective of this work is the analysis of the chemical composition of the volatile oil from leaves of *Piper regnellii*, *P. umbellatum* and *P. xylosteoides* native populations of Rio Grande do Sul. The aerial parts of the species were collected in Três Cachoeiras. The oil from the leaves was obtained by hydrodistillation, using the Clevenger-type apparatus for 4 h. Chemical analysis was carried out by gas chromatography coupled to mass spectrometry (GC/MS). Identification of compounds was based in comparison of retention indices and mass spectra with authentic samples and data from literature. The yield of volatile oil from leaves of *P. regnellii* was 0.5 %, for *P.umbellatum* was 0.1 % and *P. xylosteoides* was 0.1 %. The major compounds identified in the leaves of *P. regnellii* were the arylpropanoids dill apiole (16.7 %) and apiole (42.0 %). For *P. umbellatum* were the sesquiterpenes β-caryophyllene (25.2 %), germacrene D (36.4 %) and bicyclogermacrene (12.2 %) and for *P. xylosteoides* were the sesquiterpenes β-caryophyllene (10.4 %), bicyclogermacrene (38.2 %) and *trans*-γ-bisabolene (14.4 %).

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Acknowledgements: Capes, CNPQ

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

The effects of different culture conditions on the production of volatiles from *Lippia origanoides* Kunth (Verbenaceae).

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Keywords: *Lippia origanoides*; volatiles; micropropagation.

*Lippia origanoides*is popularly known as "salva-de-marajó", occurring mainly in Brazil, northern South America, Colombia and Venezuela.[1] This aromatic plant grows in wild habitats, obtained exclusively by extractivism. This species presents five different chemotypes (A-E), so far described: (A) *p*-cymene, α- and β-phellandrene and limonene, (B) carvacrol, (C) thymol, (D) 1,8-cineole and (E) (*E*)-methyl cinamate and (*E*)-nerolidol.[1,2] *L. origanoides* was collected in the Amazon region in order to verify the effects of growth regulators on the chemical composition of the volatiles of the *in vitro* plants, compared to the field material collected at different times. Cultures of this plant were maintained *in vitro* in basic medium,[3] either without growth regulators or supplemented with different concentrations of cytokinins (kinetin and 6-benzylaminopurine) and auxin (indolylacetic acid) combined with each other. Volatiles were obtained from 5 g of plant material by SDE for 3 h, and collected in dichloromethane, and analyzed by GC/FID and GC/MS in Shimadzu GC-2010, in a DB-5MS capillary column (30 m X 0.25 mm X 0.25 µm. Oven temperature was raised from 60 to 290 °C at 3°C min^{-1}. Mass detector was operated in electronic ionization mode at 70 eV. The percentage composition was obtained by normalization from FID. Volatile components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. The major compounds found in volatile extract of *L. origanoides* were: myrcene (trace-5.1 %), *p*-cymene (5.7-20.0 %), γ-terpinene (1.8-10.6 %), linalool (2.5-4.7 %), thymol (3.0-9.2 %), carvacrol (29.7-45.8 %) and β-caryophyllene (2.5-5.3 %). In this study, the carvacrol content was more pronounced in the wild plants (35.1-45.8 %), compared to the *in vitro* material (29.7-45.5 %). Carvacrol has been associated with the aroma of “oregano”, its high content can be considered economically interesting and important for the acceptance of this species as food for culinary purposes. The results showed similar percentage contents of volatile constituents for plants growing in in vitro culture media and field plants.

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Acknowledgements: Faperj, CNPq, CAPES, TIFF-UFRJ, IPPN-UFRJ, IQ-UFRJ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

HS-SPME/GC-MS Study of the Volatile Fraction of *Posoqueria latifolia* (Rubiaceae) Flowers

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Keywords: HS-SPME; GC-MS; volatile fraction; *Posoqueria latifolia.*

Posoqueria latifolia, a tree no higher than 7 m, belongs to the Rubiaceae family, and is native of Central America and part of South America. Its flowers are white, elongated and with an intense overnight fragrance.[1] The objective of this work was to study the chemical composition of the volatile fraction of *P. latifolia* flowers. The vegetal material was collected (N°512081 of the National Herbarium of Colombia) at the pilot agro-industrial complex of the National Research Center for Agro-industrialization of Tropical Aromatic and Medicinal Plants (CENIVAM), located on the main campus of Universidad Industrial de Santander (Bucaramanga, Colombia). Fresh *P. latifolia* flowers (2.4 g) were placed inside an amber vial, and allowed to reach thermal equilibrium (10 min) at 60 °C. The extraction was done by exposition of a solid-phase microextraction (SPME) fiber (coated with PDMS/DVB, PDMS or CAR/PDMS) during 30 min. The largest chromatographic area was obtained with the CAR/PDMS fiber. HS-SPME sampling was done in triplicate at different times of day (6:00 am; 12:00 m; 6:00 pm). Compound identification was based on data obtained with a GC 7890 (*Agilent Technologies* 6890N, Palo Alto, CA, EE.UU.) gas chromatograph equipped with a mass selective detector AT 5975C (electron impact ionization, 70 eV), split/splitless injector (split 30:1) and a data system *MSD ChemStation,* version G1701-DA, which included the ADAMS, NIST, and WILEY spectral libraries. A capillary column with polar stationary phase of poly(ethylene glycol) (DB-WAX, J&W Scientific) of 60 m X 0.25 mm X 0.25 µm was used. The main compounds identified in the fragrance of *P. latifolia* flowers were: methyl salicylate (28 %), hexenyl acetate (18 %), *trans*-β-ocimene (17 %), benzyl acetate (5 %) and isoeugenol (2 %). The release of the main compound, methyl salicylate, was lower in the morning (21 %) than at night (28 %). It has been reported in field tests, traps baited with methyl salicylate were highly attractive to adult beetles *C. septempunctata.*[2]

[1]Taylor, C.M.; Hammel, B.; Gereau, R.E. Rubiacearum americanarum magna hama pars XXVII: Six new species and a new taxonomic view of *Posoqueria*. *Novon* **2011**, *21*, 118. [CrossRef]
[2]Junwei, Z.; Key-Chung, P. Methyl Salicylate, a Soybean Aphid-Induced Plant Volatile Attractive to the Predator *Coccinella septempunctata*. *Journal of Chemical Ecology* **2005**, *31*, 1733. [CrossRef][PubMed]

Acknowledgement: Colciencias - National Autonomous Patrimony Financing Fund for Science, Technology and Innovation, Francisco José de Caldas, Contract RC-0572-2012. Industrial University of Santander.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Cytotoxic and Antioxidant Activity of Two Diterpenos - Type Abietane from Essential Oil *Tetradenia riparia* (Hochst.) Leaves

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Keywords: *Tetradenia riparia*; abietane diterpenes; cytotoxicity.

Tetradenia riparia (Hochstetter) Codd (Lamiaceae) was introduced in Brazil as an exotic ornamental plant. The samples for this work were collected in Umuarama, Paraná, Brazil (voucher at the UNIPAR Herbarium no 2502). The essential oil was extracted from the fresh leaves by hydrodistillation. Two grams of essential oil was submitted to column chromatography over a silica gel and eluted with pentane (P), P–dichloromethane (DCM) (9: 1; 8: 2; 7: 3 and 1: 1), DCM–P (3: 7); DCM, DCM–methanol (9: 1; 7: 3 and 1: 1) and methanol, resulting in 29 fractions. Fractions 16 (6.6 mg) and 17 (11.7 mg) were identified by 1H, 13C, DEPT, HSQC, HMBC and NOESY NMR techniques, and by comparison with literature data. Compound in fraction 16 was elucidated as the new compound 9β,13β-epoxy-7-abietene (**1**), an amorphous white solid with GC retention time 33.78 min. EI, *m/z* (rel. int.): 288 $[M]^+$ (20), 161(100). Compound in fraction 17 was identified as 6,7-didehydroroyleanone (**2**), already described by Kusumoto.[1] The cytotoxic activities of the essential oil and compounds **1** and **2** were determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, and by tumor cells MDA- MB- 435 (human breast carcinoma), HCT-8 (human colon), SF-295 (human nervous system) and HL-60 (human promyelocytic leukemia). The essential oil and compound **1** showed high cytotoxic potential of the cell lines SF-295 (78.06 % and 94.80 %, respectively), HCT-8 (85.00 % and 86.54 %, respectively) and MDA- MB-435 (59.48 % and 45.43 %, respectively). Compound **2** had no cytotoxic activity. The antioxidant activity was determined by 2, 2- diphenyl-1-picryl-hydrazyl (DPPH), β-carotene- linoleic acid system and 2,2'-azinobis-(3- ethylbenzothiazoline- 6-sulfonic acid) (ABTS) assays. The inhibitory concentration (IC50 in μg mL^{-1}) for essential oil and compound **2** was, respectively, 15.63 and 0.01 for DPPH; 130.1 and 109.6 for β-carotene- linoleic acid and 1524 and 1024 for ABTS. Compound **1** had no antioxidant activity. By fractioning the oil, it was possible to identify compounds **1** and **2,** and shows that **1** has a high cytotoxic potential, and **2,** a high antioxidant potential.

[1]Kusumoto, N., Ashitani, T.; Hayasaka, Y.; Murayama, T.; Ogiyama, K.; Takahashi, K. Antitermitic activities of abietane-type diterpenes from *Taxodium distichum* cones. *Journal of Chemical Ecology* **2009**, *35*, 635. [CrossRef] [PubMed]

Acknowledgements: UNIPAR, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Molluscicidal Activity of Essential Oil from the Leaves of *Eugenia patrisii* Vahl

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Keywords: *Eugenia patrisii*; essential oil; molluscicidal Activity.

Schistosomiasis, also known as bilharziasis, is the second most endemic parasitosis in all over the world which affects over than 200 million people in America, Africa and Asia. Brazil is the largest endemic area in America. However, five species of trematode can cause schistosomiasis; *S. mansoni* is the main agent in our continent. According to World Health Organization (WHO), one of the best ways to control the overspread of this parasitosis is the use of molluscicidal agents to the snail control, breaking the life cycle of trematode and avoiding its transmission by human skin. For many researchers, the plants represent an important source of low cost and safe compounds with molluscicidal activity that can be a great alternative to decrease the morbidity and mortality of this disease. *Eugenia patrissi* Vahl. (Myrtaceae) is a common species in Cerrrado biome of Brazil. This study aims to analyze the chemical composition of the essential oil from *E. patrissi* leaves, and evaluate its mollucicidal activity against *B. glabrata*. The species were harvested in Carolina-MA, Brazil in January 2013. The voucher specimen was deposited at the João Murça Pires Herbarium from Museu Parense Emílio Goeldi (MPEG) in Belem-PA-Brazil. The essential oil was obtained by hydrodistillation from dry leaves and then was characterized by gas chromatography (GC) and GC-mass spectrometry (GC-MS). The molluscicidal activity was evaluated according to standards recommended by the WHO, using several concentrations under 100 mg L^{-1}. Lethal concentrations (LC) of the essential oil were calculated by prohibit analysis. With this methodology, twenty five compounds were identified corresponding to 96.8 % of the oil. The major constituents were δ-cadinene (14.9 %), bicyclogermacrene (13.8 %) and β-selinene (11.1 %). The oil comprised sesquiterpenes hydrocarbons (86.1 %), oxygenated sesquiterpenes (10.6 %) and oxygenated monoterpenes (0.1 %). The oil showed significant molluscicidal activity against B. glabrata, with LC10, LC50 and LC90 values of 35.9; 62.1 and 83.8 mg L^{-1}, respectively after 24 h of the beginning of the test. These values are below the threshold of 100 mg L^{-1}, set down as potential molluscicidal activity by the WHO. Hence, the results presented here suggest that the essential oil of *E. patrisii* leaves possesses important toxicity against snail *B. glabrata* and is therefore a potential source of compounds for the control of schistosomiasis in endemic areas.

[1]Brunn, B.; Aagaard, J. *The social context of schistosomiasis and its control.Special Programme for Research & Training in Tropical Diseases*. World Health Organization: Geneva, 2008. [Link]

Acknowledgements: CNPq, FAPEMA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Analysis of the Chemical Composition of the Essential Oils of *Piper marginatum* and *Peperomia andrei* (Piperaceae)

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Keywords: *Piper marginat; Peperomia andrei;* essential oil

The Piperaceae family grows in tropical and subtropical climates. It is composed of 2000 species distributed among four genera, namely: *Piper*, *Peperomia*, *Sarchorhachis* and *Ottania.*[1]The volatile chemical composition of this family has been widely studied due to the presence of monoterpenes, sesquiterpenes, and arylpropanes (apiol, dillapiol, safrole and sarisan). In this work, the chemical composition of essential oils from *Peperomia andrei* and *Piper marginatum* were studied. Samples were collected in the municipality of Dagua, in the department of Valle del Cauca, Colombia. The taxonomic identifications (*Piper marginatum*, COL582360 and *Peperomia andrei,* COL582365) were made at the Colombian National Herbarium (COL). The *P. marginatum* essential oil (0.04 % yield) was extracted by microwave-assisted hydrodistillation (MWHD) (15 min x 3). The *P. andrei* essential oil (0.01 %) was obtained by steam distillation. Essential oil analysis was performed using an Agilent Technologies 6890 (Palo Alto, CA, USA) gas chromatograph coupled to an Agilent Technologies 5973 mass selective detector. Fused-silica capillary columns DB-5MS (J&W Scientific) (60 m X 0.25 mm X 0.25 µm), and DB-WAX (J&W Scientific) (60 m X 0.25 mm X 0.25 µm) were used. Helium was used as carrier gas. Compounds identification was performed by comparison of their mass spectra with database (Adams, NIST, WILEY) and of their linear retention indices with those reported in the scientific literature. The major components of the essential oil from *P. andrei* were: *trans*-β-caryophyllene (12.4 %), germacrene D (11.6 %), intermedeol (11.6 %), β-elemene (10.5 %), γ-muurolene (5.6 %) and δ-selinene (4.3 %). No chemical description on *P. andrei* was found in literature. The main component (47.9 %) of *P. marginatum* essential oil was a sesquiterpene with mass spectrum: *m/z* (%):204 (M^+, 13), 136 (9), 135 (100), 105 (5), 78 (4), 77 (21), 55 (11). *P. marginatum* oil showed piperonyl methyl ketone (32.3 %), apiol (3.8 %), hexylbenzene (2.8 %). 3,4-Methylenedioxy-propiophenone and safrol (phenylpropanoids) have been found as the major components in most of 22 samples of *P. marginatum*, reported by Andrade *et al.*[2] However, these compounds were not found in this work.

[1]Pino, N.; Meléndez, E.M.; Stashenko, E.E.Essential Oil Composition from Two Species of Piperaceae Family Grown in Colombia. *Journal of Chromatographic Science* **2009**, *47*, 804. [CrossRef][PubMed]
[2]Andrade, E.H.; Carreira, L.M.; da Silva, J.D.; Bastos, C.N.; Sousa, P.J.; Guimarães, E.F.; Maia, J.G. Variability in essential-oil composition of Piper *marginatum* sensu lato. *Chemistry & Biodiversity* **2008**, *5*, 197. [CrossRef][PubMed]

Acknowledgements: Colciencias - Contrato RC-0572-2012. Contract No. 101 for access to genetic resources and derivatives for scientific research with bioprospecting aims, between Ministerio del MedioAmbiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antimicrobial Activity of Essential Oil *Cymbopogom nardus* (L.) Rendle and its Effect in Combination with Synthetic Preservative Methylparaben

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Keywords: citronella; natural products; bacteria; synergism.

The demand for natural products is growing every day, due to the benefits they provide to health. Citronella (*Cymbopogom nardus* L. Rendle) is an aromatic plant belonging to Poaceae family, originally from Southern India. This plant is known to be taken out of their leaves an oil with repellent properties, with great application in the cosmetics industry.[1] The objective of this research was to determine the antibacterial potential essential oil of citronella and evaluate a possible synergistic effect when combined with synthetic preservative methylparaben front of pathogenic bacteria. The essential oil of citronella (lot 1286) was obtained commercially from Laszlo Aromatherapy Company. The antimicrobial activity was determined by evaluating the Minimum Inhibitory Concentration (MIC) by microdilution assay in microplate with 96 wells front of Gram-positive bacteria, *Bacillus cereus* (ATCC 11778) and *Staphylococcus aureus* (ATCC 14458) and Gram-negative bacteria *Escherichia coli* (ATCC 10536) and *Salmonella typhi* (ATCC 06539). These microorganisms were lyophilized, and acquired from the National Institute of Quality Control in Health (INCQS). A standard solution McFarland 0.5 in saline 0.85 % was used to standardize the bacterial inoculum. The association tests of essential oil with synthetic preservative methylparaben (Biotec, lot 32343) were performed by the technique of checkerboard. The results of the test were rated according to the Fractional Inhibitory Concentration Index (FICI) in the total synergism (FICI≤ 0.5), partial synergism (0.5 < FICI ≤ 0.75), indifferent (0.75 < FICI ≤ 2.0) or antagonistic (FICI ≥ 2.0). In determining the antibacterial activity the most sensitive strainto essential oil was *Bacillus cereus* having a minimum inhibitory concentration of 1.25 mg mL^{-1}, followed by *Escherichia coli* and *Staphylococcus aureus* with MIC 2.50 mg mL^{-1}. The essential oil did not inhibit the bacteria *Salmonella typhi* even at the highest concentration tested (5.0 mg mL^{-1}). In association tests with synthetic preservative methylparaben, the essential oil of citronella is effective against Gram-positive bacteria, showing a partial synergistic effect for *Bacillus cereus* (FICI 0.62) and *Staphylococcus aureus* (FICI 0.75), reducing the concentration of preservative in the use of low concentrations of the essential oil. For the Gram-negative bacterium *Escherichia coli* (FICI 0.99) was observed an indifference effect by combining the substances.

[1]Castro, L. O.; Ramos, R. L. D. Principais gramíneas produtoras de óleos essenciais. *Boletim Fepagro* **2003**, *11*, 1. [Link]

Acknowledgements: UTFPR.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Evaluation of Antifungal and Antioxidant Potential of *Piper marginatum* Jacq. (Piperaceae) Essential Oil in Santarém-PA

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Keywords: *Piper marginatum*; antifungal potential; antioxidant.

Piper marginatum Jacq. (Piperaceae), known as "caapeba cheirosa", it is popularly used in northern Brazil against liver diseases and as a tonic andantispasmodic, and its essential oil (EO) has fungicidal properties and cercaricide.[1] Chemical composition of *P. marginatum* OE classified the specimens collected in the Amazon into 10 chemotypes.[1] Here, the chemical composition, antifungal and antioxidant activities of the essential oil from two specimens of *P. marginatum* collected in Santarém-PA were studied. Aerial parts were collected in Alter-do-Chão community (PM44 - 2°30'25.0"S 54°57'02.2"W) and at the UFOPA campus (PM48 - 2°25'05.0"S 54°44'29.7"W) (voucher at Herbarium of the Federal University of Western Pará, HSTM 000089). The material was dried at 40 °C followed by hydrodistillation in a Clevenger type apparatus for 180 min. Identification of chemical constituents was performed by GC/MS. The antifungal activity against *Candida albicans* (CCCD - CC001), *C. tropicalis* (CCCD - CC002) and *C. parapsilosis* (CCCD - CC003) was performed in triplicate using the microdilution broth method and fluconazole was used as standard antifungal. The antioxidant activity was determined by the DPPH radical-scavenging. The control sample was prepared using ethanol instead of OE and trolox (1 mM) was used as antioxidant standard. The OEs yield was 0.65 % (PM44) and 1.39 % (PM48). 3,4-Methylenedioxypropiophenone was the major compound in both PM44 oils (19.6 %) and PM48 (34.4 %), although there were variations in the chemical composition of these oils. Other major compounds in PM44 oil were safrole (8.8 %), 2-hydroxy-4,5-methylenedioxypropiophenone (7.1 %), β-caryophyllene (4.8 %), elemol (5.6 %) and β-eudesmol (7.4 %), while in the PM48 oil were safrole (4.5 %), myristicin (6.0 %), elemicin (4.2 %), (*E*)-β-ocimene (7.4 %) and spathulenol (4.7 %). MIC was 0.6, 2.5 and 0.3 µL mL^{-1} (PM44); 0.6, 5.0 and 0.3 µL mL^{-1} (PM48); and 0.95; 0.95 and 0.15 µl mL^{-1} (fluconazole) for *C. albicans*, *C. tropicalis* and *C. parapsilosis*, respectively. In the DPPH assay, inhibition was 61.08 ± 0,21 % (PM44) and 13.82 ± 0.31 % (PM48), and trolox standard was 33.01 ± 0.16 %. So, the OE from PM44 showed better antifungal potential against *Candida spp*. and high antioxidant activity when compared to trolox.

[1]Andrade, E. H. A.; Carreira, L. M. M.; Silva, M. H. L.; Silva, J. D.; Bastos, C. N.; Sousa, P. J. C.; Guimarães, E. F.; Maia, J. G. Variability in essential-oil composition of *Piper marginatum* sensu lato. *Chemistry & Biodiversity* **2008**, *5*, 197. [CrossRef] [PubMed]

Acknowledgements: UFOPA, CNPQ, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of Essential Oils of Three Species Belonging to the Verbenaceae family: *Lippia alba, Lantana camara* and *Lantana canescens*

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Keywords: *Lippia alba*; *Lantana camara; Lantana canescens*.

The Verbenaceae family has been widely studied for its diversity and its applications to the pharmaceutical, cosmetic and food industries.[1] This family includes about 91 genus and 3000 species.[2] Plant materials were collected in Dagua, Valle del Cauca, Colombia. The taxonomic identifications (*Lippia alba*, COL582597, *Lantana canescens*, COL582598 and *Lantana camara*, COL582528) were made at the Colombian National Herbarium (COL). Essential oils (EO) were extracted by steam distillation from the plant aerial parts. Yields (% w/w) of 0.05, 0.01, and 0.02, were obtained for *L. alba, L. canescens*, and *L. camera,* respectively. The oils were analyzed in an Agilent Technologies (Palo Alto, CA, USA) 6890 Plus gas chromatograph, coupled to a 5973 Network mass selective detector. A non-polar DB-5MS column (J & W Scientific, Folsom, CA, USA) 60 m X 0.25 mm ID, coated with 5 % phenylpolydimethylsiloxane (0.25 µm) and a polar DB-WAX (J&W Scientific) column 60m X 0.25 mm ID, coated with polyethylene glycol (0.25 µm) were employed. The chromatographic oven temperature was programmed from 50 °C (5 min) to 250 °C (2 min) at 4 °C min^{-1}. Compound identification was based on mass spectra and retention indices. Germacrene D was common to the three oils in amounts of 20-30 %. *trans*-β-Caryophyllene was found in relative amounts of 31-35 % in the studied plants of the genus *Lantana*. The major compounds identified in *L. alba* oil were: carvone (48 %), germacrene D (21 %) and limonene (18 %). In the *L. canescens* EO they were *trans*-β-caryophyllene (35 %), germacrene D (30 %) and α-humulene (17 %), and in the *L. camara* EO, *trans*-β-caryophyllene (32 %), germacrene D (21 %) and *trans*-nerolidol (14 %).

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101 for access to genetic resources and derivatives for scientific research with bioprospecting aims,between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Plectranthus amboinicus* (Lour.) Spreng. that Grows in Santander (Colombia)

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Keywords: *Plectranthus amboinicus*; essential oil; MWHD.

Plectranthus genus (Lamiaceae) is constituted by approximately 300 species that grow in Africa, Asia, America and Oceania. One out of five of them has an ethnobotanical use.[1,2] *Plectranthus amboinicus* (Lour.) Spreng., also known as *P. aromaticus*, *Coleus amboinicus* or *C. aromaticus*, grows all across the intertropical region of the globe. It's used in traditional medicine to treat different pathologies, mostly infectious ones.[3] This work focuses on determining the chemical composition of the essential oil (EO) of *P. amboinicus* (Santander, Colombia). The leaves and stems were dried for 50 days and then reduced to 2 mm. The EO was obtained by microwave-assisted hydrodistillation (MWHD). Dried material (100 g) was subjected to MWHD in 3 periods of 15 min with 5 min of resting time between each extraction. The EO was collected in the Dean-Stark trap of a Clevenger apparatus. Compounds were analyzed on an Agilent Technologies 6890 GC with a mass-selective detector 5973 (EI, 70 eV). DB-5 and DB-WAX (60 m X 0.25 mm X 0.25 µm) capillary columns were used. The injector port temperature was 250 °C, the split ratio used was 30:1. Oven temperature was programmed from 45 to 150 °C, at 4 °C min^{-1}, then to 250 °C (5 min), at 5°C min^{-1}, and finally 275 °C (15 min), at 10 °C min^{-1}. The relative amounts were established with GC-FID normalization. Analytes in the oil were identified using ADAMS, NIST and WILEY databases. MWHD yield was 0.18 %, and 28 compounds were identified. Carvacrol (69.8 %) was the main component, followed by trans-β-caryophyllene (9.3 %), cis-α-bergamotene (4.9 %) and caryophyllene oxide (2.2 %). These results differ from the ones reported by Saïd et al. (Comoros), who hydrodistillated dry plant material, had a yield of 0.12 % and identified carvacrol (23 %), camphor (22.2 %), Δ-3-carene (15 %) and γ-terpinene (8.4 %) as abundant constituents in the EO. Carvacrol was the major compound in both studies, which is a monoterpenoid known for its antimicrobial activity.[4]

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Acknowledgments: Colciencias-Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of *Salvia aratocensis* Essential Oil and Volatile Fraction

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Keywords: *Salvia aratocensis;* essential oil; volatile fraction.

Salvia aratocensis is one of the 300 species that compose the *Salvia* genus. This plant is endemic to the Chicamocha Canyon, in Santander, Colombia. Some studies have reported positive results in the use of *S. aratocensis* in the treatment of different pathologies such as tuberculosis, Chagas disease and leishmaniosis.[1] The plant material (COL. No. 517740, Colombian National Herbarium) was collected at the CENIVAM Agroindustrial Complex (Bucaramanga-Santander, Colombia). The essential oil was obtained from the aerial parts of the plant by microwave-assisted hydro-distillation (MWHD) using a Clevenger apparatus. The volatile fraction was sampled with dynamic headspace purge and trap in dichloromethane (P&T) and headspace solid-phase microextraction (HS-SPME). The latter was performed by exposing for 30 min a SPME fiber (coated with Carboxen/PDMS) to the headspace inside an amber vial (15 mL) that contained the plant material, at 60 °C. The chromatographic analyses were performed with an Agilent Technologies 6890 (Palo Alto, CA, USA) gas chromatograph coupled to an Agilent Technologies MSD 5973 mass selective detector. Fused-silica capillary columns DB-5MS (J&W Scientific, 60 m X 0.25 mm X 0.25 µm), and DB-WAX (J&W Scientific, 60 m X 0.25 mm X 0.25 µm) were used. Helium was used as carrier gas. The GC oven temperature was programmed from 45 to 275 °C for the DB-5MS column, and from 45 to 230 °C for DB-WAX column. Quantification was performed with a gas chromatography with flame ionization detector and the same column types. Compound identification was based on the comparison of their mass spectra with those of databases (Adams, NITS, WILEY) and of linear retention indices with those reported in the scientific literature. The main components the *Salvia aratocensis* essential oil were: *epi*-α-cadinol (47.7 %), 1-*epi*-cubenol (22.7 %), and γ-cadinene (6.3 %). The analysis of the volatile fraction by P&T and SPME showed that the secondary metabolites present in greater proportion were: γ-cadinene (17.4 %); *epi*-α-cadinol (10.4 %) and 1,10-*di*-*epi*-cubenol (9.0 %).

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Aknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparison of the Volatile Fractions of *Brugmansia suaveolens* Flower Using Micro-Solid Phase Extraction (HS-SPME) and Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

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Keywords: *Brugmansia suaveolens*; HS-SPME; GC-MS.

The Solanaceae family is distributed mainly in tropical areas. It has more than 2600 species grown industrially in many countries. The genus *Brugmansia* belongs to this family. It is characterized by a strong and pleasant flower fragrance, especially at night. *Brugmansia suaveolens* is native to South America. It is a 2-4 m tall shrub with many branches and elliptical – ovate leaves. It presents pendulous hermaphrodite flowers of about 35 cm in length, with tubular calyx and corolla that can be white or pink at the base and orange toward the apex. The aim of this work is to study the chemical composition of the volatile fraction of *B. suaveolens* flowers at different times of the day (6 am, 12 pm, 6 pm). *B. suaveolens* flowers (16 g) were weighed, placed in a glass container and left to thermally equilibrate at 60 °C (10 min), before exposing a Carboxen-PDMS coated SPME fiber (30 minutes at 60 °C). The analysis was performed on a 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a 5975C mass selective detector MSD (AT, EI, 70 eV). Split (30:1) injection was used. A DB-WAX (J & W Scientific, Folsom, CA, USA) of 60 m X 0.25 mm X 0.25 µm was used. At 6 am *trans*-β-ocimene (69 %) was identified as the main component of *B. suaveolens* flower scent, followed by 1,8-cineole (9 %) and *allo*-ocimene (3 %). At 12 pm *trans*-β-ocimene (67 %), was followed by 1,8-cineole (8 %) and linalool (7 %). At night, the main components were *trans*-β-ocimene (45 %), β-nerolidol (10 %) and 1,8-cineole (9 %). The main component *trans*-β-ocimene is one of the most common monoterpenes in floral scents.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Correlation between the Chemical and Genetic Characteristics of Wild Populations of *Lychnophora pinaster* Mart. (Asteraceae)

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Keywords: *Lychnophora pinaster*; SSR markers; brazilian Cerrado.

Lychnophora pinaster is endemic to the Cerrado of Minas Gerais, Brazil, with strong cultural importance as anti-inflammatory, painkiller, and healer. In the essential oil of southern populations, anti-inflammatory agents as (*E*)-caryophyllene and α-humulene, and microbicides as (*E*)-methyl cinnamate, α-pinene, and β-pinene were seen.[1] The EO composition is affected by environmental factors and can be correlated with the genetic differentiation of populations. Leaves of three populations (75 individuals) from three different sites were collected in the summer and winter 2012. The EO of the leaves was extracted by hydrodistillation in a Clevenger-type apparatus for 2 h, and the chemical composition analyzed by GC/MS, GC/FID, and multivariate statistics. The genetic variability was analyzed using SSR markers, and a correlation between genetic and chemical computed using a Mantel test with 10,000 permutations. All evaluated populations showed similar chemical composition, and presented (*E*)-methyl cinnamate (summer average: 57.8 % - 80.0 %; winter average: 66.3 % - 80.3 %) as the major component. However, the comparison of the chemical composition by Discriminant Analysis allowed separation of the essential oils of each population into three distinct groups, correlated to the production of monoterpenes (α-pinene, β-pinene, limonene, α-terpinene, γ-terpinene, *p*-cimene, and santalone, called Group 1), sesquiterpenes (cedreanol, (*E*)-caryophyllene, α-humulene, and caryphyllene oxide, called Group 2) and (*E*)-methyl cinnamate (called Group 3). The *p*-valor of the Wilks' Lambda was 0.0001 (alpha = 0.05), and it is an indication of existence, in quantitative terms, of a significant intraspecific chemical differentiation in the essential oil of *L. pinaster*. Furthermore, the comparison of the chemical and genetic UPGMA trees revealed no obvious relation between genetic and chemical differentiation, which was reinforced by the Mantel test of relationship between the genetic and chemical distances (winter and summer data) of the populations, where an insignificant correlation was detected, r_{summer} = 0,02233, p = 0,1981 and r_{winter} = -0,009669, p = 0,3795. Since neutral markers are expected to reflect the demographic and (neutral) genetic history of the populations, the essential oils divergence observed in populations of *L. pinaster* studied tends to be a result of natural selection according to local environmental factors.

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Acknowledgements: FAPESP and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In Vitro and *In Vivo* Antifungal Activity of the Essential Oil of *Lippia alba* against *Alternaria tenuissima*, a Postharvest Pathogens of Blueberries

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Keywords: Essential oils; GC-MS; *Lippia alb;* Blueberries; *Alternaria tenuissima.*

Protection from physical damage and inhibition of infection by pathogenic fungi on fruit are crucial aspects that must be considered in postharvest. *Alternaria* spp. is one of the most important pathogens involved in the deterioration of fruits.[1] For the control of pathogens in fragile fruits, like blueberries, the use of volatile compounds is considered an advantageous option. *Lippia alba* (Verbenaceae) produces an essential oil characterized by presenting a wide variability in their chemical composition depending on the state of development of the plant and environmental growth conditions.[2] In this work, *in vitro* and *in vivo* activity of *L. alba* essential oil was studied as biological insecticide to control *A. tenuissima* in blueberries. Fresh leaves and flowers of *L. alba* were hydrodistillated in a Clevenger-type apparatus for 2 h. Analysis of the essential oils was performed using a Shimadzu GC 2010 Plus; HP-5 MS capillary column (30 m X 0.25 mm X 0.25 µm); column temperature 40 °C (4 min) rising to 180 °C at 5°C min^{-1}, then to 280 °C at 10°C min^{-1}, 280 °C (8 min). Mass detector was operated in electronic ionization mode at 70 eV. Two different *in vitro* assays were performed, in triplicate, in which *A. tenuissima* growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. In the first assay, the essential oil was incorporated into the culture medium, while in the second one, the oil was placed on the opposite side of the Petri dish in order to evaluate its activity in volatile state. The *in vivo* antifungal activity was performed using fresh blueberries inoculated with a suspension of *A. tenuissima* where, after an incubation period, the presence or absence of the disease in the wound was evaluated. For all tests, positive growth controls were performed without essential oils. The main oil components were: 2-octanol, 1,8-cineol, linalool, (*Z*) and (*E*)–dihydrocarvone, (*E*)-caryophyllene, germacrene B and germacrene D. The *in vitro* test showed high efficacy against *A. tenuissima* (70 % and 100 % inhibition). In vitro tests also fungicidal activity of the essential oil is observed, due to an inhibition of fungal growth was seen in all fruits. From the in vitro tests it can be seen an inhibition of fungal growth in all the fruits, due to the application of the essential oil.

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Acknowledgements: ANII, INIA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Phytotoxic Effects of *Schinus terebinthifolius* Raddi Volatiles on *Arabidopsis thaliana* (L.) Heynh

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Keywords: Volatiles; germination; seedling growth; genes expression.

Schinus L. (Anacardiaceae) essential oils (EO) show a wide spectrum of ecological interactions.[1] Initial evaluation of phytotoxic activity involves analysis of allelochemical interference on germination and initial growth of target species, processes that reflect effects on cellular levels. Here, we investigated the phytotoxic effects of *S. terebinthifolius* EO on *A. thaliana* germination, seedling growth and gene expression. Leaves samples from six individuals were harvested in natural vegetation in Porto Alegre, RS, Brazil (voucher no. 164707 at herbarium ICN of UFRGS). Leaves (dried and fragmented) were hydrodistillated in a Clevenger apparatus for 4 h. Thirty *A. thaliana* Columbia wild type seeds were surface sterilized and sown in Petri dishes containing 3 % sucrose, 0.8 % (w v^{-1}) agar and 0.1x MS minerals. Seeds were stratified and EO (5, 10, 15, 20 and 25 µL) were pipetted on filter paper attached to the inner face of the Petri dish. Control was a treatment without EO application. Parameters examined included germination rate (GR) and speed of accumulated germination (AS). In post-germination assays, EO was applied after emergence of the primary root. Ten seedlings remained exposed to the EO for 7 days. Photographs were taken to measure shoot length (SL) and root length (RT) using ImageJ 1.45s software. For quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis, 5 µL of the EO were applied and seedlings remained exposed to volatiles during 24 h. Roots were collected (3 repetitions of 25 mg material), frozen in liquid nitrogen and stored at -80 °C until RNA isolation. Total RNA was prepared using Plant RNA Purification reagent and DNAse I treatment. RNA quantification was carried out using a NanoDrop. First-strand cDNA synthesis was performed with reverse transcriptase (M-MLV) using 1 µg of total RNA. Gene evaluated was ANP1 *Arabidopsis* NPK1-like protein kinase 1 (ANP1). Comparisons between treatments and control were performed using one way analyses of variance (ANOVA) and a post hoc Student-Newman-Keuls (SNK) or Tukey, using SPSS 17.0 software. Differences were considered significant at $P \leq 0.05$. EO inhibited all morphometric measurements. Amounts from 10 µL reduced about 50 % the GR of *A. thaliana*. AS was reduced by 65 % and 75 % when 5 and 10-25 µL were applied, respectively. Volumes equal or higher than 10 µL of EO reduced SL by 60 %. Inhibitory effect on RL demonstrated a dose-dependent effect. EO reduced by 52 % and 81 % *A. thaliana* RT when 10 µL and up to 15 µL were applied, respectively. RT-qPCR results showed that ANP1 expression were not affected by EO. Results suggest that phytotoxic effects of *S. terebinthifolius* EO, in quantities studied seem to be explained in terms of cellular damage rather than by induction of stress-inducible genes.

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Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Volatile Profiling of Arnicão (*Lychnophora salicifolia*), a Wild Medicinal Species from Brazilian Cerrado

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Keywords: *Lychnophora salicifolia*; Arnicão; volatile fraction.

The Cerrado is a diverse Brazilian savanna with a flora with more than 12,000 species. Arnicão (*Lychnophora salicifolia*) is an endemic species that occurs in a rocky savanna type in Central and Southeast Brazil, at higher altitudes, in sandstone and quartzite soils. It shows an aggregate distribution forming spatially defined populations, in well-delimited patches, with adaptations to Cerrado environment. *Lychnophora* species was reported for anti-inflammatory, antioxidant, and UV protectant effects, and also as trypanocide, cytotoxic, analgesic, antifungic and antibacterial. Local communities use the leaves and branches of *L. salicifolia* in the preparation of traditional medicine. The aim of this work was to describe the composition of the headspace and essential oil of *L. salicifolia*, and to compare individuals harvested in preserved and non-preserved areas from four populations. Headspace–solid-phase microextraction gas chromatography-principal component analysis (HS-SPME GC-PCA) is proposed as a method to discriminate between plant populations of *L. salicifolia* from two distinct areas. Forty-eight individuals of *L. salicifolia* were randomly collected and vouchers from each population deposited at Embrapa Genetic Resources and Biotechnology herbarium (CEN). Grounded dried leaves of *L. salicifolia* were sampled by HS-SPME with a CAR/PDMS/DVB fused silica fiber. A mixture of a suitable amount of dried leaves from all individuals of each population was submitted to hydrodistillation in a microdistillation apparatus. EOs and headspace samples by HS-SPME of *L. salicifolia* dried leaves were analyzed by GC (GC/MS; GC/FID), and the results submitted to statistical analysis by PCA. The PCA obtained from *L. salicifolia* essential oil was quite similar to that of the headspace sampled by HS-SPME, both showing a similar discrimination of all populations. All samples contained a significant amount of myrcene. The most abundant compounds were tricyclene (1.3-3.4 %), myrcene (21.1-36.4 %), limonene (3.3-5.6 %), presilperfol-7-ene (0-5.2 %), butyl benzoate (1.6-12.7 %), β-bisabolene (0.9-4.8 %), γ-cadinene (4.7-12.6 %), caryophyllene oxide (1.8-3.6 %), *epi*-α-cadinol (5.2-11 %), and 14-hydroxy-9-*epi*-(*E*)-caryophyllene (0-18.2 %). The headspace showed a predominance of mono and sesquiterpenes hydrocarbons, varying from 34.5 % to 46.9 % and 15.3 to 25.2 %, respectively. It was observed a higher concentration of caryophyllene derivatives on populations from non-protected areas, usually with compounds associated to plant defense. Also, oxygenated sesquiterpenes were present in relatively high percentage (12-27 %), predominant in one population from a protected area. It is important to highlight that HS-SPME can successfully be used (also) for rapid in-field analysis of a large amount of samples of wild populations.

Acknowledgements: Embrapa.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Cytotoxicity of *Hedyosmum brasiliense* Essential Oils from Cerrado and Atlantic Forest

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Keywords: *Hedyosmum brasiliense*; cytotoxicity; Cerrado.

Hedyosmum brasiliense (Chloranthaceae) is widely distributed in Central, Southeastern and Southern Brazilian regions,[1] used to treat headache and stomach pain.[2] The aim of this work was to compare the chemical composition and cytotoxicity of *H. brasiliense* essential oils (EO) obtained from Cerrado and Atlantic Forest. EO of fresh flowers and leaves of male and female plants were extracted in triplicate by hydrodistillation (3.5 h), analyzed by GC/MS and the compounds were identified by mass spectra comparison (Wiley 275 and Adams 2007) and retention indexes. Cytotoxicity was tested towards tumor cell lines MCF-7 (breast adenocarcinoma) and PC-3 (prostate carcinoma), using doxorubicin as positive control. The statistical analysis was performed by one-way analysis of variance (ANOVA) and the differences were considered significant when $P < 0.05$ among the essential oil provenance. Cerrado leaves presented, in average, the lowest essential oil yields (0.14 %♂ and 0.13 %♀) when compared to those from Atlantic Forest (0.17 %♂ and 0.18 %♀). Besides, female flowers presented higher essential oil yields (0.19 % and 0.20 %) than male (0.05 %. and 0.07 %) in Cerrado and Atlantic Forest, respectively. Fifty-five compounds were identified from Cerrado essential oils, with predominance of sesquiterpenes, possibly due to the hot and dry Cerrado weather conditions. Some of their major compounds are myrcene (≈2-11 %), germacrene D (≈12-20 %), bicyclogermacrene (≈9-15 %), carotol (≈6-13 %), α-eudesmol (≈7-10 %) and ferula lactone I (≈5-8 %). Fifty-three compounds were identified from Atlantic Forest essential oils, with predominance of monoterpenes and hydrocarbon sesquiterpenes. Some of their major compounds are sabinene (≈5-31 %), pinocarvone (≈4-9 %), bicyclogermacrene (≈8-10 %) and (*E*)-iso-γ-bisabolene (≈7-18 %). All essential oils were cytotoxic. Atlantic Forest essential oils presented the lowest IC_{50} values against MCF-7 (from 0.018 ± 0.001 to 0.033 ± 0.008 μL mL^{-1}), when compared to those from Cerrado (from 0.061 ± 0.008 to 0.089 ± 0.007), as confirmed by statistical analysis. However, for PC-3 cell line, there was no significant difference among the oils collection sites. These results suggest that the difference on the chemical composition of the essential oils from Atlantic Forest and Cerrado have more influence against MCF-7 tumor cell lines.

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Acknowledgements: CNPq, FAPESP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

GC × GC-qMS Analysis of the Essential Oil of *Piper xylosteoides* and *Piper hemmendorffii*

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Keywords: GCxGC; essential oil; Piperaceae.

The Piperaceae family comprises from 2400 to 3600 species, mostly in tropical and subtropical areas from both hemispheres, divided in five genera: *Peperomia*, *Manekia*, *Zippelia*, *Verhuelia* and Piper.[1] The genus latter is the largest one, comprising about 1000 species worldwide, from which 260 are found in Brazil.[2] Thirty grams of leaves (LPx) and branches (BPx) from *P. xylosteoides* and 30 g of leaves (LPh) and stems (SPh) from *P. hemmendorffii* were subjected to hydrodistillation separately and in duplicate in a modified Clevenger-type apparatus for 4 h each, the yields were 7.0, 3.5, 1.7 and 1.0 % of oil, respectively. The oils were analyzed by GC/MS and GC × GC-qMS in Shimadzu GC-2010 systems, both coupled with a mass spectrometer detector Shimadzu GCMS-QP2010 Plus. GC chromatograms were obtained with a Rtx-5MS fused silica column (5 % diphenyldimethyl polysiloxane) of 30 m X 0.25 mm X 0.25 µm and helium was used as carrier gas with a flow rate of 1.0 mL min^{-1}. For the two-dimensional analysis, the first column was the same as the one-dimensional and the second one was a more polar (DB-Wax, filled with polyethylene glycol) of 1.0 m X 0.10 mm X 0.10 µm. It was used a 2 jets modulator with modulation time of 5 s. The same oven temperature (60-250 °C min^{-1} at 3 °C min^{-1}) and ionization power of mass detector (70 eV) was used. Oil components were identified by comparison of both arithmetical index (based on an homologous series of hydrocarbons from 9 to 22 carbons analyzed in the same conditions) and mass spectra with literature and spectral library resulting in the identification, by GC/MS and GC × GC-qMS respectively, of 8 and 40 substances from LPh, 4 and 40 substances from SPh, 9 and 61 substances from LPx and finally 7 and 36 substances from BPx. The main composition of *P. xylosteoides* is represented by phenylpropanoids while the essential oil from *P. hemmendorfii* is mostly composed of sesquiterpenes. This study indicates that two-dimensional gas chromatography has a superior sensitivity and resolution, making it possible to identify a larger number of compounds. This is the first study of essential oil of *P. hemmendorffii* and the first GC × GC-qMS study reported of both species.

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Acknowledgements: Fundação Araucária, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Effect of the Essential Oil from *Philodendron bippinatifidum* on the Production of Ochratoxin A by *Aspergillus carbonarius*

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Keywords: natural products; mycotoxins; fungi.

There is a growing interest in investigating plants as a source of compounds capable of minimizing the negative effects of microorganisms that cause damage to industrial foods and human health. The *Aspergillus carbonarius* (Bainier) Thom fungus, which produces ochratoxin A (OTA), has been found in foods of plant origin.[1] The objectives of this study were to characterize the chemical composition and evaluate the effect of the essential oil from *Philodendron bippinatifidum* on the production of OTA by *A. carbonarius*. The essential oil was extracted by hydrodistillation for 2 h using a modified Clevenger apparatus. The antiochratoxigenic potential of the essential oil (31.25; 15.62 and 7.81 µL mL^{-1}) was evaluated by inhibition of the production of ochratoxin A by *A. carbonarius* at 15 °C and at 25 °C. The activity of the essential oil was compared with those obtained for the pure fungus and the fungus with DMSO. The OTA extraction with methanol was performed on the 10th day of the incubation period of the fungal spores.[2] The major compounds found in the essential oil were β-bisabolene (65.3 %), *trans*-α-bergamotene (10.0 %), α-copaene (3.3 %), γ-cadinene (2.8 %) and ar-curcumene (2.0 %). The yields of OTA obtained were 8.77 g and 2.09 µg g^{-1} (yeast); 6.63 and 1.19 µg g^{-1} (fungus + DMSO); 2.37 and 0.24 µg g^{-1} (31.25 µL mL^{-1}); 2.61 and 0.56 µg g^{-1} (15.62 µL mL^{-1}); 2.78 and 1.32 µg g^{-1} (7.81 µL mL^{-1}) at 15 °C and 25 °C, respectively. Thus, the use of the essential oil from *P. bippinatifidum* caused a reduction in the production by ochratoxin by *A. carbonarius* under the conditions employed in this study.

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Acknowledgements: FAPEMIG, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Circadian Rhythm and Seasonal Analysis of the Essential Oil of *Eugenia jambolana* Lam. from Ilha do Fundão, Rio de Janeiro, RJ

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Keywords: *Eugenia jambolana*; Myrtaceae; essential oil.

Eugenia jambolana Lam., (Myrtaceae), also known as "jamelão", "azeitona" or "jambolão" is a tree about 15 m high with edible fruits, popularly used in *Diabetes mellitus* to lower blood sugar levels. The chemical profile consists mainly of flavonoids, anthocyanins and tannins with a considerable antioxidant effect. The aim of this study is to characterize the essential oil composition of *E. jambolana* leaves and to investigate the daily and seasonal variations of the volatile fractions. Fresh leaves (250 g) have been subjected to hydrodistillation in a Clevenger-type apparatus for 2 h, monthly from February until July, 2015. The oils were analyzed by GC/FID in a QP 5000 Shimadzu and by GC/MS in a GC-QP2010 PLUS Shimadzu, with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Helium was used as a carrier gas for GC-MS, with a flow rate of 1.0 mL min^{-1}. The programmed temperatures were 270 °C at the injector and 290 °C at the detector. Oven temperature was raised from 60 °C to 240 °C at 3 °C min^{-1}. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Oil yields have been 0.1 % between February and July, 2015. The main components of the oil were (*E*)-ocimene (15.4-25.8 %), α-pinene (12.3-22.2 %), (*Z*)-ocimene (8.4-10.0 %), (*E*)-caryophyllene (6.6-8.0 %), limonene (6.7-7.6 %), α-humulene (4.1-4.4 %), β-pinene (3.2-3.7 %), α-terpineol (5.0-2.6 %) and bornyl acetate (3.0-3.3 %). The seasonal analyses showed that monoterpenes are present in higher concentration during the summer season while the sesquiterpenes are the major components during the winter time. In the warmer months, the major compounds were β-ocimene and α-pinene, while α-terpineol and (*E*)-caryophyllene are the major compounds in the winter season. Besides, it is showed a balance in the production of mono- and sesquiterpenes in the autumn season. The circadian analysis has been done in July, 2015 at 7, 9, 11, 13, 15, 17 and 19 h and it was showed that α-terpineol and (*E*)-caryophyllene, were found as major compounds during the cool morning hours. The monoterpenes content was evidenced during the hottest part of the day. From 11 h there was a significant increase of α and β-pinene, limonene and (*Z*) and (*E*)-β-ocimenes in all samples analyzed with (*E*)-β-ocimene as the major volatile component from 11-17 h.

[1]Adams, R.P; *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4a. ed.,Allured Publishing Corporation: Illinois 2007.

Acknowledgements: CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Oxygen Radical Absorbance Capacity and GC-MS Identification of the Secondary Metabolites of Essential Oils Obtained by Steam Distillation of *Turnera diffusa*

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Keywords: *Turnera diffusa*; essential oils; steam distillation.

Turnera diffusa (damiana, Turneraceae) grows in Mexico, Central America, India and in some parts of South America, with relevance for making tea, alcohol flavoring, and as diuretic, afrodisiac, astringent, among other uses.[1] Here, a chemical comparison of *T. diffussa* essential oil (EO) collected in different periods was made. The *T. diffusa* plants were collected in Zapatoca town (Santander, Colombia) on two different occasions corresponding to 2014 and 2015. Damiana EOs were obtained by steam distillation from diced vegetal material (stems and leaves) with yields of 0.29 and 0.07 %, respectively. GC/MS was performed in a split/splitless injector (split 30:1), with DB-5 and DB-WAX (60m X 0.25 mm X 0.25 µm) columns. The ORAC assay[2] was implemented in a Turner biosystems Modulus II microplate reader. Different dilutions were used for each sample. The relative amounts of the major secondary metabolites in the essential oils of two collection periods were *p*-cymene (3.4 and 1.2 %), *trans*-β-caryophyllene (3.8 and 2.2 %), 4,5-di-*epi*-aristolochene (16.1 and 10.0 %) and dehydrofukinone (11.8 and 22.0 %). ORAC values for the essential oils of *T. diffusa* (2014 and 2015 collections) were 584 ± 2 and 630 ± 25 µmol Trolox® g substance^{-1}, respectively. These were higher than commonly used antioxidants: α-tocopherol (550 ± 13 µmol Trolox® g substance^{-1}) and BHT (457 ± 9 µmol Trolox® g substance^{-1}). Previous studies[3] found that *T. diffusa* showed similar strong antioxidant activities with different methods as scavenging of DPPH, lipid oxidation inhibition and the activity of the antioxidant enzymes SOD. The results obtained suggest that damiana is potential source to obtain bioactive compounds with antioxidant properties.

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[2]Ou, B.; Hampsch, M.; Prior, R. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry* **2001**, *49*, 4619. [CrossRef] [PubMed]
[3]Soriano-Melgar, L. A. A.; Alcaraz-Meléndez, L.; Méndez-Rodríguez, L. C.; Puente, M. E.; Rivera-Cabrera, F.; Zenteno-Savín, T. Antioxidant and trace element content of damiana (*Turnera diffusa* Willd) under wild and cultivated conditions in semi-arid zones. *Industrial Crops and Products* **2012**, *37*, 321. [CrossRef]

Acknowledgements: Colciencias-Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101101 for Access to genetic resources and derivatives for scientific research with bioprospectin aims, between Ministerio del Medio ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Volatile Fraction of *Erythroxylum coca* Flower by Gas Chromatography Coupled with Mass Spectrometry

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Keyword: *Erythroxylum coca;* solid phase microextraction (SPME); GC-MS.

Erythroxylum coca, or coca, is a plant native to the Andes. There have been many studies on the composition of secondary metabolites in this plant´s leaves,[1] but no studies on the fragrance of its flowers are known. The aim of this study was to identify the volatile organic components of coca flower fragrance. *In vitro* solid phase microextraction (SPME) was used to sample the volatile fraction of freshly picked coca flowers. Extraction conditions were systematically varied in order to select the SPME fiber coating, and extraction time and temperature. A Carboxen/PDMS-coated SPME fiber (intermediate polarity) was used to sample (20 min) the headspace of coca flowers placed in a vial (20 mL) maintained at 60 °C. An Agilent Technologies (Palo Alto, CA, USA) 7890/5975C GC-MS system was used to analyze the secondary metabolites collected by the SPME fiber. Splitless injection mode was used, into a SPME liner (78.5 mm X 0.75 mm X 6.5 mm). Capillary columns (60 m) with polar (DB-Wax) or non-polar (DB-5MS) stationary phase were employed. The main components in the flowers fragrance in the morning were *trans*-β-ocimene, linalool, *cis*-β-ocimene, methyl salicylate, α-farnesene and bovolide. In the afternoon, the same components were found, with an increment in ethyl salicylate; bovolide, a compound with a strong odor reminiscent of celery,[2] was not detected in the afternoon.

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[2]Lardelli, G.; Dijkstra, G.; Harkes, P. D.; Bolding, J. A new γ-lactone found in butter. *Recueil des Travaux Chimiques des Pays-Bas* **1966**, *85***,** 43. [CrossRef]

Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Acute Pain Relief Effect of Essential Oil from the Leaves of *Zanthoxylum piperitum* (L.) DC. (Rutaceae) through Glutamatergic Pathway.

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Keywords: *Zanthoxylum piperitum*; pain relief; glutamate.

Glutamate is one of the major excitatory neurotransmitters involved in the activation of nociceptive fibres. The consequence of its action affects central and peripheral activities. The essential oil from *Zanthoxylum piperitum* (ZP) exhibits a significant effect in the first phase of the formalin test, which involves neurogenic pain. Previous reports suggested that glutamate neurotransmission also plays an important role as a pain modulator during this first phase. Therefore, the aims of this study were to evaluate the potential of ZP essential oil (ZPEO) in reducing the acute pain caused by glutamate and to verify whether this effect is central or peripheral. Fresh leaves (150 g) of ZP were collected in Dublin on May 2015 and their essential oil was obtained via hydro-distillation in a Clevenger-type apparatus for 2 h. The oil was then analyzed by gas chromatography and its components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. ZPEO was given (p. o.) to Swiss Webster mice (25-30 g) at doses of 10 µl, 30 µl or 100 µl kg^{-1} and these mice were investigated using the glutamate and hot plate test. The glutamate nociception is mediated by *N*-methyl-D-aspartate (NMDA), non-NMDA receptors, and by the release of nitric oxide. This test involved intra-plantar injections of glutamate (20 µmol) into the mouse hind paw and counting the amount of time it spent licking this paw. The hot plate test (HP) mainly involves the central anti-nociception, measured through behavioural components (such as jumping, withdrawing or licking the hind paw) on a heated plate at a constant temperature (55 °C). Measurements were taken at 30 min intervals after treatment (30, 60, 90, 120, 150 and 180 min). In the glutamate test, the 100 µl and 30 µl doses of ZPEO presented significant inhibition showing licking time reduction rates of 58 % and 22 %, respectively, when compared to the vehicle group (cooking soybean oil). No significant effect was observed for 10 µl kg^{-1}. The hot plate test showed no activity for the 3 tested doses. Chemical analysis of ZPEO confirmed the presence of 29 compounds. The major components of ZPEO are beta-phellandrene (29.4 %), (*E,E*)-farnesyl acetate (14.5 %), beta-citronellol (10.3 %), alpha-pinene (9.7 %) and beta-citronellal (6.8 %). From this investigation, ZPEO was only able to inhibit glutamate-induced licking at higher doses and the fact that none of these doses were able to increase the pain threshold on the hot plate suggests that ZPEO components affects the peripheral pain pathway.

Acknowledgements: CAPES, CNPq and FAPERJ (financial support), Instituto Vital Brazil (for animal donation) and Alam Minho (technical support).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Reliable Identification of Plant and Marine Constituents by Using Dedicated MS Databases

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Keywords: fish lipids; essential oils; MS database; LRI.

Gas chromatography combined with mass spectrometry (GC-MS) is a very powerful two-dimensional method, commonly used for the identification of unknown compounds. Peak assignment is usually performed through MS database matching. However, there are drawbacks related to such an approach. In first instance, when dealing with classes of compounds characterized by similar structures (e.g., sesquiterpenes in essential oils, fatty acids in fish oils, etc.), the fragments generated by the ionization process are very similar, thus leading to the acquisition of nearly-identical spectra for different compounds. In such cases, the occurrence of incorrect cases of peak identification is, by no means, not rare. Additionally, many commonly used GC-MS databases contain thousands of compounds, the spectra of which are often of poor quality, with no hint on the applied acquisition conditions. Such issues have enhanced the need for a novel GC-MS databases, such as the "FFNSC" (Flavour and Fragrance Natural and Synthetic Compounds) and "FAMEs" (Fatty Acid Methyl Esters), constructed with spectra derived from essential oils and pure standard compounds. Each compound in the database is accompanied by one (or more) linear retention index (LRI) value. The innovative feature of the FFNSC and FAMEs databases lies in the "LRI filter" option provided by the software (GCMS solution). In fact, the software can automatically calculate LRI values for each peak in the chromatogram, and use such values interactively, as a filter, during MS database matching. Possible matches, with a non-compatible LRI value, are automatically excluded from the "hit list", greatly increasing the reliability of peak assignment. Furthermore, many other parameters can be set in the interactive windows of the software, such as the minimum degree of similarity between the target and the database spectrum. The FFNSC and FAMEs databases contain around 3,500 and 250 spectra, respectively; each is provided with CAS registered information and LRI value(s). Applications on fish lipid FAMEs and essential oils are shown and discussed.

Acknowledgements: This work has been carried out within the framework of the Research Project "PRIN 2012": assessment of quality and safety of Mediterranean seafood by "omics" sciences, supported by the Italian Ministry of University and Scientific Research, nr. 2012TLC44W.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Production of Rhizomes, Content and Composition of Essential Oil of Accessions of Turmeric in Response to Nitrogen

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Keywords: *Curcuma longa* L.; nitrogen fertilization; biomass.

Turmeric (*Curcuma longa* L.) is a perennial species of lateral growth of the same family as ginger Zingiberaceae. Its rhizomes are widely used by diverse populations in the world, both for culinary purposes for medicated with which is mainly due to active principles and other compounds present. Nutritional imbalance can affect both the production and the composition and content of essential oil as well as its composition. The present study aimed to evaluate the effect of nitrogen on the production, content and composition of essential oil from three accessions of turmeric. The experiment introduced in a randomized block design with split plot with the main factor 3 hits of turmeric (C06, C36 and C39) and 5 N rates (15, 60, 105, 150, 240 kg ha^{-1}) with 5 replications. At 270 days the total weight of the rhizome, content and composition of essential oil of turmeric were evaluated. The essential oil was characterized by gas chromatography and mass spectrometry. Three compounds were found in larger concentrations in turmeric independent of doses of N and access type. They were: *ar*-tumerone (17.3 to 22.8 %), α-tumerone (14.3 to 16.1 %) and 1,8-cineole (11.8 to 14.8 %). Overall, essential oil yield was not affected by turmeric accesses (C06 – 0.7 %; C36 – 0.5 % and C39 – 0.8 %) or N doses (15 kg ha^{-1} - 0.54 %, 60 kg ha^{-1} - 0.59 %, 105 kg ha^{-1} - 0.88 %, 150 kg ha^{-1} - 0.62 %, and 240 kg ha^{-1} - 0.60 %), just as content and composition of essential oil, except for α-phellandrene and α-pinene, which showed linear behavior in function of N doses. The three access of turmeric have equal doses production characteristics and essential oil content, it recommended that the lowest dose of N for the production of turmeric.

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[2]Umete, M. G.; Latchanna, A.; Bidgire, U. S. Growth and yield of turmeric varieties as influenced by varying levels of nitrogen. *Arecanut & Spices Journal* **1984**, *8*, 23.
[3]Shrishail, D.; Harish, H.; Ravichandra, H.; Tulsianand, G.; Shruthi, S. D. Turmeric: nature's precious medicine. *Asian Journal of Pharmaceutical and Clinical Research* **2013**, *6,* 10. [Link]

Acknowledgements: Embrapa.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Floral Volatile Profile of *Dendrobium nobile* (Orchidaceae) in Circadian Cycle by Dynamic Headspace *In Vivo*.

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Keywords: *Dendrobium nobile*; floral volatiles; dynamic headspace.

The olfactory stimulus used by flowers are composed by a complex mixture of volatile compounds, usually emitted in a well defined ratio. In addition to the ecological interactions, the complexity of floral volatiles has been an inexhaustible source of inspiration and raw material development for the perfume industry.However, due to the fragility of the plant tissue, the study of floral volatiles require suitable techniques, without use of solvent or heating, in such a way that not cause distortion of the authentic composition of the floral scent.The aim of this study was to analyze qualitative and quantitatively the variation of the floral volatile profile of *Dendrobium nobile* (Orchidaceae) in circadian cycle by dynamic headspace *in vivo*.Floral scent was collected with adsorbent tubes filled with 3mg of Porapak Q, based on the methododogy developed by Roman Kaiser. *D. nobile* flowers were sampled in the laboratory. Twenty flowers were enclosed *in vivo* within a polyester bag (15 cm X 25 cm) and the emitted volatiles trapped in an adsorbent tube through the use of a vacuum pump, rate adjusted to 60 mL min^{-1} using a power supply and a flow meter. A second pump with positive flow was used to counterbalance the vacuum. Samples were collected for 4 h in two periods of the day (8 to 12 am and 1 to 5 pm) and at 7 consecutive days with a 50 μL dichloromethane elution of the adsorbent trap, after each sampling. *n*-Octadecane was added to the extractas internal standard (12.4 μg). A 2 μL aliquot of each extract was injected in splitless mode on to Agilent 6890N gas chromatograph fitted with a HP5MS capillary column (30 m X 0.25mm X 0.25 μm), using hydrogen as carrier gas at 1.0 mL min^{-1}. Injector was kept at 250 °C and FID at 280 °C. Oven temperature ranged from 40 °C (for 5 min) up to 240 °C at a rate of 3 °C min^{-1}. The amount of each compound was calculated by relating its peak area to that of the internal standard and corrected by the response factor (RF). For GC/MS, samples were injected into Agilent 6890N gas chromatograph coupled to a 5973N mass detector. Helium was used as carrier gas (1.0 mL min^{-1}). Compounds were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. In the flowers scent, 32 substances were identified, among which four showed great relevance to the major profile: α-pinene (65.5 to 211.3 ng), *p*-cymene (24.0 to 116.6 ng), limonene (1.1 to 88.7 ng) and (*E,E*)-α-farnesene (4.1 to 70.4 ng). On average, volatiles release was much more pronounced from 8 to 12 am (388.5ng) than in 1 to 5 pm (107.7 ng). Furthermore, the ratio of the major compounds undergoes dramatic changes. Between 8 to 12 am, the sum of α-pinene and *p*-cymene is only 2.2 times the sum of limonene and (*E, E*)-α-farnesene, while in the period between 1 to 5 pm, the sum of limonene and (*E,E*)-α-farnesene is 15.6 times less than the sum of α-pinene and *p*-cymene in the volatile composition of *D. nobile* flowers ($p<0.05$).

Acknowledgements: Embrapa, Faperj, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Chemical Composition of the Essential Oils of *Lippia lasiocalycina* and *Lippia Insignis*

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Keywords: *Lippia;* essential oil; chemical composition.

The genus *Lippia* (Verbenaceae) includes approximately 160 species of herbs, shrubs and small trees distributed mainly in Africa, Central and South America. Brazil, one of the most important centers of diversity, possesses 88 species of *Lippia* and most of them are endemic and poorly studied.[1] Thus, the main objective of this study was to analyze and compare the chemical composition of essential oils of *Lippia lasiocalycina* and *Lippia insignis* from the state of Bahia. These species were grown under organic fertilizer in Horto Florestal – UEFS, and collected in October 2014. Essential oil from air-dried leaves was extracted by hydrodistillation in a Clevenger-type apparatus for 3 hours. The chemical composition was analyzed by GC/FID and GC/MS. Oil yields were 0.83 % and 1.42 % for *L. lasiocalycina* and *L. insignis*, respectively. In the essential oil from *L. lasiocalycina*, a total of 19 compounds were identified, comprising a mixture of monoterpenes (71.2 %) and sesquiterpenes (20.5 %). The major compounds were β-myrcene (23.6 %), (*E*)-ocimenone (19.1 %) and *p*-cymene (13.3 %). The *L. insignis* oil consists almost entirely of monoterpenes (91.6 %). Twenty-five compounds were identified, being *p*-cymene (21.1 %), limonene (17.2 %), β-myrcene (10.9 %), (*E*)-ocimenone (10.8 %) and thymol (10.3 %) the major compounds. Similar predominant compounds were found in both species although quantitatively distinct.

[1]O'Leary, N.; Denham, S. S.; Salimena, F.; Múlgura, M. E. Species delimitation in *Lippia* section *Goniostachyum* (Verbenaceae) using the phylogenetic species concept. *Botanical Journal of the Linnean Society* **2012**, *170*, 197. [CrossRef]

Acknowledgements: UEFS, FAPESB, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Identification of Different Essential Oils from *Pelargonium graveolens* L. (Geraniaceae)

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Keywords: *Pelargonium graveolens*; geranium; DMAA.

Dietary supplements and weight-loss products containing 1,3-dimethylamylamine (DMAA) have recently presented an increase in consumption, despite the lack of studies proving its safety and efficacy. Present in small quantities in the oil extracted from the species of *Pelargonium graveolens* (geranium, Geraniaceae), the DMAA has been added to dietary supplements indiscriminately without conclusive scientific evidences about the safe doses for consumption and its adverse effects. Thus, we highlight the importance of the identification of DMAA in the essential oil of *Pelargonium* species, especially from Brazil. Essential oils of six countries were analyzed and compared. The analyses were performed on a 5975C gas chromatograph coupled with a 7890A mass detector (Agilent Technologies, CA, USA). A silica DB-5 column, 30 m x 0.25 mm x 0.25 mm, was employed for chromatographic separation. Oven temperature was raisedfrom 60 to 300 °C at 3°C min^{-1}. Helium (1 mL min^{-1}) was employed as the carrier gas. Mass detector was operated in electronic ionization mode at 70Ev. Source temperature, quadrupole and injector were set at 230 °C, 150 °C and 220 °C, respectively. Oil components were identified by comparison of both mass spectra and linear retention indices of *n*-alkanes with literature. In the oil from the leaves, obtained by hydrodistillation, were identified between 26 and 43 compounds of oils from Brazil, China, France, Egypt (two different samples), Albania, South Africa (two different samples).The oils present mainly oxygenated monoterpenes and their esters. The major components were citronellol, geraniol, citronellylformate, geranyl formate and geranyl acetate, with citronelol (22.5-40.5 %) and geraniol (5.8-18.7 %)as the main components at oils from China, France, South Africa, Albania and Egypt. The oil from Brazil and South Africa (different producer) presents as main components the geraniol (38.7-39.8 %) instead citronellol (10.7-11.3 %). The DMAA was not identified in oil from Brazil and other countries. Until now, studies demonstrate the presence of DMAA only in oils from China. However, we need to perform more accurate sample preparation and analysis through confirmatory techniques, to prove your absence in the oils, and so, confirm that these samples obtained from six different countries, especially from Brazil and China, do not contain DMAA in your composition.

Acknowledgements: CAPES and FAPERGS for financial support and Lazlo, Verbhena and Ferquima for donation of essential oils.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study by Solid-Phase Micro-Extraction (HS-SPME) and Gas Chromatography Coupled to Mass Spectrometry of the Volatile Fraction of *Petrea volubilis* L. (Verbenaceae) Flowers at Different Times of Day

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Keywords: Flowers, *Petrea volubilis* L., HS-SPME.

Flower secondary metabolites play important roles in plant survival, mainly related to chemical defense. *Petrea volubilis* L. is a woody shrub, belongs to the Verbenaceae family, and is native to Mexico, the Caribbean and Central America. The *P. volubilis* L. flower is lilac-blue in color.[1] Here, we identified the compounds present in the volatile fraction of *P. volubilis* L. flowers. The plant material (COL No. 569628, Colombian National Herbarium) was collected from experimental plots at the Pilot Agroindustrial Complex of CENIVAM, on the main campus of Universidad Industrial de Santander. Whole *P. volubilis* L. flowers (1.5 g) were placed in an amber vial (15 mL) and allowed to reach thermal equilibrium (10 min, 60 °C). Volatile compounds were sampled by exposing a solid-phase microextraction fiber (coated with PDMS, PDMS/DVB or CAR/PDMS) to the headspace inside the vial for 30 minutes. Sampling was performed at 6 am, 12 m and 6 pm, in triplicate. The analyses were carried out with a GC 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a mass selective detector AT 5975C (electron ionization 70 eV), with MSD ChemStation data system, G1701-DA version, including spectral libraries (ADAMS, NIST and WILEY). A polar chromatographic column (DB-WAX, J & W Scientific), with stationary phase of poly(ethylenenglycol) (60 m X 0.25 mm X 0.25 um) was used. The largest total chromatographic area was obtained with the CAR/PDMS coating. The largest volatile compound emission was registered at 6 am. The main compounds identified by GC/MS were: *trans*-β-ocimene (11.6 %), (3Z)-hexen-1-ol (2.2 %), 1-octen-3-ol (28.2 %), *cis*-3-hexenyl-2-methylbutanoate (2.1 %), linalool (34.4 %), 1-octanol (0.8 %) and geraniol (1.0 %). There are no reported studies on the volatile profile of *P. volubilis* L. flowers. Compounds found have different applications; *trans*-β-ocimene, (3Z)-hexen-1-ol, linalool, 1-octanol, geraniol and cis-3-hexenyl-2-methylbutanoate principally used in the fragance and flavor industry and 1-octen-3-ol is a chemical that attracts insects.

[1]Drewes, S. I.; Martínez, S. Morfología de las inflorescencias en *Verbenaceae verbenoideae* II: tribu petreeae. *Darwiniana* **1999**, *37*, 209. [Link]

Acknowledgements: Colciencias Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación Francisco José de Caldas Contrato RC-0572-2012. Universidad Industrial de Santander, CENIVAM & CROMMASS.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparison of the Presented Biological Activities of Essential Oils Extracted from Herbs: Parsley (*Petroselinum crispum*) and Celery (*Apium graveolens*)

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Keywords: Essential oil; *Apium graveolens; Petroselium crispum.*

Aromatic plants need more research on biologically active substances, to determine the beneficial effects in order to offer effective products which contain them. Parsley (*Petroselinum crispum*) and Celery (*Apium graveolens)* may present antioxidant and antibacterial activity, stimulating the interest of food, pharmaceutical and cosmetic industries. The microbiological experiments were performed by the standard methodology of diffusion in disk and recommended by NCCLS.[1] Tests were performed with the following bacteria: *Escherichia coli; Staphylococcus aureus* and *Bacillus cereus*. Three concentrations of oil were tested of oil: 5 %, 10 % and 20 %. DMSO was used as solvent and 10 μL were applied on a disk at a concentration of 10% (v/v). It was used one plate for each essential oil with 5 discs, one disc with nothing being applied (negative control) and a disc with tetracycline antibiotic (positive control). There was no apparent inhibition for all of the oils and DMSO when compared to the positive control (*E. coli* and *B. cereus* 18.0 mm; *S. aureus* 20.1 mm). Apparently, a 20 % solution of the celery oil at 50 °C for 25 h and the green parsley had a very low initial effect around the disk S. *aureus* (6.2 mm), but no significant effect. For the evaluation of the antioxidant activity of both essential oils, it was evaluated the capture capability of free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Four concentrations were tested for each sample: 5; 2.5; 1.25 and 0.625 mg mL^{-1}, all diluted in methanol. In 3 test tubes 0.1 mL of each dilution and 3.9 mL of 60 μM DPPH solution in methanol were added, and the volume completed to 4 mL. A test tube with a control was also prepared. The absorbance readings were made in a spectrophotometer (UV/VIS Shimadzu 1601PC) at 515 nm. The higher inhibition concentrations were presented in the following treatments: 5 μL mL^{-1} was the treatment at 50 °C for both essential oils, celery and parsley (6.23 and 1.34) respectively. On the other hand, in the concentrations of 2.5, 1.25 and 0.625 μL mL^{-1} for parsley occurred in the 45 °C treatment (2.61, 2.03 and 1.15) respectively. For the essential oil of celery in the green plant the inhibition showed 0.93 0.40 and 0.53, corresponding to the concentrations. The drying process influenced the biological characteristics of the essential oils of parsley and celery. Among the oils, the parsley one provided greater inhibitory potential index.

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Acknowledgements: CAPES, Araucaria Foundation, UTFPR.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of the Reduction of Acute Toxicity of "Priprioca" Hydrolate (C*yperus articulatus* l. Var. *Nodosus* - Cyperaceae) in Young Tambaqui Fish (C*olossoma macropomum* Cuvier) as a Function of Storage Time of the Product

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Keywords: *Cyperus articulates*; *Colossoma macropomum;* toxicity.

Priprioca (*Cyperus articulates* var. nodosus) is used by cosmetic industries and in folk medicine. Priprioca hydrolate is produced during essential oil process, and so the aim of this work was evaluate its acute toxicity as a function of storage time in young tambaqui fish *(Colossom amacropomum)*. Two acute toxicity tests (letal concentration in 96 h – $LC_{50\text{-}96h}$) were done with one year interval, using the hydrolate of the same extraction [1], as also chemical. Water/hydrolate proportions were used as: 5/95, 10/90, 15/85, 20/80, 25/75, 50/50 (%) and 6/94, 9/91, 12/88, 15/85 e 18/82 (%), in the first and second assays, respectively (negative control: water, tests in triplicate), with sample number of 15 young fish (4.48 cm ± 1.05 and 1.27 g ± 0.09/ 4.6 ± 0.5 cm e 1.4 ± 0.5 g). The animals remained in solution during 96 h and the physicochemical parameters of water (temperature, pH, dissolved oxygen and conductivity) were daily monitored. Physicochemical parameters showed acceptable limits for the species, except conductivity in the proportion of 50 % hydrolate (higher than 500 µS cm^{-2}). By probit analysis (program SPSS16) values of $LD_{50\text{-}96h}$ in the first and second bioassays were 12.1 and 14.8 %, respectively. Chemical analysis of hydrolate showed at first analysis following major components: *trans*-sabinol (22.4 %), myrtenol (16.2 %) and verbenone (28.8 %); second analysis of hydrolate one year after the extraction (hydrolate was kept in the dark under refrigeration) showed following composition: *trans*-pinocarveol (25.4 %), myrtenol (20.1 %) and verbenone (33.4 %). These data showed that during the one year period chemical alterations of hydrolate occurred. Probably, *trans*-sabinol transformed itself into *trans*-pinocarveol, its diastereoisomer ($C_{10}H_{16}O$). This molecular rearrangement occurred by hydrate migration looking for the more stable conformation for the molecule. This modification on chemical composition of the hydrolate diminished its toxicity for young tambaqui fish. More studies related to the effects of the chemical components of priprioca hydrolate in fishes or aquatic environment are necessary to study potential biological impacts .

[1]Silva, A. D. R.; Santos, R. B.; Bruno, A. M. S. S.; Soares, E. C. Cultivo de tambaqui em canais de abastecimento sob diferentes densidades de peixes. *Acta Amazônica* **2013**, *43*, 517. [CrossRef]

Acknowledgements: FAPESPA, CNPq, CAPES and Unicamp.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of the Anti-Inflammatory and Antinociceptive Activities of *Marsypianthes sp* Essential Oil

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Keywords: *Marsypianthes sp*; anti-inflammatory; antinociceptive effect.

The genus *Marsypianthes sp.* is uniquely American and has six species (*M. chamaedrys*, *M. montana*, *M. foliosa, M. arenosa*, *M. burchellii*, *M. hassleri*).[1] The aim of this work was to evaluate the possible anti-inflammatory and antinociceptive activities of *Marsypianthes sp.* essential oil (EO). Samples of the plant were collected in November/2013, in Siriema's and Cariocas' trails, at National Park of Chapada dos Veadeiros (Goiás, Brazil) and received the registry CEN 88269. EO was obtained by hydrodistillation using Clevenger apparatus. Mice (n = 6-8) were pretreated with (1, 10, 30 or 100 mg kg^{-1}, p.o.) 1 h before each experimental model and vehicle group received tween 80. Antinociceptive and anti-inflammatory effects were evaluated in formalin-induced licking of paw and subcutaneous air pouch (SAP) models.[2] Statistical analysis was performed by ANOVA followed by Bonferroni's test (*p <0.05). Results are expressed as mean ± SD. Protocols for animal use received number #DFBCICB015-04/16 (COBEA/UFRJ/Brazil). EO significantly reduced 1st and 2nd phases of formalin-induced licking: 1st phase: vehicle-treated group = 53.8 ± 9.4 sec, 1 mg kg^{-1} = 56.5 ± 11 sec, 10 mg kg^{-1} = 38.5 ± 4.7, 30 mg kg^{-1} = 26.1 ± 3.9* sec, 100 mg kg^{-1} = 28.4±2.8* sec; 2nd phase: vehicle-treated group = 209.4 ± 5 sec, 1 mg kg^{-1} = 200.3 ± 57.2 sec, 10 mg kg^{-1} = 122.9 ± 47.9* sec, 30 mg kg^{-1}= 109.4 ± 41.3* sec, 100 mg kg^{-1} = 94.3 ± 50.5* sec. Cell migration into SAP was also inhibited: PBS in SAP group = 0.9 ± 0.3 cells 10^6 mL^{-1}, carrageenan in SAP group = 91 ± 11.7 cells 10^6 mL^{-1}, 1 mg kg^{-1} = 76.9 ± 16.7 cells 10^6 mL^{-1}, 10 mg kg^{-1} = 59.2 ± 3.9*cells 10^6 mL^{-1}, 30 mg kg^{-1} = 54.9 ± 7.6* cells 10^6 mL^{-1}, 100 mg kg^{-1} = 57.2±25.2* cells 10^6 mL^{-1}. Our results suggest that EO from *Marsypianthes sp* has significant antinociceptive and anti-inflammatory effects. The mechanism of action is under investigation.

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[2]Raymundo, L. J. R. P.; Guilhon, C. C.; Alviano, D. S.; Matheus, M. E.; Antoniolli, A. R.; Cavalcanti, S. C. H.; Alves, P. B.; Alviano, C. S.; Fernandes, P. D. Characterisation of the anti-inflammatory and antinociceptive activities of the *Hyptis pectinata* (L.) Poit essential oil. *Journal of Ethnopharmacology* **2011**, *134*, 725. [CrossRef] [PubMed]

Acknowledgements: CAPES, CNPq, FAPERJ (financial support), Instituto Vital Brazil (for animal donation), Alan Minho (technical support).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antinociceptive Effect of Essential Oil from the Leaves of *Zanthoxylum piperitum* (l.) Dc. (rutaceae)

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Keywords: *Zanthoxylum piperitum*; essential oil; pain relief.

Zanthoxylum piperitum (ZP) has been traditionally used in Asia for treating the symptoms of diarrhoea and abdominal pain. Previous studies reported the antibacterial and anti-inflammatory effects of ZP, and chemical studies previously published showed that ZP essential oil (ZPEO) contains monoterpenes, such as phellandrene, as its main component although some authors have shown that ZPEO components can vary according to the season. The aim of this study was to evaluate the possible antinociceptive and/or anti-inflammatory properties of ZPEO. Fresh leaves (150 g) of ZP were collected in Dublin May 2015 and they were exposed to hydrodistillation in a Clevenger-type apparatus for 2 h. The oil was analyzed by GC/MS and linear retention indices with spectral library and literature. ZPEO was given (p. o.) to Swiss Webster mice (25-30 g) at doses of 10 μl, 30 μl or 100 μl kg^{-1}, and these mice were investigated using the formalin test, which consists of injecting formaldehyde (2.5 %) into the hind paw (plantar). The time that each animal spent licking their hind paw was recorded. The response to formalin (paw licking) involves an early phase (0-5 min after injection) and a late phase (15-30 min after injection). The early phase is known to involve the C-fibre suggesting a peripheral pain stimulus, whereas the late phase seems to engage in an inflammatory process and affects the activity in the dorsal horn of the spinal cord. Acetylsalicylic acid (ASA) and morphine were used as reference drugs. Both drugs show significant effect in both phases, however morphine has a stronger effect in the first phase whereas ASA is more potent in the second phase. All ZPEO doses tested presented significant inhibition in the first phase of the formalin test with a reduction rate of 36, 41 and 49 % of the licking time, respectively, when compared to the vehicle group (cooking soybean oil). No significant effect was observed in the second phase for ZPEO. The drugs morphine and ASA presented a 54 % and 36 % licking inhibition in the first phase and 39 % and 58 % in the second phase, respectively. Chemical analysis of ZPEO showed the presence of 29 compounds, among them 14 compounds have already been reported as having some antinociceptive effects. The major components of ZPEO are beta-phellandrene (29.4 %), (*E,E*)-farnesyl acetate (14.5 %), beta-citronellol (10.3 %), alpha-pinene (9.7 %) and beta-citronellal (6.8 %). The fact that ZPEO was only able to inhibit licking in the first phase suggests that its components have effect on peripheral pain but they were inactive against inflammatory pain. A literature survey shows that the essential oil of ZP has been previously reported to being used as an anti-inflammatory treatment, however when comparing the chemical composition of the essential oil obtained in this current study with that reported in the literature, they have distinctly different composition with few common compounds.

Acknowledgements: Faperj, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Toxicity Evaluation in *Artemia salina* of the Essential Oil of Pataqueira (*Conobea scoparioides* Cham. & Schltdl) Benth

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Keywords: *Conobea scoparioides*; essential oil; *Artemia salina*.

EOs usually have potential for pharmacological use, cosmetic and industrial properties. *Conobea scoparioides* is popularly known as "pataqueira", an aromatic plant native to Amazon rainforest which grows wild in semi-flooded areas used in aromatic baths and in the treatment of beriberi. *Artemia salina* is a microcrustacean species used as a toxicity biomarker. Here, the toxicity of essential oils obtained from whole fresh and dry material of *C. scoparioides* in *A. salina* was evaluated. Plant material was collected in March 2015, in Santarém - Pará, and 400 g of material was dried in an oven at 40° C for 48 h. Essential oil extraction was performed in a Clevenger type apparatus, for 3 h, through the standard methodology of hydrodistillation, being used 150 g of whole dried plant material and 300 g of whole fresh material. Chemical analysis was performed by gas chromatography coupled with to a mass spectrometer (GC/MS). The bioassay with *A. salina* was held as adapted from Meyer et al. (1982).[2] The concentrations used were: 1, 10, 100 and 1000 µL mL^{-1}. The bioassay was performed in triplicate. The count of the nauplii was performed after 12 hours. Comparative analysis between the treatments employed the statistical software PRISM (version 3.0), to determine the LD50 (lethal dose to 50 % of test organisms). The degree of toxicity was classified according to mortality: 0-9 % = not toxic; 10-49 % = slightly toxic; 50-89 % = toxic; 90-100 % = highly toxic. The essential oils of *C. scopariodes* presented the following major constituents for whole plant dry (PS) and fresh (PF): Methyl thymol (35 %; 28 %) and thymol (60 %; 69 %), respectively. At concentrations of 100 and 1000 µL mL^{-1}OE from dried plant mortality rate was 100%, in concentrations of 1 and 10 µL mL^{-1}, the mortality rate was 33 % and 73 %, respectively. For the fresh plant, only the concentrations of 1000 and 100 µL mL^{-1} presented 100% mortality rate, while with 10 µL mL^{-1} 50 % lethality was found, and the concentration of 1 µL mL^{-1} presented 33 % lethality. The high toxicity is directly related to variations of the content of major components thymol and methylthymol which are substances considered toxic compounds, allowing the conclusion that OE of "pataqueira" has high toxicity in both dried and fresh plant.

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Acknowledgements: Biotechnology laboratory UFOPA and Phytochemistry Laboratory UFOPA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of Antinociceptive Activity of the Essential Oil of *Stevia elatior* Kunt

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Keywords: *Stevia elatior* Kunt; antinociceptive activity.

Stevia elatior Kunth (Asteraceae) grows in Central Americ, Mexico and South America.[1] Here, we evaluated the antinociceptive activity of its essential oil (EO). Aerial parts were collected in September/2014, in San José Chacayá, Sololá, Guatemala, hydrodistillated using a Clevenger-type apparatus for 2 h. Swiss *Webster* mice (20-25 g, n=6) were orally pretreated with 10, 30 or 100 µL kg^{-1} doses and evaluated in formalin- or capsaicin-induced licking response and hot plate test. One hour after treatment mice received intraplantar injection (20 µL) of formalin (2.5 %) or capsaicin (5.2 nmol paw^{-1}). The time (s) that animal spent licking the injected paw was counted with a stopwatch during the first 5 minutes (1st phase) and between 15 and 30 min (2nd phase) or during 5 min, after formalin or capsaicin injection, respectively. In hot plate test, animals were placed on a plate set at 55 ± 1 °C. At 30 min intervals between 30 and 180 min, after EO or vehicle administration, the reaction time was recorded when the animals licked their fore- and hind-paws and jumped. Antinociception was calculated by the area under the curve (AUC) of responses between 30 and 180 min after drug administration. EO significantly reduced 1st and 2nd phases of formalin-induced licking, 1st phase: vehicle-treated group = 42.4 ± 7.7 s *versus* 23 ± 10.4* s (45.7 %); 18.1 ± 3.6* s (57.3 %) and 16.2 ± 7.1* s (61.9 %) to 10, 30 and 100 µL kg^{-1}, respectively; and 2nd phase: vehicle-treated group = 224.7 ± 25.7 s *versus* 195.5 ± 16.6 (12.1 %); 127.7 ± 31.3* s (43.2 %) and 77.6 ± 26.3* s (65.5 %) to 10, 30 and 100 µL kg^{-1}, respectively. Higher doses of EO also reduced capsaicin-induced licking: vehicle-treated group = 57.8 ± 7.8 s *versus* 41.6 ± 9.9 s (28 %); 25 ± 10.2* s (56.8 %) and 18.1 ± 6.2* c (68.7 %). EO also demonstrated significant effect at the hot plate model. Doses of EO significantly increased the AUC values when compared with vehicle-treated group. Vehicle-treated group = 986.5 ± 193.1; 10 µL kg^{-1} = 1,725.5 ± 370 (74.9 % increase); 30 µL kg^{-1} = 1,229.5 ± 152.2 (24.6 % increase); 100 µL kg^{-1} = 1,868.5 ± 339.1 (89.4% increase). Our results are the first evidence that EO from *S. elatior* Kunt produce peripheral and central antinociceptive effects.

[1]Quattrocchi, U. *CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology*. Boca Raton, FL, USA: CRC Press. 2012.

Acknowledgements: CAPES, CNPq and FAPERJ (financial support), Instituto Vital Brazil (for animal donation), Alan Minho (technical support).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical composition of the essential oil of *Croton argyrophyllus* Kunth leaves (Euphorbiaceae)

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Keywords: *Croton argyrophyllus*; essential oil; Brazilian caatinga.

Croton is the second largest genus of Euphorbiaceae family, with about 1200 species, of which 350 occur in Brazil and 68 are found in Caatinga. Several species of this genus are used in folk medicine and some of them had their anti-inflammatory, healing and antimicrobial activities proven. *Croton argyrophyllus* Kunth, popularly known as marmeleiro, is traditionally used as tranquilizer, to treat heart diseases and influenza.[3] The aim of this study was compare the yield and the chemical constituents present in the essential oil of *C. argyrophyllus* leaves submitted to two different extraction methods. Samples of plant material were collected in May 2015, in Chapada São José, Catimbau National Park, Buíque (PE) (voucher at IPA herbarium no. 93,393). For essential oil extraction, 50 g of fresh leaves were subjected to hydrodistillation in Clevenger apparatus for 2 h and, simultaneously, 200 g of fresh leaves were subjected to steam distillation in a Linax D2 extractor for 2 h. The essential oil analyses were performed on an AgilentGC/MS and GC/FID, and an Agilent J&W HP-5MS (30 m X 0.25 mm X 0.25 µm). The oven temperature was programmed at 70 °C with an increase of 4 °C min^{-1} until 280 °C was reached and then maintained for 15 min. The carrier gas was helium, with a constant flow rate of 1.4 mL min^{-1}. The temperature of the ionization source was maintained at 280°C, ionization energy at 70 eV, and ionization current at 0.7 kV. Mass spectra were recorded from 30 to 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database by using the Wiley L-Built library and by comparing their retention indices and fragmentation patterns with those of the NIST. The oil yields, obtained by hydrodistillation and steam distillation, were 0.53 % and 0.63 %, respectively. In the oil obtained by hydrodistillation 39 compounds were identified, representing 85.4 %, while in the oil obtained by steam distillation 42 compounds were found, corresponding to 74.8 %. The first oil showed up high in sesquiterpenes (73.7 %), most of which were oxygen (39.5 %), and the main compounds were elixene (15.8 %), β-caryophyllene (10.7 %), β-dihydroagarofuran (7.0 %), β-elemene (3.8 %) and hedycariol (2.9 %). The latter oil, in turn, was rich in sesquiterpenes (53.5 %) and monoterpenes (46.5 %), which main compounds were elixene (15.6 %), β-caryophyllene (9.5 %) β-dihydroagarofuran (7.5 %), (-)-spathulenol (5.3 %) and β-elemene (4.6 %).

[3]Ramos, J. M. O.; Santos, C. A.; Santana, D. G.; Santos, D. A.; Alves, P. B.; Thomazzi, S. M. Chemical constituents and potential anti-inflammatory activity of the essential oil from the leaves of Croton argyrophyllus. *Revista Brasileira de Farmacognosia*, **2013**, *23*, 644. [CrossRef]

Acknowledgements: CAPES and FACEPE.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of the Compounds Present in Essential Oil of the *Dalea cliffortiana* (Santander, Colombia)

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Keywords: *Dalea cliffortiana;* essential oil; MWHD.

Dalea cliffortiana is a plant belonging to the Dalea family, is an herb ranging from 0.1 m to 0.7 m, with angled, erect, simple, branched stems; leaves 1.5 cm to 4.7 cm long; conical cylindrical pins: White or pinkish petals, commonly from 3 to 5 mm long.[1,2] Native from Mexico, however, it can be found in Nicaragua, Panama, Ecuador and Colombia. The main goal of this work is to study the chemical composition of *D. cliffortiana* essential oil using gas chromatography coupled with mass spectrometry; the specimen was collected in the municipality of Jordan (Santander, Colombia). Botanic sample was identified at Universidad Nacional de Colombia Herbarium, voucher number 579 427. Essential oil was obtained from fresh vegetal material by microwave-assisted hydrodistillation (MWHD), with 45 min extraction time with three lots. Volatile compounds identification was performed using a GC 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatography, coupled to a 5975C mass spectrometer (EI, 70 eV). Compounds separation was done with two capillary columns, one with non-polar stationary phase [5% phenyl-poly(methylsiloxane) DB-5MS (J&W, Scientific, Folsom, CA, USA) 60 m X 0.25 mm X 0.25 µm] and one with polar stationary phase [DB-WAX (J&W, Scientific, Folsom, CA, USA) de 60 m X 0.25 mm X 0.25 µm]. Injection volume was 2 µL, with Split injection 30:1 (250 °C). Oven temperature went from 4 °C to 150 °C at 4 °C min^{-1}, and then to 250 °C at 5 °C min^{-1}, and finally to 275 °C at 10 °C min^{-1}. Tentative identification of volatile compounds was made by comparing their mass spectra and linear retention indices for each column, with data-bases (ADAMS, Wiley, NIST). Major identified components were: methyl-eugenol (69 %; it presents insecticidal, acaricidal, antimicrobial and repellent against *Aedesaegypti* L.), *trans*-β-caryophyllene (15 %; it presents antileishmanial effect, antioxidant and antibacterial activity), germacrene D (5 %), caryophyllene oxide (3 %) and α-humulene (1 %).

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Acknowledgment: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contrato RC-0572-2012. Collectinglicense: contrato de acceso a recursos genéticos y productos derivados para investigación científica con fines de prospección biológica No. 101 entre el Ministerio del Medio Ambiente y Desarrollo Sostenible y la Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development and Yield of Essential Oil of Basil (*Ocimum basilicum* L.) under Successive Cuts and Application of Growth Regulators

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Keywords: *Ocimum basilicum* L.; auxin; cytokinin; gibberellin; biostimulant.

The cultivation of medicinal plants, aromatic and culinary grows every year. The state of Paraná has featured in the cultivation of these plants, accounting for 90 % of Brazil.[1] Lamiaceae family species have great economic importance for producing essential oil in their glandular trichomes. The use of active substances which enhance the growth and yield of vegetable species has great economic interest. The objective of this study was to evaluate the development and the essential oil yield of basil plants when they are subjected to successive cuts using different biostimulants and plant growth regulators applied in isolation and combination. The experiment was conducted under field conditions, Londrina, PR, Brazil. Seedlings were obtained from seeds. The experimental design was a randomized complete block in a factorial 2 x 2 x 2 + 3 (IBA (IBA) 10 mg L^{-1}; gibberellic acid (GA 3) 10 mg L^{-1}; kinetin (KIN) 18 mg L^{-1}; AIB 10 mg L^{-1} + GA3 10 mg L^{-1}; AIB 10 mg L^{-1} + KIN 18 mg L^{-1}; GA3 10 mg L^{-1} + KIN 18 mg L^{-1}; AIB 10 mg L^{-1} + GA3 10 mg L^{-1} + KIN 18 mg L^{-1}; control (distilled water); Biostimulant; witness with foliar application of macro and micronutrients (TAF) 1 %; witness with ammonium sulfate topdressing (TAC) (250 kg ha^{-1}) and five replications. Three cuts were made during the development of the experiment at 40, 80 and 120 days after transplanting. Each repetition was evaluated for plant height, leaf area, dry mass of leaves and yield essential oil. The collected data were submitted to variance analysis and the comparison of means was performed by Tukey test at 5 % probability. All variables of basil plants exhibited maximum peaks in the 1st cut. The average yield of essential oil was 2,72 L ha^{-1} (1^{st} cut), 2,29 L ha^{-1} (2^{nd} cut) and 1,58 L ha^{-1} (3^{rd} cut). The application of treatments was not able to keep the development and yield of essential oil from plants on the 3^{rd} cut.

[1]Corrêa Júnior, C.; Scheffer, M. C. As plantas medicinais, aromáticas e condimentares e a agricultura familiar. *Horticultura Brasileira* **2014**, *32*, 376. [CrossRef]

Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Biomass and Essential Oil of Lavender under Protected Cultivation and Organic Fertilization

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Keywords: Lavender; organic fertilizers; essential oil; harvesting.

Lavandula dentata (Lamiaceae) is a species cultivated in the fields of Europe, North America, Central and South America, to industrial scale production of essential oil with high quality.[1] Among its main features, lavender is resistant to drought and has high rusticity, requiring good soil drainage and average rainfall.[2] For lavender cultivation and another aromatics plants, are needed more explications about advantages and disadvantages to using organic or mineral fertilizers, aiming more security in production and quality of their chemical compounds. The objective of this study was to assess the effects of greenhouse and organic fertilization in lavender biomass and essential oil. The experimental design was a randomized complete block design with four replications in split-plot, and those relating to fertilizers plots and subplots concerning flower crops. The treatments consisted of different doses of this fertilizer formulated in NPK (10-10-10) with 8 % organic carbon from the recommendation of 500 kg ha^{-1}, 100 % (T1), 80 % (T2) 60 % (T3), 40 % (T4) and 20 % (T5), and the recommended mineral fertilization of 500 kg ha^{-1} formulated in NPK (10-10-10) (T6). Three harvests of flowers were performed at 100, 145 and 180 days after planting. Was evaluated fresh flowers, content, yield and chemical composition of essential oil. The flowers were harvested early in the morning, to 30 cm of soil and taken to the laboratory. The essential oil extractions were made by hydrodistillation with modified Clevenger apparatus. The chemical composition was performed by gas chromatography coupled to mass spectrometry. The results showed that mass flowers was no significant difference between fertilizers. Yields of biomass were obtained close to 4 t ha^{-1}. It was observed a significant increase in mass of flowers between the first and second crop for all fertilizers, as well as a decrease of production in the end of the harvest. Successive crops also resulted in gains in essential oil yield of up to 20 kg ha^{-1}. Average contents of essential oil of 0.60 % were obtained. The dose of 60 % of organic fertilization showed the best performance in biomass and essential oil yield, at the three crops of flowers. There were found more than 20 substances in the essential oil of *L. dentata* and the majority were 1,8-cineol, fenchone and camphor. The two types of fertilizer provided increments the production of 1,8-cineole. The chemical composition of essential oil of lavender is affected by the conditions of protected cultivation and the seasons of harvest.

[1]Verma, R. Essential oil composition of *Lavandula angustifolia* Mill. cultivated in the mid hills of Uttarakhand, India. *Journal of the Serbian Chemical Society* **2010**, *75*, 343. [CrossRef]
[2]McNaughton, V. *Lavender: the grower's guide*. Portland (USA): Timber Press, 2006. 192 p. [Link]

Acknowledgements: Fapemig, CNPq, CAPES, Geociclo.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Repellency and Irritability of Essential Oil Thymol Chemotype of *Lippia gracilis* and its Major Compound on *Cryptolestes ferrugineus*

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Keywords: Cucujidae; botanical insecticides; monoterpenes.

The beetle *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) is a secondary pest of greatest importance among the insects that attack stored products. Substances from botanical origin have been identified as viable alternatives to pest management because they have advantages such as fast action, degradation and selectivity to non-target organisms. *Lippia gracilis* Schauer is a shrub native of northeastern Brazil and has rich scent leaves essential oil. The essential oil of this plant is composed of monoterpenes and sesquiterpenes, which has medicinal properties. This study aimed to evaluate the behavioral effects of essential oil from *L. gracilis* genotype LGRA106 and its major constituent thymol against *C. ferrugineus*. Behavioral bioassays, repellency and irritability, using the essential oil and its major compound thymol were performed in 1% solution following the adapted methodology described by Cordeiro *et al.*[1] The essential oil was obtained from leaves dried in an oven at 40 °C for 5 days, by using a Clevenger apparatus. Thymol, was purchased from Sigma-Aldrich. The identification of compounds was performed by GC/MS/FID, and 20 constituents were identified. Thymol (43.8 %) was the major component of this genotype, characterizing it as thymol chemotype, followed by carvacrol (15.7 %), methyl thymol (8.1 %), γ-terpinene (6.9 %), β-caryophyllene (6.5 %) and *p*-cymene (6.4 %). All treatments caused repellency and irritability to *C. ferrugineus*. Generally, insects passed more than 85 % of the total time (10 minutes) in the untreated side. The essential oil was the most repellent treatment (76 %) and increased irritability rate (93.75 %). Thus, these results demonstrate the potential of the essential oil from *L. gracilis* and its major compound thymol for the development of new products to control stored product pests.

[1]Cordeiro, E. M. G.; Corrêa, A. S.; Venzon, M.; Guedes, R. N. C.Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. *Chemosphere* **2010**, *81*, 1352. [CrossRef] [PubMed]

Acknowledgements: UFS, CNPq, CAPES, FAPITEC/SE and FINEP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Analysis and Lethal and sub-Lethal Effect of the Essential Oil of Aristoloquia trilobata on Cutting Ants.

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Keywords: Aristolochiaceae; fumigation; repellency.

Leaf-cutting ants of *Atta* and *Acromyrmex* genus (Hymenoptera: Formicidae) are common insects in the Neotropics region and an important pests in agricultural environments. These organisms are often controlled using organosynthetic insecticides. However, mostly products are inefficient. This problem has generated a growing demand for environmentally safe products to control these pests. Thus, this work aimed to analyze the chemical composition of the essential oil of *Aristolochia trilobata* and test lethal and sub-lethal effect on ants *Atta sexdens* and *Acromymex* balzani. Acute toxicity and behavioral bioassays were performed, being these effects evaluated in repellency (evasion without contact with the essential oil) and irritability (evasion after contact) in 1% solution. The essential oil of *A. trilobata* was obtained by hydrodistillation of crushed dried stems and the identification of compounds was performed by GC/MS/FID. We identified 25 compounds in the essential oil of *A. trilobata*, and mostly were monoterpenes. The major chemical constituents were sulcatyl acetate (25.6 %), limonene (24.8 %), *p*-cymene (10.4 %) and linalool (9.5 %). The essential oil of *A. trilobata* was highly toxic by fumigation for *A. balzani* and *A. sexdens*. The lethal concentration to kill 50 % of population of *A. balzani* and *A. sexdens* was 3.76 and 5.48 $\mu L\ mL^{-1}$ respectively. The essential oil of *A. trilobata* was repellent and caused great irritability to both species. The insects passed over 96 % of the total time in the untreated side after contact. Thus, these results show the great potential of the essential oil from *A. trilobata* for the development of new insecticides.

Acknowledgements: CNPq, CAPES, FAPITEC/SE, FINEP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Antioxidant Activity of *Tagetes caracasana* Essential Oil

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Keywords: *Tagetes caracasana;* essential oil; antioxidant activity.

Plants of the genus *Tagetes* (Asteraceae family), commonly known as marigolds are grown as ornamental plants and can be grown in a variety of climates. The *Tagetes* genus comprises about 30 species, distributed in South America, Central America and Mexico. Bioactive extracts from different parts of the plants belonging to this genus exhibit nematicide, fungicide and insecticide activity.[1]Two specimens of *T. caracasana* were collected in the municipalities of Aratoca and Zapatoca, Santander, Colombia. These were identified in the National Herbarium of Colombia, with Voucher N° 560984 (T1, Aratoca) and 579244 (T2, Zapatoca). The essential oil (EO) extraction was performed by microwave radiation-assisted hydrodistillation. Characterization of essential oils was performed with GC/MS (Agilent). DB-WAX and DB-5MS (60 m X 0.25 mm X 0.25 µm) columns were used. Oven temperature was programmed from 45 °C to 150 °C at 4 °C min^{-1} (7 min), 150 °C to 230 °C at 4 °C min^{-1} (40 min). The oxygen radical absorption capacity (ORAC) assay was performed in a multiplate reader (Turner Biosystems). Essential oil extraction yields of 0.1 % (T1) and 1.49 % (T2) were obtained. The chemical characterization was based on mass spectra and LRI. The major components for the Aratoca collected species were: limonene (14 %), diosphenol (13.5 %), terpinolene (7.1 %), piperitone (5.7 %), *p*-cimen-8-ol (5.2 %) and a not identified sesquiterpenoid ($C_{10}H_{14}O_2$, 9.3 %). The major components for the Zapatoca collected species were, *trans*-ocimenone (16.9 %), dihydrotagetone (7.4 %), *cis*-tagetone (7.2 %), and *cis*-ocimenone (7.1 %). Armas*et al*. studied the chemical composition of plants that belong to this genus, the main components were: *T. caracasana*, *trans*-ocimene (64 %) and *cis*-tagetone (14 %); *T. erecta*, piperitenone (36 %) and terpinolene (22 %); *T. subulata*, terpinolene (26 %), piperitenone (13 %), limonene (11 %).[2] The ORAC antioxidant activity values of the studied EO were 1930 ± 15 µmol Trolox® g^{-1} sample (T1) and 1120 ± 67 µmol Trolox® g^{-1} sample (T2), which were superior to those of the reference substances, α-tocopherol (550 ± 13 µmol Trolox® g^{-1} sample) and BHT (457 ± 9 µmol Trolox® g^{-1} sample).

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101 for access to genetic resources and derivatives for scientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of Essential Oils Isolated by Steam Distillation of *Croton ferrugineus*, *Hyptis pectinata* and *Calea sessiliflora*

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Keywords: *C. ferrugineus; H. pectinata; C. sessiliflora*.

The species *Croton ferrugineus* (Euphorbiaceae), *Hypti spectinata* (Lamiaceae) and *Calea sessiliflora* (Asteraceae) are distributed through the andean and pacific regions of Colombia. *C. sessiliflora*is endemic of the American continent, *C. ferrugineus*is a native plant that grows in Cauca, Cundinamarca, Huila, Nariño, Quindío, Tolima and Valle del Cauca departments, between 500-2000 meters above sea level. *H. pectinata*is a cosmopolitan plant.[1] In this work, *C. ferrugineus, H. pectinata* and *C. sessiliflora* (COL 582569, 582529, 582603) essential oils (EO) were obtained from dry plant material using steam distillation and were analyzed by gas chromatography-mass spectrometry (*Agilent Technologies* 6890 Plus GC witheither5973 or 5975 MSD). DB-WAX (60 m X 0.25 mm X 0.25 µm) and DB-5MS (60 m X 0.25 mm X 0.25 µm) capillary columns were employed. Injection volume was 2 µL in *split* (1:30) injection mode. Compound identification was carried out by comparison of their mass spectra and linear retention indexes with those from databases (Adams, Wiley, and NIST) and scientific literature. *C. ferrugineus*, *H. pectinata* and *C. sessiliflora* EO yields were 0.01, 0.01 and 0.06 % (w/w), respectively. The major compounds identified in *C. ferrugineus* oil were *trans*-β-caryophyllene (36.8 %), dill apiole (22.8 %), germacrene D (13.2 %), *cis*-chrysantenyl acetate (7.3 %), α-humulene (3.2 %), and bicyclogermacrene (2.6 %). The main compounds found in *H. pectinata* oil were *trans*-β-caryophyllene (37.5 %), germacrene D (27.5 %), bicyclogermacrene (11.1 %), β-cubebene (3.2 %), α-copaene (3.0 %), and caryophyllene oxide (2.6 %); EO was rich in sesquiterpenes, according to other authors.[3,4] The main compounds of *C. sessiliflora* oil were identified asα-zingiberene (34.5 %), germacrene D (16.9 %), *ar*-curcumene (12.6 %), *trans*-β-caryophyllene (7.1 %), and β-sesquiphellandrene (3.5 %). No other research about the chemical composition of *C. ferrugineus* and *C. sessiliflora* essential oils has been reported.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101 for access to genetic resources and derivatives for scientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of Antifungal Capacity of Essential Oils from *Baccharis dracunlifolia* and *Lippia sidoides* on *Aspergillus flavus* Growth and Production of Aflatoxin B1 and B2

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Keywords: *Lippia sidoides; Aspergillus flavus;* aflatoxin.

The objective of this research was to evaluate the antifungal capacity of essential oils (EO) from leaves of *Baccharis dracunculifolia*, *Lippia sidoides* and their blend (50 %). The growth of *Aspergillus flavus* (CCT 7638) fungus and its production of aflatoxin B1 and B2 were evaluated when different concentrations of EOs or their blend were present. The *in vitro* evaluation was carried out on microplates with visual determination of Minimal Inhibitory Concentration (MIC), and also by measurement of fungal radial growth.[1] The concentrations checked were 31.25, 62.5, 125.0, 250.0 and 500.0 ppm. The aflatoxin B1 and B2 production was evaluated by HPLC in the treatment with best efficiency and *in vivo* trial evaluated only with the best oil for *in vitro* evaluation (maize with and without sterilization- both with *A. flavus* inoculums- as substrate for fungal). EOs were evaluated in the *in vivo* trial, 500 and 800 ppm (control: one was sprayed acetone (EO solvent for spraying), and the other sprayed nothing). The treated maize grains (50 g) were poured in 12 beckers of 100 mL and, they were put into a plastic box at 89 % of relative humidity. The plastic boxes were stored at 28 ± 2 ^{o}C without light for 15 days. The boxes were opened and 6 beckers used for mould count and other 6 for aflatoxin measurement. In the *in vitro* tria, *L. sidoides* oil showed the best results for both methodologies with MIC of 200 and 500 ppm. For *B. dracunculifolia* and the oil blend no MIC was observed. Aflatoxin production follows an indirect dose dependent relationship with EOs concentrations. In the *in vivo* trial was observed a decrease of mould count for both concentrations when was used sterilized maize but, when not sterilized maize was used, only the highest dose (800 ppm) was able to decrease it. The aflatoxin B1 production was decreased as the oil dose was increased when maize sterilized was checked but, aflatoxin B2 production was higher in 800 ppm than in 500 ppm dose. When not sterilized maize was used none dose was able to decrease aflatoxins production and they may has stimulated it.

[1]Alvarez-Castellanos, P. P.; Bishop, C. D.; Pascula-Villalobos, M. J. Antifungal activity of the essential oil of flower heads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* **2001**, *57*, 99. [CrossRef] [PubMed]

Acknowledgements: FAPESP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Evaluation of Stability and Antimicrobial Activity of a Topical Formulation Containing Essential Oil of *Lippia alba* (Mill.) NE Brown and *Lippia origanoides* Kunth (Verbenaceae)

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Keywords: essential oil formulation; *Lippia alba; Lippia origanoides;* antibacterial activity.

Lippia alba and *L. origanoides* are aromatic plants (Verbenaceae) with high essential oil content (OE). Their essential oils showed pharmacological properties as antiprotozoal, antifungal, antibacterial, among others.[1,2] Considering the biological potential OE of these species the aim of this study was to develop formulations containing essential oil of *L. alba* and *L. origanoides*, evaluate stability and antibacterial activity against a strain of *Staphylococcus aureus*, a gram-positive bacterium, known clinically as cause skin infections and provide resistance against the treatment with commercial antibiotics. Samples of *Lippia* were collected in the growing Productive Arrangement Medicinal Plants and Herbal located in Santarém city, Pará (S 02º26'35.7 "and 054º54'54.2 W"). The OE of species was obtained from aerial parts by hydrodistillation in Clevenger type apparatus for 2 h. The formulations were prepared with the incorporation of an OE base LANETTE® anionic type, in concentrations of 10 and 20 g of OE per 100 g of base. It is obtained two formulations containing the OE of *L. alba* and one with the OE of *L. origanoides*. The formulations were submitted to stability test and evaluation of the antibacterial activity by disc diffusion method on agar front S. aureus ATCC 25923. The OE yields were 3.5 % and 2.3 % for *L. alba* and *L. origanoides* respectively. In stability tests in different temperatures (5 °C, 25 °C and 40 °C) the formulation containing *L. alba* OE presented pH 7.6, viscosity 33,250 MPas at 10 rpm, torque 27.1 % and 65 Spindle. For the formulation of *L. origanoides* the pH was 5.67 and viscosity 24,200 MPas at 10 rpm, Torque 20.2 % and Spindle 65. In centrifuge test no change was observed in homogeneity of both formulations. In bacterial activity in the inhibition zones were 45 mm for both formulations at a concentration of 20 % OE, 15 mm for the concentrations of 10 % *L. origanoides* and 11 mm for the same concentration of *L. alba*. The results demonstrate that the formulations are stable under the temperatures and evaluated have considerable antimicrobial activity with *Staphylococcus aureus* halos of inhibition greater than 10 mm.

[1] Borges, A. R.; Aires, J. R. A.; Higino, T. M. M.; de Medeiros, M. G. F.; Citó, A. M. G. F.; Lopes, J. A. D.; de Figueiredo, R. C. B. Q. Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil. *Experimental Parasitology* **2012,** *132*, 123. [CrossRef] [PubMed]
[2] Gomes, S. V.F.; Nogueira, P. C. L.; Moraes, V. R. S.Aspectos químicos e biológicos do gênero Lippia enfatizando Lippia gracilis Schauer. *Eclética Química* **2011**, *36*, 64. [CrossRef]

Acknowledgements: CNPq and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antimicrobial Activity of Essential Oils from *Piper hispidum* and *Piper hispidinervum*

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Keywords: Essential oil; *Piper hispidum; Piper hispidinervum.*

The genus *Piper* comprises around 700 species, among 130-400 species are part of the rainforest flora of the Amazon region. The essential oil from these plants presents various biological activities such as larvicide, antimicrobial, among others.[1] The objective of this work was to evaluate the antimicrobial activities of two plants from the genus *Piper*: *Piper hispidum* and *Piper hispidinervum*. The essential oils were obtained by hydrodistillation from leaf samples from both species. The *essential oils* were*extracted*using a *Clevenger*modified apparatus for three hours. After extraction, the oils were evaluated for antifungal activity with the fungus *Ceratocystis paradoxa*. Forantibacterial *activity*, it was used the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebisiella pneumoniae* and *Hafniaalvei*. For the antifungal activity, it was used disk diffusion technique. For theantibacterial *activity,* three techniques were used: disk diffusion, essential oil incorporation into the agar medium and broth dilution. The fungus *Ceratocystis paradoxa* showed resistance to both essential oils, preventing the continuation of the antifungal study. The bactericidal assay demonstrated that only *P. hispidum* oil presented activity, revealing that its chemical composition has antimicrobial activity against all bacteria used in the study. The minimum inhibitory concentrations (MIC) obtained were: 33 mg mL^{-1} for *Escherichia coli*, 28 mg mL^{-1} for *Pseudomonas aeruginosa*, 7 mg mL^{-1} for *Hafniaalvei* and 32 mg mL^{-1} for *Klebisiella pneumoniae.* It is necessary to continue the experiments to isolate from the essential oils the compounds responsible for the activity.

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Acknowledgements: EMBRAPA, FAPEAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antifungal Activity of *Baccharis dracunculifolia* and *Baccharis psiadioides* Essential Oils against Tomato Phytopathogenic Fungi

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Keywords: Tomato diseases; natural product; phytopathogens; leaf spot.

Essential oils are among natural products and provide an alternative, effective and safe products against phytopathogens. They have presented antimicrobial action proved, enabling their use in phytopathogen control, and low environmental impact when compared to conventional pesticides. Essential oils of *Baccharis dracunculifolia* and *B. psiadioides* were tested against the phytopathogens *Alternaria solani*, *Septoria lycopersici* and *Stemphylium solani* which causes early blight, septoria leaf spot and grey leaf spot on tomatoes, respectively, and are responsible for great economic losses regarding production. Leaves of *B. dracunculifolia* and *B. psiadioides* were obtained from plants located at the cities Caxias do Sul and Porto Alegre, RS. The essential oils were extracted by hydrodistillation method from the dried plant leaves for 1h in a Clevenger-type apparatus and analysed by GC/MS for chemical identification. The antifungal action of essential oils was tested *in vitro* using potato dextrose agar medium with essential oil concentrations ranging from 0.1 to 20.0 µL mL^{-1}. Oils extracted from dried leaves of *B. dracunculifolia* and *B. psiadioides* yielded 0.19 % and 0.89 % (mL 100 g^{-1} of dried leaves), respectively. The major constituents of *B. dracunculifolia* essential oil was β-pinene (26.6 %) and limonene (11.0 %). Also β-pinene (22.5 %) was identified in *B. psiadioides* oil, besides ar-curcumene (6.7 %) and spathulenol (6.1 %). Both essential oils were efficient in inhibiting the fungi tested and vary with their concentration and target species. *B. dracunculifolia* essential oil proved to be the most effective inhibitor for all fungi tested, showing inhibition between 88.85 % and 100 % at the 20.0 µL mL^{-1} concentration and essential oil of *B. psiadioides* demonstrated inhibition percentages between 46.33 % and 97.10 % for the same concentration. In conclusion, the essential oils tested in this study presented fungicidal action against *A. solani*, *S. lycopersici* and *S. solani*.

Acknowledgements: CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil from Priprioca (*Cyperus articulatus* var. *nodosos*): Chemical Characterization and Phytocosmetic Development

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Keywords: Essential oil; priprioca; liquid crystals;phytocosmetic.

Priprioca (*Cyperus articulatus* var. *nodosus*) is a Cyperaceae with immeasurable social and economic value in Amazon, since essential oils can provide an excellent odor, olfactory and fixative, used for decades by regional perfumery companies as an important ingredient in the composition of perfumes and cosmetics. Beyond its pharmacological properties, pripioca essential oil can be used to obtain some dashing liquid crystallinesystems, applied to the development of cosmetics with physicochemical properties suitable for topical use. It is therefore, pertinent to use this product as a sustainable source, in order to improve scientific advancement, technological and sustainable socio-economic development for the region. As a result, this study aimed to develop and evaluate liquid crystalline systems containing essential oil of priprioca (*Cyperus articulatus*). *C. articulatus* rhizomes were collected in Tabocal region, Santarém (-54 ° 43'00,10 "W and -02 ° 37'41,10" S), and subjected to extraction by steam distillation at Beraca industry. For chemical analysis, 1 µL of the essential oil was analyzed by gas chromatography coupled with mass spectrometry (GC-MS Agilent 6890). The chemical components of the oil were identified by comparing the calculated retention indices with those from the literature. To obtain the samples, 7 liquid crystalline systems were prepared having as component murumuru butter, Procetyl and distilled water with a low concentration, medium and high amount of priprioca essential oil. After 24 h, the presence of liquid crystalline phases was investigated by polarized light microscopy (Linkam THMSG600). The essential oil chemical composition presented the following major components: Cadalene (10.6 %), β-selinene (8.4 %), ciclocolorenone (7.0 %), α-copaene (6.6 %) and α-pinene (4.8 %). These results were similar to components described in the literature,[1] which denotes the identity of plant material as *C. articulatus*, fundamental stage for quality control to develop phytocosmetics. When analyzed by polarizing light microscopy, the structure of both formulations with different proportions of pripioca oil / murumuru butter / surfactant / water showed bi-refringency with geometric and streaky striated texture. Thus, we concluded that pripioca oil, coming from the Amazon region, can be used as new raw materials to develop of liquid crystalline systems in cosmetics.

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Acknowledgements: CAPES, CNPQ, UFOPA, UNICAMP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Profile of Flower Hop Essential Oils and its Chemometric Classifications

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Keywords: Volatile; terpenes; gas chromatography.

The Hops (*Humulus lupulus* L.) belongs to the botanical family Cannabaceae and plays an important role in the brewing industry history, giving the product durability, characteristic odor and bitterness. It can be said that these features occur due to high levels of essential oils and resins existing in its flowers.[1] Despite its importance, essential oils belonging to this plant are poorly studied. The plant material was obtained commercially in Pato Branco - PR and after dried and ground, the essential oil has been obtained through hydrodistillation using a Clevenger apparatus. The extraction was done in triplicate using 30 g for each sample. The extraction time was 4 and the essential oil was collected using diethyl ether, and dried with anhydrous sodium sulfate. Each essential oil sample was stored in vials and kept refrigerated until analysis, which was done using a GC/MS (Varian). One µL of each sample was injected at 250 °C, with a column flow of 1.2 mL min^{-1}. The column temperature ranged from 50 to 240 °C. The initial temperature during first minute was of 50 °C, the slope used corresponded to a 3 °C min^{-1} over the following 3 minutes, and 3.5 °C for the remainder of the analysis. The final temperature was maintained unchanged for 14.5 minutes resulting in a total analysis time of 70 min. The identification of hop essential oil constituents was based on retention indexes, obtained by co-injection of a mixture of *n*-alkanes standards, and by comparison of their mass spectra. The results were classified by the principal coordinates analysis (PCoA), for this purpose the software PAST was used.[2] Specific compounds were selected from the resulting chromatograms and an average of 63.1 % were identified among all three assays. It was observed that among the 45 compounds identified in the three hop flower samples, 12 compounds were present in all of them, namely: *p*-methyl-acetophenone (0.7 %), verbenone (0.4 %), carvacrol methyl ether (0.4 %), perilla aldehyde (0.3 %), 2,3,4-trimethyl-benzaldehyde (0.9 %), α-humulene (2.2 %), cadina-1-(6)-*trans*-4-diene (5.9 %), β-selinene (10.5 %), α-selinene (6.1 %), δ-amorphene (5.1 %), *trans*-calamenene (3.3 %) and selina-3,11-dien-6-α-ol (4.8 %). The main compounds among the samples were *epi*-α-muurolol (29.9 %), humulene epoxide II (28.6 %), β-selinene (10,5 %), 1-epi-cubenol (9.0 %) and *allo*-aromadendrene epoxide (7.7 %). The principal coordinates analysis showed lack of uniformity among the assays.

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Acknowledgements: Central de Análises – UTFPR Pato Branco.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Agronomical Assays, Chemical Composition and Brine Shrimp Toxicity Assay of Essential Oil of *Pogostemon cablin* Benth

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Keywords: *Pogostemon cablin;* essential oil; brine shrimp assay.

Pogostemon cablin Benth. (Lamiaceae) popularly known as patchouli is worldwide cultivated for extraction of essential oil which is rich in mono and sesquiterpenes and has medicinal properties such as antiemetic and antifungal. In Amazon patchouli is known as "oriza" and used in the perfume industry. The aim of this work was evaluate the yield, the chromatographic profile of the essential oil and verify their toxicity by brine shrimp assay (*Artemia salina*). Sprouts of 20 cm from Belém-PA were cultivated in Santarém-PA and submitted to three different agronomical treatments using as substrates: “terra preta”- biochar-rich soil found in the Amazon region, “terra preta” and poultry litter and “terra preta”, carbonized rice and sawdust.[1,2] After 100 days of cultivation the leaves of the plants were collected and dried, followed by hydrodistillation using Clevenger-type apparatus during 3 h. Essential oils were submitted to chromatographic analysis by GC/MS (Agilent), column HP-5MS (30 m X 0.25 mm X 0.25 µm), injector 220 °C, detector 250 °C, column 60 °C, 3 °C min^{-1}, 240 °C (20 min). Compounds were identified by comparison with NIST-05 library, LRI and comparing with literature. *A. salina* eggs were incubated in artificial saline solution during 24 h under illumination until the hatching. Ten nauplii were transferred to tubes containing saline solution and samples in the following concentrations: 1, 10, 100 and 1000 ppm. The assay was done in triplicate and the mortality rate of the nauplii was performed before 24 h. The yield of essential oils obtained from T1, T2 and T3 were respectively 1.8, 1.0 and 1.0 %. Major compounds of the essential oils were, respectively, patchoulol 66.5 %, 73.4 % and 71.7 %, α-guaiene 3.1, 6.1 and 4.1 %, α-bulnesene 3.6, 7.6 and 4.9 %, seychelene 1.7, 1.5 and 2.1 %, α-patchoulene 1.4, 2.1 and 2.0 %, β-patchoulene 1.3, 0.6 and 0.5 %. The essential oils showed 100 % mortality at the concentrations of 100 and 1000 ppm, showing the acute toxicity in nauplii of *A. salina.*

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Acknowledgements: PIBITI- CNPq, CPQBA-UNICAMP.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Phytochemical Evaluation of Essential Oil Pataqueira (*Conobea scoparioides* Cham. & Schltdl) from Santarém, Pará, Brazil

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Keywords: *Conobea scoparioides;* pataqueira; essential oil.

Conobea scoparioides Cham. & Schltdl (Scrophulariaceae), known as "pataqueira" is an herbaceous aromatic plant occurring in the bed of small streams and wetland of the Amazon region. It is used locally as a fragrance in baths, and to treat "beriberi".[1] Here, the essential oils (EO) of *C. scoparioides* from dry and fresh plant material were studied. Plant material was collected in Santarém – PA and separated in two samples. One was dried (48 h at 50 °C in heating oven) and the other used fresh. The samples hydrodistilled in a Clevenger type apparatus for 3 . EOs were analyzed in CPQBA – Unicamp by GC/MS (Agilent) with a HP-5MS (30 m X 0.25 mm X 0.25 μm) capillary column, with temperatures: injector 220 °C, detector 250 °C, column at 60 °C, 3 °C min^{-1} to 240 °C (20 min). Helium was the carrier gas (1.0 mL min^{-1}). Compounds were identified by comparison with the NIST-05 library and the calculated retention indices with literature data. The yields of the oils were 1.3 % and 0.2 % for the dried and fresh materials, respectively. Five compounds were identified in the essential oil from the dried sample: thymol (59.9 %), methylthymol (34.6 %), α-phellandrene (3.4 %), 3-octanone (1.2 %) and *p*-cymene (0.7 %). Seven compounds from the fresh sample: thymol (68.3 %), methylthymol (27.5 %), α-phellandrene (1.7 %), 3-octanone (1.1 %), *p*–cymene (0.4 %), eugenol (0.4 %) and linalool (0.2 %). Thymol, methylthymol and α-phellandrene were major constituents in both samples analyzed. Differences in percentage of the chemical composition of the essentials oils in dry and fresh material were observed. Methylthymol and α-phellandrene percentage increased when the material was subjected to drying (27.5 % and 1.7 % for fresh; 34.6 % and 3.4 % for dried, respectively). Moreover, thymol showed a decrease in their content when comparing fresh sample with dried sample (68.3 % for fresh; 59.9 % for dry). According to studies by Venskutonis,[2] the loss of volatile constituents in herbs and spices depends mainly on the type of drying and biological characteristics of plants. Based on the assessment of dried and fresh plant material, it was concluded that no influence on the content and chemical composition of essential oil *C. scoparioides*.

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Acknowledgements: Laboratory of Plant Biotechnology UFOPA, Laboratory of Research and Development of Bioactive Natural Products of UFOPA, University of Campinas - CPQBA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antifungal Property of *Lippia origanoides* Kunth Essential Oil

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Keywords: *Lippia origanoides;* essential oil; antifungal activity.

Lippia origanoides Kunth. (Verbenaceae), also known as "Salva-de-Marajo", is of great importance in the Brazilian traditional medicine. The aim of this study was to evaluate the antifungal activity of the essential oil of *L. origanoides* in relation to *Candida* yeast species. The essential oil (EO) from *L. origanoides* (collected in Santarém, Pará State, Brazil) was obtained by hydrodistillation and analyzed by GC and GC/MS. Carvacrol (46.1 %) and thymol (11.8 %) were the main components found. Antifungal activity against *Candida* yeast species was unraveled by disk diffusion and micro-dilution assay. The minimum inhibitory and minimum fungicidal concentration (MIC and MFC) were 0.62, 1.25, 0.31 µL mL^{-1} and 5.0, 2.5, 0.62 µL mL^{-1} for *Candida albicans, C. tropicalis* and *C. parapsilosis*, respectively. The combined use of the EO with Fluconazole has been tested on *Candida* yeasts and the strategy resulted in a synergistic enhancement of the antifungal action of the azolic chemical product. Indeed, in association with 0.125 µL mL^{-1} of *L. origanoides* EO, the fluconazole MICs dropped from 1.05, 1.05, and 0.12 down to 0.03, 0.03, and 0.01 mg mL^{-1} for *C. albicans, C. tropicalis*and *C. parapsilosis*, respectively. The combinatorial use of *L. origanoides* EO as chemosensitizer agent should contribute to enhance the efficiency of conventional antifungal drugs, reducing their negative side effects.

Acknowledgements: UFOPA, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Growth Control of the Fungi *Pythium* spp. and *Thielaviopsis* spp. Through Volatile Compounds of the Essential Oil of *Eucalyptus staigeriana*

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Keywords: Alternative control; root rot; phytopathogen.

Pythium spp. and *Thielaviopsis* spp. fungi are the main cause of root rot in various cultures, including lettuce crops.[1] An alternative to control plant pathogens is the use of essential oils, which have antifungal action by inhibiting the mycelial growth and spore germination. Here, we evaluated the effect of the essential oil (EO) of *Eucalyptus staigeriana* in the control *in vitro* of *Pythium* spp. and *Thielaviopsis* spp. Leaves of *E. staigeriana* were harvested from plants located in Caxias do Sul - Brazil and the EO was extracted by steam distillation from dried plant leaves for 1 h and analysed by GC/MS. *Pythium* spp. was isolated from a hydroponic system for lettuce production and *Thielaviopsis* spp. from hydroponic cultivated arugula roots. The major constituents from EO of *E. staigeriana* were geranial (21.4 %), limonene (18.9 %), 1,8-cineole (18.3 %) and neral (15.5 %). For the evaluation of volatile compounds, *Pythium* spp. mycelial discs of 5 mm (Ø) were placed into Petri dishes of 9 cm (Ø) with Potato-Dextrose-Agar medium, while for *Thielaviopsis* spp. it was used Carrot-Potato-Dextrose medium, and both were maintained at 25 °C in a 12 h photoperiod. The EO was emulsified with Tween 20 in the concentrations: 0, 12.5, 18 and 25 %. At the same time that the fungi were inoculated, the cover of each Petri dish had a small piece of cotton fixed on it, in which was applied a 100 µL of each (EO). The Petri dishes were sealed with plastic film to keep the volatiles inside. The radial mycelial growth of the colonies was measured from the 3rd to the 14th days. On the 7th day half of the plates had its piece of cotton removed and received a new piece with EO applied with the same concentrations. After 14 days, transfer experiments were performed in order to make a distinction between the fungistatic and fungicidal effects of the essential oils on the phytopathogen. For that, discs that did not grow were transferred to PDA and their viability and growth was assessed at the 14th day. The results were similar for both fungi and it was observed some growth at one application of the concentration 12.5 %. However, when it was reapplied the colonies were unable to continue their development. The concentrations of 18 and 25 % applied once showed lack of mycelial growth until the 14th day, but presented growth on the transfer experiments. Sothe EO of *E. staigeriana* may be used in alternative control of *Pythium* spp. and *Thielaviopsis* spp.

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Acknowledgements: Universidade de Caxias do Sul and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Rectification of Palma Rosa Essential Oil, *Cymbopogon martinii*

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Keywords: *Cymbopogon martini*; geraniol; essential oil.

Cymbopogon martini (palma rosa) is an herbaceous plant of the genus *Cymbopogon* and the family of grasses that grows in tropical areas. Palma rosa essential oil (EO) is rich in geraniol. Geraniol is a monoterpene alcohol which gives to the oil its aromatic character and commercial interest for its use in the production of repellents, fragrances and cosmetics.[1] Palma rosa EO extracted from the plant cultivated in the municipality of Barbosa, Santander, Colombia was obtained by steam distillation. The commercial value of palma rosa essential oil depends on its content of geraniol. Because of this, the EO was subjected to a process of fractional distillation using a spinning band column *B/R Instruments 800* (BR Instruments, Easton, Maryland, United States) with 15 theoretical plates, at a pressure of 8 Torr.[2] Four fractions were obtained, three at the column top and one at the bottom. For each fraction, the equilibrium temperature was measured at the boiling flask and the top of the column. For the first fraction the equilibrium temperature was obtained at 123 °C and 60 ± 5 °C and it was composed mainly of (*E*)-β-ocimene (54.2 %), (*Z*)-β-ocimene (16.8 %), and eucalyptol (6.0 %); the second fraction was obtained at 124 °C and 80 ± 5 °C and it was composed mainly of geraniol (78.5 %) and geranyl acetate (2.3 %); the third fraction at 124 °C and 95 ± 5 °C was composed of geraniol (82.5 %) and geranyl acetate (2.2 %); the fourth fraction was obtained from the bottom of the column with composition mainly of geraniol (83.5 %) and geranyl acetate (2.4 %). The mass percentage for each fraction was ~4.8, ~9.2, ~ 22.4 and ~56.3 with losses of ~ 7.3 %. Geraniol relative amounts for each fraction were 0, 78.5, 82.5 and 83.5 %. Fraction characterization was performed on an Agilent Technologies 7890A gas chromatograph coupled to an Agilent Technologies 5975C mass spectrometer with quadrupole analyzer. DB-5MS and DB-WAX capillary columns were used; split injection (30:1) was employed. For geraniol quantification, a calibration curve was made and samples were analyzed on an HP 5890 Series II GC with flame ionization detector and a DB-Wax (60 m) column.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract **RC-0343-2013.**

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Biological Activities of *Myrcia minutiflora* and *Myrcia magnolifolia*

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Keywords: *Myrtaceae*; essential oil; bactericidal; cytoxicity.

The Amazon Rainforest biome has several Angiosperms families. In the Reserva Florestal Adolpho Ducke, a representative sample of the Terra-Firme ecosystem, 67 identified species of the Myrtaceae family are found, predominantly arboreal. Almost 15 species are from *Myrcia* genus.[1]. Therefore, the volatile chemical compositions of the leaves from *M. minutiflora* and *M. magnolifolia* were characterized, as well as the antibacterial and cytotoxicity activitys of these essential oils were evaluated. Dried leaves (250 g) of *M. minutiflora* and *M. magnolifolia* previously identified (voucher numbers: 4774 and 1384, respectively) were hydrodistillated in a modified Clevenger type apparatus (4 h). The crystalline solidwas obtained from the oil of *M. magnolifolia*, which was separated and identified by GC/MS and NMR (^{1}H and ^{13}C). Essential oils were analyzed by GC/FID and GC/MS [Shimadzu, GC2010 and GCMS - QP2010, using capillary column DB-5 and DB-5MS (30 m X 0.25 mm X 0.25 mM), respectively; carrier gas: helium for both previous systems (1 mL min^{-1}); temperature program: 60 to 240 °C (3 °C min^{-1}); electronic ionization: 70 eV]. The contents of compounds were obtained by normalizing the data acquired by GC-FID. The identification of compounds was carried out by comparing mass spectra existing in databases Wiley 7th and Adams (2007), as well as by comparative analysis of the Kovats indices. Antibacterial and cytotoxic activities were carried out by applying methods of measurement of theinhibition zone and the alamar blue assays (time: 72 h; human cell lines: Skmell 3, ACP02, and MRC5). The yields obtained of oils were 0.35 % and 0.08 % for *M. minutiflora* and *M. magnolifolia*, respectively. The crystals were identified as 7-eudesm(11)-en-4-ol. From *M. magnolifolia* oils were identified 47 compounds (100 %), consisting of mono- and sesquiterpenes, whose majority are: α-pinene (28.6 %), β-pinene (8.6 %), limonene (4.6 %), α-terpineol (12.0 %) and (*E*)-caryophyllene (11.4 %). About *M. minutiflora*, 41 compounds were identified (98.3 %), composed mainly by sesquiterpenes: β-elemene (11.6 %), (*E*)-caryophyllene (15.6 %), sesquiterpene (7.2 %) *cis*-β-guaiene (6.5 %), δ-cadinene (5.2 %) and viridiflorol (10.5 %). These oils have shown high antibacterial action (Ø >1.7 cm) against *Staphylococcus aureus*. The essential oil of *M. minutiflora* has showed high cytotoxicity to non-neoplastic line MRC5 (10.74 %). Therefore, the biological potentials of these Amazon Rainforest species have shown to be promising.

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Acknowledgements: CAPES, CBA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Analysis of the Essential Oils from Leaves and Branches of Five Varieties of *Aniba rosaedodora* (Rosewood) Employed in the Industry

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Keywords: *Aniba rosaedodora;* essential oil.

The genus *Aniba* has 44 species, taxonomically divided in two groups, *affinis* and *guianensis*. Inside the *guianensis* group has four subgroups such as the *panurensis* (14 species) in which the *Aniba rosaedodora* species is part. Morphologically, these species can be differentiated by characteristics of the flowers and also, some times, by the fruits. However, the recurrent overlap of the floral morphology makes it impossible the taxonomic analysis. *Aniba rosaedodora* stands out principally on the perfume industry and also it was target of intense exploration for the essential oil commercialization. The objective of this project was to evaluate the composition differences of the essential oil on five rosewood varieties commercially used. Magaldi Company located in Maués, Amazon, Brazil, gently provided the samples of leaves and branches, in July 2015. Approximately 30 g of dry leaves, fresh leaves and dry branches were submitted to hydrodistillation separately in a modified Clevenger-type apparatus for 4 h each. The oils were analysed by GC/FID in and Shimadzu GC-2010 system, with DB-5 fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Helium was used as carrier gas for GC/FID, with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 180 °C at 3 °C min^{-1} and later was raised from 180 to 290 °C at 20 °C min^{-1}. The comparison between the chromatograms and literature data allowed to classify the samples in two groups. The major characteristic of the first group was the higher concentration of linalool (63.1 - 84.9 %). The second group was marked by the presence of two major substances: linalool (35.6 – 52.7 %) and caryophyllene oxide (9.4 – 38.5 %). Other minority substances were detected in both groups, characterizing the rosewood oils, as the presence of caryophyllene, pinene and selinene. The fresh and dry leaves of the five varieties showed similar chromatographic profiles. The dry branches profile of the second variety analysed showed a higher number of substances when compared with the other samples.

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Acknowledgements: FAPEAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition, Antimicrobial Activity in Vapour and Liquid Phase and Cytotoxicity from the Essential Oil of *Hesperozygis myrtoides* (St.Hil. ex Benth.) Epling (Lamiaceae)

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Keywords: *Hesperozygis myrtoides*; essential oil; antimicrobial activity.

Essential oils (EO) in liquid or vapour phases have been used for their antimicrobial properties since ancient times. In the literature, this activity is commonly tested in the liquid phase, although currently the vapour phase activity has gained interest. *Hesperozygis myrtoides* (St.Hil. ex Benth.) Epling is a small aromatic bush that is used for treating respiratory diseases. Thus, the aim of the present work was to analyse the *H. myrtoides* EO composition, comparing its antimicrobial activity in the vapour and in liquid phases from plants collected in Campos do Jordão (São Paulo, Brazil). Cytotoxicity was performed with cancer cells breast (MF-7) and prostate (P3). The EO was obtained by hydrodistillation for 4 h, and the component identification was performed by GC/MS. The antimicrobial activity of the EO vapours was evaluated by the inverted plate method and in the liquid phase by microplate method against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231).[1] The average EO yield was 1.7 % (w/w), presenting as major components pulegone (31 %), isomenthone (16 %), neo-isomenthyl acetate (12 %), neo-isomenthol (10 %) and menthone (6 %). The EO vapours were able to inhibit the growth of *S. aureus* and *C. albicans*, with Minimum Inhibitory Concentrations (MIC) of 392 µg L^{-1} and 833 µg L^{-1}, and respectively 19 mg L^{-1} and 94 mg L^{-1} for the liquid phase has MIC. The first tests IC_{50} indicated higher values 50 mg L^{-1} and 230 mgL^{-1} are toxic to breast and prostate tumor cells, respectively. These results indicated that the vapours were much more active than liquid phase. The EO vapours have the advantage as sanitizers because they can treat large areas without requiring direct application on surfaces, which is suitable for the use as room disinfectants and air decontaminants even in inhabited areas due to their lower toxicity in relation the dose.

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Acknowledgements: CAPES, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Bath Products Lineation with Essential Oil of *Lippia gracilis* Schumof Amazonian Origin

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Keywords: *Lippia gracilis;* cosmetics; formulation.

The species *Lippia gracilis* Schum, popularly known as "alecrim-da-chapada" or "alecrim-do-tabuleiro", is a deciduous, branched shrub with brittle stems, up to 2 m high.[1] In addition, there are several studies of volatile constituents of this plant, consisting mainly of thymol, carvacrol and 1,8-cineole, and these volatile components have strong antimicrobial, antioxidant and anti trypanomicide activities. The development of biocosmetics appropriate to the desired effect, place and intensity, is a step forward in research on the use of essential oils as components in cosmetic products. The developed formulations must have appropriate physicochemical and sensory characteristics, as well as efficacy, safety and quality. Physicochemical tests were performed to evaluate pH, apparent viscosity, organoleptic characteristics, foam and density. This work aimed at the development and evaluation of the stability of biocosmetic formulations used in the bath such as shampoo and liquid soap, from incorporation of essential oils of *L. gracilis* through pre-formulation studies and preliminary and accelerated stability tests.[2] Leaves of *L. gracilis* were collected in the National Park of Chapada das Mesas, municipality of Carolina/MA. The oil was extracted from dry leaves by hydrodistillation and the chemical constituents were identified by GC/MS. The oil showed a yield of 4.7 % and the analysis by GC/MS led to the identification of 23 compounds. Major constituents found were thymol (77.0 %), *p*-cymene (7.4 %) and thymol methyl ether (4.7 %). During the preliminary and accelerated stability studies, it was observed that in the refrigerator (5.0 ± 0.5 °C) and oven (4.0 ± 0.5 °C), only the liquid soap type formulations had changed in pH a reduction from baseline of 10 to 8.5, viscosity of 59,816.9 mPa.S to 26,663.9 mPa.S and sensory characteristics. The shampoo type formulations remained unchanged. At the end of the study, the formulations kept at room temperature maintained the initial characteristics.

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Acknowledgements: UFMA, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Influence of Seasonality in Chemical Composition of the Essential Oil of *Copaifera duckei* and Evaluation of Toxicity Against *Artemia salina*

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Keywords: *Copaifera duckei;* essential oil; seasonality.

Copaifera duckei, known popularly as "copaiba", produces "copaiba" oil or balm, with anti-inflammatory, healing and antiseptic action potential. Few data releases of seasonal influence to *C. duckei* led to this study, proposed to evaluate the influence of seasonality on chemical composition and evaluation of toxicity in essential oil (EO) of *C. duckei* against *A. salina*. The oilresin were collected in the Tapajós National Forest for two periods (summer and winter), then 40 mL of oil resin was subjected to hydrodistillation for 6 h obtaining a volatile fraction. It was analyzed by gas chromatography coupled to mass spectrometry (GC/MS) to identified the variation in the percentage of chemical compounds.The volatiles fractions of *C. duckei* presented the major constituents: β-bisabolene (summer period 34.3 %; winter period 28.2 %), β-caryophyllene (summer period 23.5 %; winter period 15.9 %), β-selinene (summer period 7.1 %; winter period 11.4 %) and α-humulene (summer period 1.0 %; winter period 1.3 %), respectively. The cysts of *A. salina* were placed in artificial saline for 24 h under lighting. After ten larvae were transferred to test tubes containing saline solution and samples of EO in concentrations: 1, 10, 100 and 1000 µL mL^{-1}. The bioassay was performed in triplicate. The count of the larvae was performed after 24 h. The mortality rate was determined by dividing the number of larvae killed by total number of individuals and multiplying the result by 100. The degree of toxicity was classified according to mortality: 0-9% = not toxic (NT); 10-49% = slightly toxic (ST); 50-89% = toxic (T); 90-100% = highly toxic (HT). At concentrations of 10, 100 and 1000 µL mL^{-1} OE in summer period the mortality rate was 100% (AT), with the exception of 1 μlml^{-1}, which showed a mortality rate of 13.3 %. OE in winter period, only concentrations of 1000 and 100 μlml^{-1} presented 100% mortality rate, the concentration of 10 µL mL^{-1} presented in the rate of 53.3 % larvae dead and 1 µL mL^{-1} concentration only 23.3% in the mortality rate. According to the methodology of Harwig and Scott,[1] the EO of *C. duckei* presents toxic, with the highest mortality rate in the dry period, where the concentration of compounds is larger than in the rainy season. Preliminary results with *A.salina* demonstrate that EO of *C. duckei* presents toxicity, suggesting sequential studies.

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Acknowledgements: UFOPA, Laboratório de Pesquisa e Desenvolvimento de Produtos Naturais Bioativos (PDBio), FAPESPA, ICMBIO, CoomFlona

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Yield in Developed Leaves of *Pereskia aculeata*

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Keywords: *Pereskia aculeate;* chemical composition; essential oil.

Unconventional vegetables, used as functional foods, are important for food security, conservation and management of biodiversity and cultural heritage for future generations. The functionality is determined by reference to bioactive compounds and ethnomedicinal use. *P. aculeata* Mill. (ora-pro-nobis), Cactaceae, is an unconventional vegetable species native to the Americas, taking place from Florida (USA) to Argentina. As a rustic plant, it adapts better to the tropical and subtropical climate and prefers well-drained soil, which is, in Brazil, characteristic in the region from the Northeast to Rio Grande do Sul. It is popularly used in anti-inflammatory processes, iron deficiency anemia, antioxidant activity,[1] among others. Its leaves are a rich source of protein and minerals such as calcium and iron. The production of these compounds is influenced by plants physiological factors. This study aims to quantify the essential oils leaves yield from *P. aculeata* grown in dense system (three installment with 10 plants per m²). The plants were grown since February 2011, subjected to successive crops, which the last was in February 2015. Thus, most of its leaves were found well developed in size and thickness, characterized as old leaves. One thousand grams of fresh leaves per installment were collected forming composite samples (3000 g). The leaves were processed and dried in a forced air oven at 40 °C until reach constant weight. The extraction of the essential oil obtained from *P. aculeata* was performed according to the procedure described in European Pharmacopoeia,[2] where 100 g of dried leaves were subjected to hydrodistillation for 3 h, with three replications. The results were expressed in mL kg^{-1} of dried plant. The essential oil yield from developed leaves was 0.6 mL kg^{-1} under the conditions of this experiment.

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Acknowledgements: FAPEMIG and CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Evaluation of the Antioxidant Potential of Essential Oils of Species *Myrcia* Collected in Santarém-PA

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Keywords: *Myrcia sylvatica; M. multiflora; M. amazonica*.

The genus *Myrcia*, one of the largest Neotropical Myrtaceae, with 750 species, is especially rich in Brazil, occurring mainly in areas of savannas. In the Amazon region the species of this genus, known popularly as "murta" or "pedra-ume-caá",[1] are abundant and diverse. In general, oils of aromatic species *Myrcia* are rich in cyclic sesquiterpenes mainly of cadinane, caryophyllane and germacrane groups.[2] The aim of this study was to identify the chemical composition and evaluate the antioxidant potential of essential oils *M. sylvatica* (G. Mey) DC., *M. multiflora* (Lam.) DC. and *M. amazonica* DC., which occur naturally in the Santarém-PA region. Samples (aerial parts) of *M. sylvatica* specimens (Ms01, Ms02), *M. multiflora* (Mm01) and *M. amazonica* (Ma01) were collected in savanna regions in Santarém in the dry season, and its voucher specimens were deposited in the Herbarium of Santarém (HSTM), under the registry numbers 000077, 000083 and 000095, respectively. The oils were obtained from the air-dried material followed by hydrodistillation in Clevenger-type apparatus for 2 h and chemical analysis was performed by GC/MS. The antioxidant activity was determined by the DPPH radical-scavenging assay, adding 10 µL oil, 40 µL of ethanol and 1950 µL DPPH (0.25M in ethanol), with absorbance reading (517 nm) after 30 min of reaction in the dark. The control sample was prepared using ethanol and trolox (1 mM) was used as antioxidant standard. High yields were obtained from oils of specimens Ms01, Ms02 (1.1%), Mm01 (1.4 %) and Ma01 (0.92 %). The major compounds identified were cyclic sesquiterpenes, such as δ-cadinene (8.5 %), β-selinene (8.3 %) and *epi*-1-cubenol (6.9 %) in the Ms02 oil, bicyclogermacrene (10.8 %), germacrene D (10.6 %) and δ-cadinene (7.7 %) in the oil Mm01 and *epi*-1-cubenol (16.6 %), germacrene B (15.7 %) and germacrene D (12.6 %) in the Ma01 oil. However, in the oil from the specimen *M. sylvatica* Ms01 predominated the monoterpenes *trans*-pinocarveol (7.3 %), myrtenol (7.2 %) and α-pinene (6.8 %), indicating a chemical variability of this species, in the study region. In the DPPH assay, inhibition of the oils were 37.4 ± 1.7 % (Ms01), 18.8 ± 0.3 % (Ms02), 42.5 ± 1.6 % (Mm01) and 11.9 ± 0.3 % (Ma01), and the trolox standard was 42.8 ± 0.5 %. These results indicated that the oils from the specimens *M. sylvatica* (Ms01) and *M. multiflora* showed significant antioxidant activity when compared to trolox.

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Acknowledgements: CNPq and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Varronia curassavica* Jacq. (Boraginaceae) from Instituto Vital Brazil Farm.

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Keywords: *Cordia verbenacea;* essential oil; medicinal plant.

Varronia curassavica Jacq. (Boraginaceae) is a shrub native to most of Brazil, abundant in open coastal areas and pastures. Known as "erva-baleeira", it is used in folk medicine with anti-inflammatory, anti-arthritic and analgesic actions.[1] The essential oil (EO) of its leaves has been scientifically validated for use as anti-inflammatory, alpha-humulene and beta-caryophyllene being identified as main active constituents.[2] Clinical assessment of efficacy from *Varronia curassavica* Jacq. (syn. *Cordia verbenacea* DC) was performed with standardized extract containing 2.3-2.9 % alpha-humulene.[3] Here, the EO chemical composition of leaves from *V. curassavica* cultivated at the Instituto Vital Brazil farm localized in Cachoeiras de Macacu / RJ was studied, for the use in horses employed in the production of hyperimmune serum. Fresh leaves of were collected in March and July 2015 (voucher at the herbarium of UFRJ, no. 39924. EO was extracted for 2 h by hydrodistillation, using a Clevenger-type apparatus, and analyzed by GC/MS (Agilent) (70 eV, *m/z* 50–700), with DB-5MS fused silica capillary column (30 m X 0.25 mm X 0.25 µm). Helium was used as carrier gas with a flow rate of 0.5 mL min^{-1}. The injector temperature was maintained at 250 °C. The oven temperature program was as follows: 70 (5 min) to 300 °C (10.5 min) at a rate of 4 °C min^{-1}. Oil components were identified by comparison of mass spectra with spectral library and literature. The area percentage composition was obtained by peak area normalization. Oil yields were 0.15 % and 0.1 7%, respectively. The GC/MS analysis revealed the presence of at least 27 components, among them the following constituents of interest: alpha-humulene (3.9 % and 4.8 %); beta-caryophyllene (16.3 % and 16.5 %)**;** alpha-pinene (52.3 % and 37.9 %); alpha-santalene (3.0 % and 10.6 %). The results show that the essential oil analyzed in terms of its active components has a good potential for medicinal use.

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Acknowledgements: Antonio Siani and Leandro Rocha for the use of Clevenger apparatus.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Identification of the Essential Oil Obtained from Leaves and Twigs of *Eucalyptus viminalis* Waste Collected in the Southwest Region of Paraná - Adding Value to the Production Chain of Wood.

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Keywords: Residual biomass; hydrodistillation; multivariate analysis.

The *Eucalyptus* genus comprises a large number of species, with different adaptive characteristics in relation to climate and soil. *Eucalyptus viminalis* is native of Australia and is usually found in high altitudes and cold places, and is well to some regions of Brazil.[1] Its use in the plywood and furniture industry is growing, causing an increased the amount of waste. Gums, resins, essential oils may have good potential for various applications. Here, a factorial design to optimize the conditions for obtaining its essential oil via hydrodistillation was done using the leaves and twigs from residual *E. viminalis*. Five essential oil samples were obtained, their chemical components were identified and quantified. The extraction time was 4 h and the essential oil was collected using diethyl ether, and dried with anhydrous sodium sulfate. GC/MS (Varian) was performed. One µL of each sample was injected at 250 °C, with a column flow of 1.2 ml min^{-1}. The column temperature ranged from 50 to 240 °(3 °C min^{-1}), and 3.5 °C for the remainder of the analysis. The final temperature was maintained unchanged for 14.5 minutes resulting in a total analysis time of 70 minutes. The identification essential oil constituents was based on retention indices, obtained by co-injection of a mixture of *n*-alkanes standards, and by comparison of their mass spectra. Helium was used as the carrier gas with 1 mL min^{-1} flow. The statistical treatment was conducted using factorial design, cluster analysis and principal component analysis (PCA). As a result, 74 components were obtained and identified (80.7 %). Eucalyptol wit minimum and maximum percentages equal to 47.7 % and 72.2 %, was found in all samples and was the major component in all cases. In addition to eucalyptol, in most samples other hydroxylated sesquiterpenes such as α-cadinol (0.2 % to 0.9 %) were found. Hydroxylated monoterpenes represented by *trans*-myrtenol (0.01 % to 1.6%), and sesquiterpenes represented by 6,9-guaiadiene (0.4 % to 1.5 %) and *cis*-β-guaiene (0.7 % to 1.3 %) were also present. Regarding the acquisition of higher eucalyptol concentrations per sample (72.2 %), the most favorable conditions were 48 h of drying time for the leaves and branches, 3 h of essential oil extraction and 40 g of biomass with an average of contact surface 8 cm, enabling the procurement of 0.5 mL of essential oil.

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Acknowledgements: Central de Análises – UTFPR Pato Branco.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Advances in the Analysis of Aromatic Compounds in Hop "nugget" from Patagonia

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Keywords: Hop; *Humulus lupulus;* nugget.

In the manufacture of beer malt addition with small and varying amounts of two groups of hops (*Humulusl upulus* L.) which provide the bitter taste and the flavor profile of the beverage depending on the composition of its hundreds of volatile constituents. Overall, it is considered that there are four types of scents provided by the different hop varieties: 1, fats and green notes for (aldehydes); 2, citrus chords (nerol and linalool); 3, fruity or floral notes (alcohols and esters); 4, herbaceous and spicy notes (sesquiterpenes and oxygenated).[1] (*E*)-caryophyllene and α-humulene are two key sesquiterpene compounds in most of the varieties. Its percentage ratio allows differentiating between varieties. Other important sesquiterpenes compounds are of farnesene and selinene nuclei. Furthermore, the presence of myrcene is undesirable for giving a "chemical or solvent" aroma; however, this compound is easily volatilized during processing of the beverage. The presence of sulfur compounds is also of utmost organoleptic importance for its low odor thresholds that have been studied by several authors. New varieties of hops with new aromatic profiles are prized as a way to produce novel types of beer. In Argentina, "Nugget" is a variety characterized among the bitter ones (high humulones). This hop was obtained from Patagonia, Argentina, and the EO (hydrodistillation) analyzed by GC/FID/MS. One hundred and two constituents were identified, being the main: myrcene: 62.4 %, α-humulene: 11.7 %, (*E*)-caryophyllene: 4.6 %; methyl *cis*-4-decenoate: 1.6 %, methyl 4,8-decadienoate: 1.5 % and linalool: 1.0 %.Hop Nugget from Patagonia presented high myrcene content compared with hops from the same variety of other regions; however, this monoterpene disappears to a great extent during brewing. Besides, minimal amounts of 2-nonanol and methyl 3,6-dodecadienoate were detected, both regarded as typical markers of this variety. The ratio α-humulene/(*E*)-caryophyllene, used to characterize different varieties, was 2.5, similar to the values found with Nugget from Spain (1.6) and from the United States.

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Acknowledgements: To the National University of Patagonia "San Juan Bosco", to the 2° Convocatoria de Proyectos de Asistencia Exportadora "Manuel Belgrano" (SPU, Min. de Educación) and to the University of Buenos Aires (UBACyT 20020130200057BA and 20020130100169BA).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Analysis of the Essential Oils of Two Species of the Genus *Lantana* (Verbenaceae) from La Cumbre, Valle del Cauca, Colombia.

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Keywords: *Lantana alba; Lantana colombiana;* essentia loils.

The genus *Lantana* (Verbenaceae) contains approximately 314 described species, originally from tropical and subtropical regions of America.[1] They were introduced in Europe as ornamental plants, fact that explains their presence in some countries of Asia and Africa. Different studies have verified that the essential oils of some species of the genus *Lantana* present biological activity, as tetanus, malaria, rheumatism, bronchitis, among others.[2] The essential oils of *L.a alba* (known also as *Lippia alba*), and *L. colombiana* were obtained by microwave-assisted hydrodistillation (MWHD). Oil yields were 0.02 % (w/w) in both cases. The oils were analyzed by GC/MS (Agilent) with DB-5MS and DB-WAX capillary columns (60 m X 0.25 mm X 0.25 µm), injector at 250 °C. A 30:1 split injection was used. Oven temperature was raised from 45°C to 150°C (4°C min^{-1}), to 250°C (5 min) at 5 °C min^{-1}, and to 27 5°C (15 min) at 10°C min^{-1}. Oil components were identified by comparison of mass spectra and LRI. The main components of the essential oil of *L. alba* and *L. colombiana* were: germacrene D (35.2 % and 32.0 %, respectively); α-humulene: (23.8 % and 8.8 %); *trans*-β-caryophyllene (16.2 % and 27.2 %), and bicyclogermacrene (6.9 % and 20.2 %). In a study made in Brazil with *L. alba* collected in Chaves (Pará State), that contained neral (13.7 %), geranial (22.5 %), germacrene D (25.4 %) and *trans*-β-caryophyllene (10.2 %),there is some similarity with those of *L. alba and L. colombiana* collected in Colombia.[3] La Cumbre (Colombia) and Chaves (Brazil) have a medium altitude and a subtropical weather and the compositional difference between them was the presence of main components neral and geranial in *L.alba* from Brazil.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101 foraccess to genetic resources and derivatives fors cientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Geraniol Content Enhancement in *Cymbopogon martinii* Essential Oil

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Keywords: Essential oils; hydrolysis; *Cymbopogon martinii.*

Cymbopogon martinii belongs to the Poaceae family. It is native to Southeast Asia. It is grown mainly in India, Brazil, Paraguay, Madagascar, Guatemala, and Indonesia. The essential oil (EO) of *C. martinii* (palmarosa) has over 70 constituents, among which geraniol (78-85 %) and geranyl acetate (3-12 %) are the most abundant.[1] Geraniol is an acyclic monoterpenic alcohol. It is one of the most important molecules in the flavor and fragrance industries and is a common ingredient in consumer products of these industries. This project seeks to increase the geraniol concentration in palmarosa EO using catalytic hydrolysis of geranyl acetate. Geranyl acetate hydrolysis was tested in treatments (reflux, 2 h) whit hydrochloric acid (5 N), sulfonated silica (5 N), potassium hydroxide (5 N), and calcium hydroxide (5 N). Compound identification was performed on a gas chromatograph (Agilent Technologies, AT, 7890A) coupled to a quadrupole mass spectrometer (AT, 5975C). DB-5MS (60 m) and DB-Wax (60 m) capillary columns were used, with oven temperatures programmed from 50 to 280 °C and 50 to 200 °C, respectively. A 30: 1 split injection (1 µL) was employed. Quantification was based on chromatographic areas obtained with a gas chromatograph (HP 5890 series II) with FID and a DB-Wax (60 m) column. Calibration curves were made with certified standards of geraniol and geranyl acetate. The hydrolysis in acid medium (HCl and sulfonated silica) had no selectivity for the formation of geraniol. The hydrolysis in heterogeneous phase with $CaOH_2$ permitted a conversion of 11 % and a selectivity of 88 %. One reason for the limited use of heterogeneous basic catalysts is the rapid deactivation in the presence of air. The calcium-based catalysts have strong basic sites, but need a pretreatment at a high temperature to remove adsorbed CO_2 from the atmosphere.[2]Though, the selectivity was high towards the formation of geraniol. Calcium-based catalysts have been used in the production of biodiesel for their low cost and ease of preparation.[2] The highest conversion (34 %) and selectivity (96 %) were obtained with the potassium hydroxide treatment. Further improvements of basic catalyst materials results are under way.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para laCiencia, laTecnología y laInnovación, Francisco José de Caldas, Contract RC-0343-2013.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

MDGC Analysis and Biological Evaluation of Essential Oil from *Cymbopogon flexuosus* (Steud) Wats Cultivated at PAF/FIOCRUZ (RJ)

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Keywords: *Cymbopogon flexuosus;* antinociceptive activity; cytotoxicity.

Cymbopogon flexuosus, popularly known as lemongrass (Poaceae) comprises approximately 500 genus and 8,000 herb species. Chemical profile of essential oil (EO) from *C. flexuosus* cultivated at Agroecologica Platform of Phytomedicines (PAF/FIOCRUZ) allowed the identification of 13 compounds from fresh and frozen leaves samples, including two major compounds (neral and citral). In the writhing assay induced by acetic acid (0.8 %), to check the antinociceptive activity, the two samples of the essential oil at the dose of 1mg kg^{-1} presented significant inhibition of writhings: 66.44 % and 54.48 %, respectively, compared to control. The in vitro cytotoxicity bioassay of both samples showed toxicity at the dose of 50 µg mL^{-1}. The EO extraction was done in a modified Clevenger apparatus. The Shimadzu MDGC system consisted of two gas chromatographs (GC1 and GC2), and a quadrupole MS. In GC1 we used an HP-FFAP 25 m X 0.20 mm X 0.33 µm (Agilent) and in GC2 a Rtx-5MS 30 m X 0.25 mm X 0.2 5µm (Restek) as columns. All data were collected by the GC Solution software (Shimadzu) for the FID (GC 1) and by the GC-MS solution software for the MS (GC 2). MS: Ion source: 250 °C; interface temp: 250 °C, interval scan: 40-400 *m/z*; scan speed: 2000 $amus^{-1}$. The analgesic activity was evaluated through Collier's method. The major compounds in the volatile oil samples obtained from the fresh and frozen leaves were quantified through standardization of the areas in the chromatogram obtained by analysis MDGC in the GC 1 (FID) were neral (36.9 and 37.1 %) and geranial (42.7 e 44.0 %), followed by myrcene (3.1 and 2.3 %). Our study have showed that the sample oil from fresh and frozen have different concentration composition and they present a potential antinociceptive activity.

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Acknowledgements: Farmanguinhos, Fiotec, CNPq, CAPES, FIOCRUZ, IME.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Physicochemical Properties and Chemical Composition of Essential Oils Extracted by Hydrodistillation of Culinary Herbs: Parsley (*Petroselinum crispum*) and Celery (*Apium graveolens L.)*

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Keywords: Essential oils; *Apium graveolens; Petroselium crispum.*

Parsley (*Petroselinum crispum*) and celery (*Apium graviolens L*) (Apiaceae), two aromatic leafy vegetables and condiments, have interesting essential oils. Therefore, it was performed a study of physicochemical properties of the essential oil obtained from these plants for the identification of substances and comparison thereof. Whereas both these vegetables are widely used in cooking and have a great visual similarity and belongs to the same family. The two plants were dried in air circulating oven under two conditions (45 °C for 36 h and 50 °C for 24 h). It was used 30.0 g of fresh plants and dried plants for each extraction. The essential oil was obtained by hydrodistillation, using the Clevenger SL-76 equipment, for 2 h. The yields parsley at 50 °C and 45 °C were respectively 0.22 % and 0.16 %. Besides, for the celery at 50 °C and 45 °C the yields were respectively 0.25 % and 0.18 %. The oils were analyzed by GC-MS Agilent 5977A and Agilent 7890B system with capillary column of fused silica (30m X 0.25 mm X 0.25 µm). The necessary conditions for analysis were performed according to the method described by Santos.[1] The components of the oil were identified by comparing the spectra with NIST database and linear retention indices. In parsley oils, in all treatments (in nature, 50 °C and 45 °C) it was found that they are rich in apiol, with 49.0 %, 50.6 % and 56.5 %, respectively, besides containing the following compound in common: 1,3-benzodioxole-4-methoxy-6-(2-propenyl) (26-36 %). The celery oils proved themselves rich in limonene being: in nature (64.2 %), 50 °C (71.0 %) and 45 °C (66.6 %). Celery characteristic aroma is associated to 3-butyl-3a,4,5,6-tetrahydro-3H-2-benzofuran-1-one, sedanolide, which was also found (18-25 %). It is possible to observe that the yields obtained from the essential oils of celery and parsley presented significantly difference among the three treatments used. The yields of celery oil were higher than the parsley oils. Each oil presented different characteristically compounds in their chemical compositions.

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Acknowledgements: CAPES, Araucaria Foundation, UTFPR.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Relationship Between the Leaf Mineral Composition of Lemongrass Fertilized With Different Nitrogen Sources and Essential Oil Production

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Keywords: *Cymbopogon citratus; Cymbopogon flexuosus;* citral.

Lemongrass is an aromatic and medicinal plant used as raw material in the preparation of tea and essential oil extraction rich in citral. The plant grows forming a clump with abundant leaf system that assumes high demand for nutrients, especially nitrogen because it is a grass. This study aimed to analyze the leaf mineral composition in two species of lemongrass *Cymbopogon citratus* (DC) Stapf and *Cymbopogon flexuosus* (Nees ex Steud.) Will. Watson, after nitrogen fertilization with composting of bovine manure, goat manure, poultry manure and urea. The mineral composition of the leaf was related with essential oil production, at 160 and 220 days after planting (DAP). The nutritional contents were not affected by nitrogen sources. The species showed differences in macro and micronutrient content and was in the following order for *C. citratus* C> N> K> Ca> P> Mg> Fe> Zn>Mn> Cu and *C. flexuosus* C> N> K> Ca> P> Mg> Fe>Mn> Zn> Cu. At harvest at 160 DAP, *C citratus*accumulated higher content of P, K, Ca, Al, Fe and Zn as *C. flexuosus* accumulated more Mg and Cu content. Already at 220 DAP, *C. citratus* presented the highest C, P, K, Ca and Fe and *C. flexuosus* higher Mg content, Cu and Mn. Treatments did not affect of dried biomass of *C. flexuosus* nor *C. citratus* at 160 and 220 DAP (2601.2-3913.8 kg ha $^{-1}$ and 593.7-592.1 kg ha $^{-1}$ respectively), oil yield (26.4-31.7 and 10.6-12.5 kg ha $^{-1}$ respectively), nor the oil content (10.0-8.4 and 17.7-21.3 g kg $^{-1}$). The nutrient content was higher by *C. flexuosus* as a result of high biomass production. The Mg and Mn appear to be related to the high content of citral in the essential oil of *C. flexuosus* that was 80.0% to 160 DAP and 80.8% to 220 DAP while *C. citratus* was 70.6 % to 160 DAP and 71.8 % to 220 DAP. The nitrogen content does not provide differences to the production of biomass and citral.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In Vitro Evaluation of Antifungal Activity from Some Essential Oils Against Papayas anthracnose Causative Agent *Colletotrichum gloeosporioides*

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Keywords: *Carica papaya* L.; anthracnose; antifungal activity.

Papaya (*Carica papaya* L.) is a native fruit from tropical climates. Brazil is a major producer and consumer of it, but annually, tons of fruits are lost due to poor logistics, bad quality control, and especially post-harvest diseases like anthracnose, caused by *Colletotrichum gloeosporioides* fungus. Antifungals has been used to control this post-harvest disease, and the essential oils (EOs) have been studied as a natural alternative. However, as EOs show different compositions and different species of fungus can show variable degree of susceptibility for a specific EOs, they should be tested for a specific use. The objective of this study was to evaluate in vitro the essential oils of *Eucalyptus staigeriana, Lippia sidoides* and *Pimenta pseudocaryophyllus* individually and blended to control *Colletotrichum gloeosporioides* fungus. The in vitro evaluation was performed using the EOs individually and in binary and ternary blends as follows: M1 (*L. sidoides* + *E. staigeriana*), M2 (*L. sidoides* + *P. pseudocaryophyllus*), M3 (*E. staigeriana* + *P. pseudocaryophyllus*) and M4 (*L. sidoides* + *E. staigeriana* + *P. pseudocaryophyllus*). The bioanalytical method was carried out checking the mycelial growth of *C. gloeosporioides* on basal medium, where different concentrations (0; 31 ppm; 62 ppm; 125 ppm; 250 ppm and 500 ppm) of EOs or blend were dissolved.[1] The Minimum Inhibitory Concentration (MIC) was considered as the lowest dose in which no growth of *C. gloeosporioides* was visible by naked eyes. The results showed that essential oils of *L. sidoides*, *E. staigeriana* and *P. pseudocaryophyllus* had inhibitory activity against *C. gloeosporioides* with a MIC of 125 ppm, 500 ppm and 500 ppm, respectively. Among blends, M1 and M2 show a MIC of 250 ppm, while for M3 and M4 the MIC was of 500 ppm. In view of the data obtained, the study suggests that all the essential oils and the blends had antifungal activity against *C. gloeosporioides*, but *L. sidoides* essential oil individually had the highest inhibitory activity therefore it should be considered for future in vivo studies on Papaya fruits.

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Acknowledgements: Farmanguinhos, Fiotec, CNPq, CAPES, FIOCRUZ, IME.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Environmental Impact Generated by the Effluents from the Extraction Process by Steam Distillation of Essential Oils

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Keywords: steam distillation; aromatic plants; effluents.

The extraction process by steam distillation of essential oils is the most widely used process in the industry of this segment, mainly because of its simplicity and low cost.[1] Studies involving this process analyze and evaluate chemical compositions, quality and the applications of various plant sources. Nonetheless, the extraction process under discussion has not been evaluated with environmental focus yet. The globalized world has the need for studies on the variable environmental for its survival.[2] Therefore, this study aimed at studying the environmental impact caused by effluents from the extraction process by steam distillation of the essential oil from two aromatic plants: *Rosmarinus officinalis* L. and *Pinus elliottii* Engelm. The effluents are one of the critical issues in environmental impact assessment, since their generation is inherent in the activity or production processes.[3] The effluents are generated in two stages of the process: an effluent is located within the extraction vessel after the end of the process; the other one is a result of the liquid-liquid separation in the final stage of the essential oil processing, also known as hydrolate. The effluents were characterized by analysis of environmental standards according to CONAMA 430/2011 and 128/2006 CONSEMA resolution, as well as CONAMA 357/2005. Hence, it was possible to divide these analyses by studying the following physico-chemical parameters: pH, dissolved oxygen, chemical oxygen demand, biochemical oxygen demand, total organic carbon, color, turbidity, electrical conductivity, ammoniacal nitrogen, nitrate, nitrite, suspended solids, dissolved solids and total solids. The results show that the parameters linked to organic matter (COD, BOD and TOC) of the effluents do not meet the requirements of current legislation, thus demystifying the fact that the waste of processes applied to natural products are not pollutants, since they present negative impacting potential on the environment. The experimental results of COD, BOD, and TOC found to effluents of rosemary and pine steam distillation processes were 9.812 mg L^{-1}, 1.463 mg L^{-1}, 750 mg L^{-1} and 20.928 mg L^{-1}, 4.500 mg L^{-1}, 2.100 mg L^{-1}, respectively. Eventhough the hydrolate does not meet the environmental requirements, it is also possible to say that it does not characterize as an environmental problem, since some products have commercial application, such as the rosemary and pine hydrolates.

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Acknowledgements: CAPES, Tekton Óleos Essenciais.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Analysis of Essential Oils from Breu Samples Collected in Alto Erepecuru Region, Brazilian Amazon

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Keywords: *Protium;* essential oil; *breu*.

Species belonging to Burseraceae family are characterized by production of oleoresins known in Brazil as *breu*, which is called black or white *breu* according to its organoleptic characteristics.[1] These oleoresins are employed by traditional communities for headaches treatment by burning and subsequent inhalation. in this study, we characterized the essential oils (eo) obtained from *breu* samples collected in the Alto Erepecuru region, Oriximiná, Pará. Aerial parts of each individual were also collected for identification purposes and vouchers were deposited in the INPA Herbarium, Manaus-AM. EOs were obtained by hydrodistillation using a modified Clevenger-type apparatus. Refractive index and optical rotation of each essential oil were measured. Chemical characterization was performed by GC/MS and GC/FID using an Agilent system with a HP-5 column. In addition, essential oils were also analyzed by INNOWAX polar column to separate co-eluted major compounds. Identification was made by comparison of the mass spectra with Wiley library and linear retention indexes. Relative quantification was based on the FID signals. Ten plant samples were collected and nine were identified, of which eight belonged to *Protium* genus, and one to *Tetragastris*. EOs yields ranged from 0.8 % to 5.4 % w/w, while the refractive indices ranged from 1.4673 to 1.4937 and optical rotation from -0,668 º to +3,664 º. Yield variation can be associated with the time and type of oleoresin exposure to the environment as well as its chemical composition, which will also influence the physicochemical parameters. A total of 126 compounds were identified. δ-3-carene/ iso-silvestrene mixture represented the main compounds in *P. heptaphyllum* and *P. aracouchini*, ranging from 40.9 % to 79.5 %. *P. opacum* and the unidentified species showed *p*-cymene as the major component and a chemical composition rich in monoterpenes. *P. spruceanum* was rich in sesquiterpenes, mainly γ-cadinene (14.4 %). *T. panamensis* also presented a high amount of sesquiterpenes, but major compound was *p*-cymene (16.4 %). *P. unifoliolatum* presented limonene/ β-phellandrene mixture (41.1 %) and α-terpineol (30.9 %) as major compounds and was the only sample with a high content of oxygenated terpenes (36.3 % of the identified compounds). *P. strumosum* was rich in monoterpenes (94.5 %) as α-pinene (57.7 %) and limonene/β-phellandrene mixture (10.8%). So, five groups could be formed by different species according to the similarity in their composition and the differentiation between white and black breu established by the Quilombolas is more visual than chemical.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

In Vitro Single and Combined Antibacterial Effect of *Eucalyptus globulus* and *Pimenta pseudocaryophyllus* Essential Oils on Multi-drug resistant *Enterococcus faecalis* and Probiotic *Lactobacillus rhamnosus*.

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Keywords:Antibacterial activity; multi-drug resistant bacterium; probiotic bacterium.

Antibiotics have been used in the animal nutrition as growth promoters. However, several consequences have arisen of this, principally, the emergence of multi-drug resistant pathogens like *Enterococci* bacteria. Similarly, several *Lactobacillus* species have been used as probiotics in poultry and pig nutrition to compete against detrimental bacteria and promote the growth. Therefore, the aim of this research was evaluate the effect of single and combined antibacterial effects of *Eucalyptus globulus* and *Pimenta pseudocaryophylllus* essential oils on multi-drug resistant pathogenic bacterium *Enterococcus faecalis* and probiotic bacterium *Lactobacillus rhamnosus*. To evaluate the antibacterial effect of single oils, microdilution method was used to determine the Minimal Inhibitory Concentration (MIC) through two-fold serial dilutions from 14.80 to 0.116 $mgmL^{-1}$. MICs were evaluated by the resazurin test. Minimal Bactericidal Concentration (MBC) was evaluated by plating. The results showed that for *E. globulus* oil the MIC was 14.80 $mgmL^{-1}$ for both bacteria, *E. faecalis* and *L. rhamnosus*. To *P. pseudocaryophylllus* oil, was 7.4 mg $mL^{-1}$to *E. faecalis* and 3.7 mg $mL^{-1}$to *L. rhamnosus*. The *E. globulus* oil was bactericidal at 14.8 mg mL^{-1} to *E. faecalis* and to *L. rhamnosus* and *P. pseudocaryophyllus* oil was bactericidal at 7.4 mg mL^{-1} to *E. faecalis.* The combined antibacterial effect for these two oils was evaluated by the determination of Fractional Inhibitory Concentration (FIC) by resazurin test and by calculation the FIC index values. *E. globulus* oil was ranged from MIC to 1/8 x MIC and *P. pseudocaryophylllus* from 2 x MIC to 1/32 x MIC for *E. faecalis* and *L. rhamnosus*. Two combinations (1/8 x MIC *E. globulus* + MIC *P. pseudocaryophylllus* and MIC *E. globulus* + 1/32 x MIC *P. pseudocaryophylllus*) were inhibitory to *E. faecalis* and FIC index values were 1.125 and 1.035 for the two combinations, respectively. These values indicate an indifferent (non-interactive) effect of these two oils to *E. faecalis*. The combination conformed by 1/2 x MIC *E. globulus* + 1/2 x MIC *P. pseudocaryophylllus* was inhibitory to *L. rhamnosus* and the FIC index value was 1.0 indicating an additive effect of these essential oils. Hence, these results showed that it was not possible to find a synergic effect to combat the pathogenic bacteria *E. faecalis*. On the other hand, the results obtained to single *E. globulus* oil showed a selective effect on the probiotic bacterium *L. rhamnosus*, although it was inhibitory to this bacterium, it was not bactericidal to it, compared with *E. faecalis.*

Acknowledgements: Ministerio de Educación del Perú-Programa Nacional de Becas y Crédito Educativo (PRONABEC) for Carmen M. Sinche Ambrosio's scholarship.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Chemical Composition of the Essential oil from *Wedelia calycina*

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Keywords: *Wedeliacalycina;* antioxidant activity; essential oil.

Wedelia genus comprises about sixty species distributed among tropical zones and warm regions, including countries such as India, China and Japan. Several of these species, have been used through the years by small communities for the treatment of different ailments and infectious diseases.[1] Research on *Wedelia* species essential oils is relatively new, however, the chemical constituents of *Wedelia calycina* have not been described. *W. calycina* was collected in two different municipalities in Santander, Barichara (COL559439) and Zapatoca (COL578353), and a third one in Palmira, Valle del Cauca (COL582605). Essential oils from Santander-collected plant material were obtained by microwave-assisted hydrodistillation (MWHD), while steam distillation was used for Valle del Cauca-collected plant. Essential oils analysis was carried out on an Agilent GC-MS system (EI, 70 eV), using two capillary columns DB-5MS DB-WAX. Helium was used as carrier gas at 1 mL min^{-1}. Compound identification was performed by comparing linear retention indices and mass spectra with a built-in library data base. Oils from *W. calycina* collected from Barichara (W1) and Zapatoca (W2), showed a very similar chemical composition, with limonene (28 % and 31 %, respectively) as the main metabolite; followed by α-pinene (14 % and 55 %, respectively). β-pinene (25 %) appeared in W1 and in a lesser extent in W2 (2 %); *E*-caryophyllene, also appeared in W2 (7 %), but in a lesser extent in W1 (4 %); *W. calycina* from Valle del Cauca (W3) showed a different chemical composition, with α-pinene (20 %) as the major metabolite, followed by β-pinene (14 %), α-phellandrene (15 %) and germacrene D (1 5%). This oil did not contain any limonene in its chemical composition. The antioxidant activity (expressed as µmolTrolox g^{-1} substance) of the obtained essential oils were: 600 ± 60 (W1), 710 ± 60 (W2) and 880 ± 50 (W3). The results obtained by the ORAC method showed that all essentials oils studied presented antioxidant activities higher than those of BHT (459 ± 9) and α-tocopherol (550 ± 13), typical standard antioxidants which were used as reference compounds.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101for access to genetic resources and derivatives fo rscientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization of *Achyrocline satureioides* (Asteraceae) Essential Oils from Colombia by GC-MS

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Keywords: *Achyrocline satureioides;* essential oil; GC-MS.

Achyrocline satureioides (Asteraceae family), is a plant native to South Africa and distributed in tropical regions. Their inflorescences are used in the treatment of a variety of medical pathologies in alternative medicine. This species has been shown to have anti-inflammatory, antispasmodic, analgesic and sedative properties.[1,2] The plant material was collected in Zapatoca (Santander, Colombia). Extraction was performed by microwave-assisted hydrodistillation (MWHD) with a Clevenger-type apparatus placed inside a domestic microwave oven (Whirlpool, 1000 W, 2.45 GHz) with a side orifice, through which an external glass condenser joined the 2-L round flask that contained the plant material (*ca*. 300 g) and water (*ca*. 0.5 L). The oven was operated for 45 min (3 x 15 min) at full power, which caused water to boil vigorously and reflux. Essential oil was decanted from the condensate and dried with anhydrous sodium sulfate. The analysis was performed on an Agilent GC/, split/splitless injector (split 30: 1) and a ChemStation data system, G1701-DA, which included the ADAMS, NIST and WILEY spectral libraries. Fused-silica capillary columns DB-5MS [(J&W Scientific) of 60 m X 0.25 mm id, coated with 5% phenyl poly(dimethylsiloxane) (0.25 µm film thickness)] and DB-WAX [(J&W Scientific) of 60 m X 0.25 mm id, coated with poly(ethyleneglycol) (0.25 µm film thickness)] were used. The GC oven temperature was programed from 45 to 150 °C (5 min) at 4°C min^{-1}, then to 250 °C (5 min) at 10 °C min^{-1} and to 275 °C (15 min) at 10 °C min^{-1}. The compounds identified by GC-MS include *trans*-β-caryophyllene (25 %), followed by caryophyllene oxide (13 %), γ-muurolene (8 %), γ-cadinene (7 %), and α-pinene (7 %). These compounds displayed marked inhibitory effects in different inflammatory experimental models in mice and rats.

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Acknowledgements: Colciencias - Patrimonio Autónomo Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas, Contract RC-0572-2012. Contract No. 101for access to genetic resources and derivatives for scientific research with bioprospecting aims, between Ministerio del Medio Ambiente y Desarrollo Sostenible and Unión Temporal Bio-Red-CO-CENIVAM.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Volatile Fraction of *Medinilla myriantha* (Melastomataceae) Flowers by Solid-Phase Microextraction and Gas Chromatography Coupled to Mass Spectrometry

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Keywords: HS-SPME; GC-MS; volatile fraction; *Medinilla myriantha.*

The *Medinilla* genus of the Melastomataceae family includes about 375 species. The plant *Medinilla myriantha*, native to Indonesia,[1] grows to 160 centimeters tall. The small flowers of this species are characterized by their pink and shiny appearance. These are produced in large panicles in hanging stems. The objective of this work was to study the chemical composition of the volatile fraction of *M. myriantha* flowers, which were collected (N°583901 of the National Herbarium of Colombia) at the pilot agro-industrial complex of the National Research Center for Agro-industrialization of Tropical Aromatic and Medicinal Plants (CENIVAM), located on the main campus of Universidad Industrial de Santander (Bucaramanga, Colombia). Fresh *M. myriantha* flowers (1.5 g) were put inside an amber vial (15 mL), and brought to thermal equilibrium (10 min) at 60 °C. The sampling was done by a solid-phase microextraction (SPME) fiber (coated with PDMS/DVB, PDMS or CAR/PDMS) in the vial during 30 min. The largest chromatographic area was obtained with the CAR/PDMS fiber. Triplicate sampling was performed at different times of day (6:00 am; 12:00 m; 6:00 pm). Compound identification was based on data obtained under split (30:1) injection with a GC 7890 (*Agilent Technologies* 6890N, Palo Alto, CA, EE.UU.) gas chromatograph equipped with a mass selective detector AT 5975C (electronic ionization, 70 eV), and a data system (MSDChemStation version G1701-DA), which included mass spectral libraries (ADAMS, NIST, WILEY). A capillary column with polar stationary phase of poly(ethylene glycol) (DB-WAX, J&W Scientific) of 60 m X 0.25 mm X 0.25 µm (d_f) was used. The main components of *M. myriantha* flower fragrance were 1-octen-3-ol (53 %), nonanal (6 %), octan-3-ol (4 %), and octan-3-one (3 %). The main compound, 1-octen-3-ol, was less abundant (22 %) at 6 pm. When used alone, 1-octen-3-ol has been a good mosquito attractant for only a few species. However, there appears to be a synergistic response of species of the genera *Aedes, Anopheles, Coquillettidia, Psorophora*, and *Mansonia* to the combination of 1-octen-3-ol and CO_2.[2]

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Acknowledgements: Colciencias - National Autonomous Patrimony Financing Fund for Science, Technology and Innovation, Francisco José de Caldas, Contract RC-0572-2012. Industrial University of Santander.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Study of the Volatile Fraction *Persea americana* and *Veitchia merrillii* Flowers by Solid Phase Microextraction

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Keywords: *Veitchia merrillii; Persea americana;* solid phase microextraction (SPME).

Veitchia merrilli (Arecaceae) is a species of palm native Philippines. It is a palm to the widely used in gardening for its colorful fruit, and its flower attracts many insects. Avocado (Lauraceae), originally *Persea americana* species from Central America, is cultivated today in tropical and subtropical climates. In traditional medicine it is used to treat conditions such as menorrhagia, hypertension, bronchitis, diarrhea and diabetes.[1] The aim of this study was to determine the compounds that may make them so appealing to insects. The monitoring was conducted at the morning and in the afternoon. The metabolites were extracted from flowers, collected by solid phase microextraction, SPME and analyzed on a gas chromatograph coupled to a mass selective detector. To improve the reproducibility and increase the sample transfer to the chromatographic column, liner 78.5 mm X 0.75 mm X 6.5 mm O.D., and splitless injection mode were used. The extraction efficiencies for three fibers, CAR-PDMS, PDMS and PDMS-DVB, with different polarity, were compared. The exposure temperature for the fibers (40 and 60 °C) was evaluated with 30 min. The metabolites were in DB-Wax, and DB-5MS columns. For *V. merrillii*, the main compounds found were *trans*-β-ocimene, *cis*-β-ocimene, linalool, *allo*-ocimene, methyl salicylate and eucalyptol. For avocado, the main compounds present in the flower fragrance during the morning were *cis*-3-hexenol, 3-methylbutan-1-ol and limonene. In the afternoon, the compounds were similar but linalool appeared as the main compound. Eucalyptol is used as an insecticide and insect repellent,[2] linalool is used in some mosquito-repellent products; however, the EPA notes that a preliminary screen of labels for products containing linalool indicates that efficacy data on file with the Agency may not support certain claims to repel mosquito.[3]

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Acknowledgements: Colciencias - National Autonomous Patrimony Financing Fund for Science, Technology and Innovation, Francisco José de Caldas, Contract RC-0572-2012. Industrial University of Santander.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Antioxidant Activity of *Calycolpus moritzianus* Essential Oil

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Keywords: *Calycolpus moritzianus;* essential oil; arrayán.

Calycolpus O. Berg is a genus of about 15 species found from Central America to Brazil. *C. moritzianus, Psidium Psidiopsis moritziana caudatum* are commonly known in Colombia as "myrtle" or "cinaron". It is a tree about 15 meters high, with green and elliptical leaves.[1] Two specimens of *C. moritzianus* were collected in the municipalities of Los Santos and Zapatoca, Santander, Colombia, identified in the National Herbarium of Colombia (voucher n. 560251 (C1, Los Santos) and 578360 (C2, Zapatoca). The extraction of essential oil (EO) was performed by microwave-assisted hydrodistillation. Characterization of essential oils was performed with an Agilent GC/MS. Polar [DB-WAX, 60 m X 0.25 mm X 0.25 µm, with stationary phase of poly(ethylene glycol)] and nonpolar [DB-5MS, 60 m X 0.25 mm X 0.25 µm, with stationary phase of 5 % phenyl-poly(methylsiloxane)] capillary chromatographic columns were used. Oven temperature was programmed from 45 to 150 °C at 4 °C min^{-1}, maintained for 7 min, and then from 150 to 230 °C at 4 °C min^{-1} and held for 40 min. The oxygen radical absorption capacity (ORAC) assay was performed in a multiplate reader (Turner Biosystems). Essential oil extraction yields of 0.25 % (C1) and 0.49 % (C2) were obtained. The chemical characterization was based on mass spectra (EI, 70 eV) and retention indices. These two species showed a very similar chromatographic profile. The major components for the Los Santos collected species were: limonene (36.0 %), 1,8-cineole (10.9 %) and α-terpinene (7.0 %), while for the Zapatoca collected species were: limonene (33.0 %), 1,8-cineole (15.9 %) and α-terpinene (7.9 %). Diaz *et al*.[2] found that for these species the major components were *trans*-β-caryophyllene (22 %), α-pinene (11 %) and viridiflorol (10 %). The ORAC antioxidant activity values of studied EO were 970 ± 77 µmol Trolox®/g sample (C1) and 570 ± 62 µmol Trolox®/g sample (C2), which were superior to those of the reference substances, α-tocopherol (550 ± 13 µmol Trolox®/g sample) and BHT (457 ± 9 µmol Trolox®/g sample).

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Acknowledgements: Colciencias - National Autonomous Patrimony Financing Fund for Science, Technology and Innovation, Francisco José de Caldas, Contract RC-0572-2012. Industrial University of Santander.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemotaxonomic Evaluation of Mandarin Orange Hybrids

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Keywords: Essential oils; polymethoxyflavonoids; mandarin-orange hybrids.

The citrus fruit production is one of the most important agricultural activities in Uruguay, where 52 % of the fruit production is represented by oranges followed by mandarins (36 %).[1] Citrus can be propagated, in addition to natural forms of propagation, by artificially crossed cultivars created by citrus breeders. In Uruguay, these activities are developed by the E.E.F.A.S–INIA citrus development program, which is based on hybridization strategies. In mandarins, activities are focused on widening the harvesting period, thereby allowing for a more extensive fruit production season, providing fresh fruit to the market all year. As a consequence of genetic improvement, the essential oils (EOs) usually present different chemical and organoleptic profiles.[2] The main goal is creating new fruit varieties that fulfill different consumer quality requirements, relating to fruit composition and sensory characteristics, thickness of albedo, size, color, and aroma.[2] In this work, a chemotaxonomic approach was used to differentiate mandarin hybridization assays through their EO composition. Seven commercial varieties (Clementina de Nules, Nova, Mandarina Común, Afourer, Murcott, Page, Ortanique) and seven experimental hybrids (CCMIII, VII-3, A 172, A 201, B 47, M 9, M19) were selected for analysis. EOs were manually extracted from the skins and then analyzed using gas chromatography–mass spectrometry (GC-MS). Eighty-nine components were identified, many strongly associated with mandarin aroma (thymol, methyl *N*-methyl anthranilate).[3] Many oxygenated compounds showed differences for several different hybrids (B47, A201, IV-13, VII-3) and for two commercial varieties (Nules, Murcott). Typical citrus volatile compounds (neral, geraniol, citronellol) were detected in 4 of the hybrids (ccm-III, M9, B47, IV-13) and Ortanique. The use of long-time-temperature GC/MS programs allowed identifying five polymethoxyflavonoids (PMFs): nobiletin, tangeretin, sinensetin, hexamethoxyflavone, and heptamethoxyflavone. Common Mandarin, Nova, and VII-3 showed all five PMFs in their oil composition. Through a chemometric analysis, the parental-hybrids relationships were evaluated using the volatile compositions studied. Three clusters were identified, showing the Common mandarin as a unique variety, while A172, B47, and ccm-III were defined as Satsuma mandarin-orange crossing derivatives.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Dental Products with *Lippia gracilis* Schum Essential oil and Antimicrobial Evaluation Action Against *Streptococcus mutans*

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Keywords: *Lippia gracilis*; toothpastes; *Streptococcus mutans.*

Essential oils are composed by volatile organic compounds. Lipophilic, mainly extracted from the leaves, they can be found in various parts of the plants and are characterized by their strong aroma found in plants, presenting themselves as a promising source of new antimicrobials. Efforts have been realized aiming the prospection, isolation and characterization of active principles to be used in formulations. The tooth decay is caused by acid actions that come from the food and beverage decomposing due bacteria that live in human mouth, which causes the tooth enamel erosion and subsequent corrosion. The essential oil extracted from *Lippia gracilis* Schum, a deciduous and branched shrub, contains in their terpenic composition some monoterpenes, and as major components thymol, carvacrol and *p*-cymene. They present pharmacological, fungicide and antimicrobial activities. The oil was extracted from the dried leaves by hydrodistillation and the constituents were identified by GC/MS. The obtained oil presented 4.7 % yield and the GC/MS analysis led to the identification of 23 compounds. Major compounds were thymol (77.0 %), *p*-cymene (7.4 %) and thymol methyl ether (4.7 %). Incorporation *L. gracilis* essential oil in a formulation did not change its physical characteristics, neither influence the evaluated parameters in the stability tests. Through evaluation of the effectiveness of the formulation against *Streptococcus mutans*, it was possible to establish the Minimum Inhibitory Concentration (MIC) at $0{,}625 \times 10^{-3}$ µg mL^{-1} and the Minimum Bactericidal Concentration (MBC) at $0{,}625 \times 10^{-3}$ µg mL^{-1} for both, of the formulation with *L. gracilis* essential oil. The developed product showed a bactericidal action and, therefore, is effective for preventing the onset of caries.

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Acknowledgements: CAPES, Bionorte, UFMA, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Characterization and Antimicrobial Activity of Essential oils from Brazilian Cerrado Species.

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Keywords: Asteraceae; essential oil; brazilian Cerrado.

Brazilian biodiversity, which comprises *circa* one-sixth of total plant species is divided into important biomes such as the Amazon rainforest, the Atlantic Forest and a savanna area in Central Brazil, known as Cerrado. Cerrado is the second largest Brazilian biome, but the most threaten by anthropic pressure. Only a small fraction of the 12000 known botanical species were chemically investigated, making Cerrado a very promising source for flavor and fragrance applications.[1] A project in order to investigate the chemical composition of the rich flora from the Cerrado is going on and this study shows three species of the family Asteraceae and there antimicrobial activities. The Asteraceae is the botanical family with the largest number of species, about 50000. *Hoehnephytum* and *Baccharis* species were collected in Brasilia, Brazil from different places: Ermida Dom Bosco, IBGE and Cooperbraz Farm. Samples of these species have been deposited in the herbarium of Embrapa Genetic Resources and Biotechnology. The leaves of the bushes were subjected to hydrodistillation separately in a Clevenger-type apparatus for 2 h each. The oils were analyzed by GC/FID and GC/MS (Agilent), both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen (GC/FID) and helium (GC/MS) were used as carrier gas. Oven temperature was raised from 60 to 240 °C at 3°C min^{-1}. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. In the oil from *Hoehnephytum* (RFV2479) the major components were spathulenol (25.1 %), caryophyllene oxide (9.7 %) and limonene (5.2 %). In *Baccharis* (RFV2495) the major components were spathulenol (25.2 %), limonene (11.9 %) and kaurene (6.8 %). In *Baccharis* (RFV2466) the major components were bicyclogermacrene (17.8 %), limonene (10.9 %) and spathulenol (9.9 %). The antimicrobial activity in *C. albicans* (ATCC 10231) with samples oils of *Hoehnephytum* (RFV2479) and *Baccharis* (RFV2495) showed MIC (minimal inhibitory concentrations) of 156 µg mL^{-1}. For *Baccharis* (RFV2466), a MIC of 312 µg mL^{-1} was recorded. In conclusion, the essential oils tested presented a low antimicrobial activity against *C. albicans*.

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Acknowledgements: Embrapa, Faperj, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Topical Anti-inflammatory Activity of *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae) Essential Oil

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Keywords: *Plectranthus amboinicus*; essential oil; ear edema.

Plectranthus amboinicus is known as malvariço, malva-do-reino, hortelã-graúda, and is widely used in folk medicine for the treatment of inflammation, cancer, and respiratory infections.[1] The aim of this study was to analyze the chemical composition of *P. amboinicus* essential oil and investigate its topical anti-inflammatory activity. Fresh leaves (500 g) were collected at 7:00 am in the Medicinal Garden of the Federal University of Pernambuco and extracted by hydrodistillation in a Clevenger apparatus for 2h. Chemical analyses were done by GC/MS (Agilent), with a capillary column J&W HP-5MS (30 m X 0.25 mm X 0.25 µm). The oven temperature was from 70 °(4 °C min^{-1}) until 280 °C, then maintained for 15 min. The carrier gas was helium, with a constant flow rate of 1.4 mL min^{-1}.. Mass spectra were recorded from 30 to 450 u, and compounds identified by matching mass spectra with database and LRI. Topical anti-inflammatory activity was evaluated by croton oil-induced ear edema in mice. Briefly, mice were anesthetized with halothane and received 20 µL of croton oil 2 % in acetone at the right ear. After drying, animals received 3 µL of essential oil diluted in acetone. Left ear received acetone and was used as control. The animals were euthanized after 6 h and samples of 6 mm of diameter were taken and weighted for edema measurement. The results were expressed as mean ± SEM and analyzed by ANOVA with posttest of Bonferroni, $p<0.05$. Essential oil extraction yielded 0.015 %. CG/MS analyses identified 17 compounds, corresponding to 97.0 % of the oil. Of these compounds, 76.3 % were monoterpenes (55.3 % oxygenated), and 20.1 % were sesquiterpenes (1.2 % oxygenated). The major compounds found were carvacrol (53.9 %), *p*-cymene (12.8 %), β-caryophyllene (10.0 %), α-bergamoptene (5.4 %), γ-terpinene (5.2 %), and α-humulene (2.9 %). Topical administration of *P. amboinicus* essential oil had no effect on the croton oil-induced ear edema, while dexamethasone (0.1 mg ear^{-1}) inhibited the ear edema by 81.3 %. This animal model is used as a screening method for topical active anti-inflammatories. This data shows that the essential oil of *P. amboinicus* does not have topical anti-inflammatory activity in the used dose.

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Acknowledgements: UFPE, FACEPE, CNPq, and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparison of Different Methods and Extraction Times on Essential Oil Yield of *Piper gaudichaudianum* Leaves (Piperaceae) from Paraná State, Brazil

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Keywords: Bioprospecting; *Piper* species; extraction protocol.

Species of the Piperaceae family are widely used for medical purposes. Therefore, the essential oil of this family has been used in different sectors of the pharmaceutical, chemical and cosmetic industries. However, studies to optimize the extraction methods of essential oils from this plant group are still scarce and commonly follow protocols established for other botanic families. Thus, the aim of this study was to test different times and extraction methods to obtain the highest yield of essential oil of fresh leaves from *Piper gaudichaudianum*. The plants were collected in São José dos Pinhais/PR on July 12th 2015 and were identified in the herbarium of Botanical Garden of Curitiba/PR. For essential oils extraction of *P. gaudichaudianum* from the leaves three methods were used: whole leaves, chopped leaves (with scissor), and triturated leaves (with blender). We also tested different extraction times for each type of leaf preparation (1h 00m, 1h 30m, 2h 00m, 2h 30m, 3h 00m, 3h 30m and 4h 00m). Three extractions (repetitions) were performed per treatment (extraction time x leaf type). Each extraction consisted of 100 g of leaves into 1 L of water placed in a glass flask of 2 L. All essential oils were obtained using a modified Clevenger-type apparatus. A completely randomized design with treatments in a factorial scheme (3x7) was used. We tested the interaction between type of leaves (x:1) and extraction times (x:2). Data were submitted to variance analyses (ANOVA), normality and Tukey tests at 5 % probability. The average yield of essential oils varied between 0.68 % for triturated leaves with 1-hour extraction and 1.84 % for chopped leaves with 4 hours of extraction. The results showed no interaction between the tested factors (Df= 12; F= 1.4214, P= 0.1945; C.V.= 14:56%). However, there was significant difference for the isolated factors (extraction time - Df= 6; F= 20.3300, P< 0.001 and type of leaves - Df= 2, F= 10.2917, P= 0.0001). Whole and chopped leaves yielded statistically more essential oils than the triturated leaves. From 2h 30m and more there was no significant increase in essential oil yield. This data is important because it reduces almost in half the extraction time commonly used for Piperaceae species (4 hours). So, we suggest that for *P. gaudichaudianum* use of chopped leaves and extractions time of 2h30m, because they provided the best yield (oil quantity). In addition, we highlight the necessity of studies for compare the main chemical constituents obtained in these extraction methods, and verify if the quality of the extracted oils are significantly similar between the different processing of leaves and extraction times.

Acknowledgements: UFPR, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of *Baccharis dracunculifolia* Essential Oil and its Larvicidal Activity Against *Lucilia cuprina* (Diptera: Calliphoridae) Using Ethanol as a Vehicle.

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Keywords: Myiases; biological control; botanical insecticide.

Essential oils are complex, volatile natural compounds produced by herbs. Native from Brazil, *Baccharis dracunculifolia* (Asteraceae), popularly called "field rosemary consists on the biological origin of green propolis. The acaricide activity of *B. dracunculifolia* essential oil was recently proven[1] and suggests that this species also has the potential for research as an insecticide. *Lucilia cuprina* arouses attention because there is evidence of primary myiasis caused by these flies. Thus, the present study evaluated the essential oil activity of *B. dracunculifolia* grown in the state of Santa Catarina, on the third instar of *L. cuprina* under "in vitro" conditions. The essential oil was extracted from the shoots of approximately 50 plants using a Clevenger apparatus and its chemical composition was determined by GC/MS. For mortality evaluation groups of 20 larvae (F2) were exposed in bottles (9 x 4 cm) containing filter paper impregnated with 0.3 mL of the essential oil solubilized in absolute ethanol at concentrations of 357.8, 173.9, 87.2, 41.1, 18.9 mg mL^{-1}, equivalent to 40, 20, 10, 5 and 2.5 % oil, respectively, using four replicates for each treatment/concentration. The larvae were maintained at room temperature with 27 ° C ± 1 ° C and relative humidity of 70 ± 10 % observing mortality between 24 and 48 h. Essential oil of *B. dracunculifolia* analysis identified 101 compounds by comparing the mass spectra and retention indices. The major constituents detected were β-pinene (9.9 %) limonene (9.5 %), nerolidol (7.9 %) and caryophyllene (7.6 %). Lethal dose DL_{10}, DL_{50} and DL_{90} after 24 and 48 h of exposure were 41.63, 82.38, 123.13 and 38.15, 75.69, 113.23 mg mL^{-1}, respectively. Thus, it can be concluded that the essential oil of *B. dracunculifolia* shoots when diluted with absolute ethanol as a vehicle has activity on of *L. cuprina* larvae, demonstrating potential use as an insecticide.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Anti- Inflammatory Activities of Essential Oil from the *Campomanesia adamantium* Fruit

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Keywords: Inflammation; pleurisy, oedema; limonene.

Cerrado region is the second largest biome in Brazil, only after Amazonia. This ecosystem comprises more than 7.000 plant species. [1] Due to its diverse flora, research interest has been increased and endemic medicinal plants from Cerrado have been a source of bioactive compounds. “Gabiroba”, “guabiroba-do-campo” or “guavira”, the fruit of *Campomanesia adamantium* (Cambess.) O. Berg, is widely found and used in areas of Cerrado, mainly in the Midwest region and Atlantic Forest in the Southeast and South regions of Brazil. *C. adamantium* belongs to Myrtaceae family and its fruit, as well as fruits from other species of *Campomanesia*, are traditionally used in the production of homemade liqueurs, juices, and sweets and also employed in folk medicine as anti-rheumatic, antidiarrheal, hypocholesterolemic, anti-inflammatory and to the treatment of cystitis and urethritis. The present study evaluated the anti-inflammatory activities of essential oils from seed (EOS) of *C. adamantium* fruits in animal models. Essential oil was obtained from 200 g of dried seeds from *C. adamantium* by hydrodistillation (3 replicates) using a Clevenger-type apparatus for 3 hours. Samples obtained by hydrodistillation was analyzed by GC/qMS (Shimadzu P2010 plus. Shimadzu Tokyo, Japan) equipped with an auto injector split/splitless. Different groups were treated with doses of 100 and 300 mg kg^{-1} and the inflammatory parameters were evaluated in carrageenan induced paw edema and leukocyte migration in pleurisy model. The major constituents of EOS were was limonene (20.9 %), β-pinene (11.5 %) and α-pinene (8.5 %). Paw edema was inhibited at all times, and maximal inhibition was at the dose of 100 mg kg^{-1} at 2 h after carrageenan injection with 74 ± 2 % for EOS. It was observed significant decrease ($P < 0.01$) in leukocyte migration at the dose of 300 mg kg^{-1} of EOS, with maximal inhibition of 80 ± 6 % for EOS. This is the first evaluation of the anti-inflammatory effects of the essential oils of fruits (EOS) of *C. adamantium* in animal models. Analyzes using chromatography (GC/MS) indicated the presence of limonene that can be characterized as being a contributor to the anti-inflammatory effects, suggesting their use as nutraceutical or pharmacological agent.

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Acknowledgements: Rede Pró Centro-Oeste, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Seasonal Study of Essential oils of *Baccharis mesoneura* DC Leaves.

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Keywords: *Baccharis mesoneura*; seasonality; PCA.

The genus *Baccharis* (Asteraceae)comprises about 500 species, from which 120 are found in Brazil. Many of these species are used in folk medicine to treat many diseases as inflammation, diabetes, anemia, among other purposes. However, it is estimated that only 15 % of the species of this genus have phytochemical studies and its bioprospecting potential evaluated. [1, 2] The objective is to study the seasonal chemical composition of essential oils of *Baccharis* abundant in southern and southeastern Brazil and still little investigated. In the present work, seasonal study of the essential oils of *Baccharis mesoneura* was evaluated. The leaves were collected in Piraquara - PR and a voucher specimen was deposited in the Herbarium of Museu Botânico Municipal de Curitiba. The dried leaves (70 g) were grounded and submitted to hydrodistillation in a Clevenger-type apparatus for 4 hours, in triplicate. The oils were analyzed by GC/MS in Shimadzu GC-2010 systems coupled with a mass spectrometer detector Shimadzu GCMS-QP2010 Plus. The GC/MS measurements were performed using a non-polar capillary column Rtx-5MS (5 % diphenyl - 95 % dimethyl polysiloxane, 30 m X 0.25 mm X 0.25 µm) operated under a temperature-programmed condition from 60 to 250 °C at 3 °C min^{-1}. The carrier gas was helium with a flow rate of 1.02 mL min^{-1}, injection volume of 1.0 µL in split mode (ratio 1:10). Oil components were identified by comparison of both arithmetical index (based on a homologous series of hydrocarbons from 9 to 22 carbons analyzed in the same conditions) and mass spectra with literature and spectral library. The Principal Component Analysis (PCA) was performed using Excel® and Matlab 7®. The yields of essential oils were 0.50, 0.69, 0.34 and 0.23 mL 70 g^{-1} for the summer, spring, autumn and winter samples, respectively. The analysis by GC-MS resulted in the identification of almost 50 compounds in each sample (approximately 90 % of the total oil), mainly comprising: α-tujene, α-pinene, β-pinene and spathulenol and smaller abundances of caryophyllene oxide and (*Z*)-caryophyllene in all samples, except for the autumn sample, which stood out (*E*)-caryophyllene, germancrene D and globulol. PCA analysis showed similarity between the oils obtained in the spring and summer where the main composition is hydrocarbon monoterpenes (> 40 %), followed by oxygenated sesquiterpenes (> 20 %). In winter it was found a predominance of oxygenated sesquiterpenes and hydrocarbon monoterpenes (> 30 %). However, the autumn oil showed most distinct profile, that was rich in sesquiterpenes (hydrocarbon sesquiterpenes > 41 % and oxygenated sesquiterpenes >25 %).

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Are Chemical Differences on the Essential Oils of Two Species of Genus *Ocotea* Responsible for their *in vitro* Antisnake Venom Activity?

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Keywords: oxygenated sesquiterpenes; electrophoretic SDS-PAGE; *Bothrops* sp.

Ocotea acutifolia (Nees.) Mez. and *O. diospyrifolia* (Meisn.) Mez., Lauraceae, are species well known by their use as phytotherapeutics in the northeastern Argentina. As far as we know, only the volatile constituents of *O. acutifolia* has been previously investigated, being caryophyllene oxide (57 %) and calarene epoxide (12 %) the main components found. [1] As a part of our research on the essential oils from species traditionally used as antisnake venoms, we report here the essential oil composition of two *Ocotea* species gathered in Corrientes Province (Argentina) at the same vegetative stage: *O. acutifolia* (I) from San Isidro and *O. diospyrifolia* (II) from Paso de la Patria. Aerial parts of both *Ocotea* species were gathered and voucher specimens were deposited at herbarium CTES (IBONE). The components of the oil were analyzed by GC and GC/MS. Once the *Ocotea* specimens were botanically identified, and considering potential misuses of this plant material by local population when it is identified as *Nectandra* sp., the potential hemolytic and anti-hemorrhagic effect of the essential oils obtained from I and II were evaluated against *Bothrops diporus* snake venom. Among the compounds present in the essential oils, 97.6 % were identified for *O. acutifolia* (I) and 98.2 % for *O. diospyrifolia* (II). The oils were characterized by high percentages of sesquiterpenes oxygenated, the amounts varied according to the species considered: 49.1 % for (I) and 62.5 % for (II). However, the chemical profiles were different for each species, being atractylone (22.0 %), β-selinene (14.6 %), β-caryophyllene (5.6 %) and spathulenol (4.6 %) the main components for I. Carotol (30.0 %), germacrene D (12.1 %), *epi*-globulol (9.7 %), longifoliol (8.6 %) and germacrene D-4-ol (7.1 %) showed the higher percentages in II. When the antisnake venom activity of the oils was evaluated, the *O. acutifolia* oil showed high activity by SDS-PAGE against *Bothrops diporus* venom, inhibiting completely the proteolytic activity on casein (1:100) and 50 % of the clotting activity (1:5). *O. diospyrifolia* oil showed activity inhibiting proteolysis of casein, but in a lesser extent than *O. acutifolia* oil, being restored only 15 % of the clotting time even at higher ratio (1:10). In brief, significant differences were observed in the chemical composition for both *Ocotea* species. However, the antisnake venom activity was much lower than for *Nectandra* sp., suggesting the need to inform the public to avoid confusions arising in the implementation of these medicinal plants.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Analysis of Volatile Fractions from *Piper tectoniifolium* Kunth (Piperaceae)

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Keywords: *Piper tectoniifolium*; hydrodistillation; SPME.

The Piperaceae family consists of 12 genera with about 2.000 species with a great distribution in the world. A wide range of volatile components can be found in *Piper* species including monoterpenes, sesquiterpenes, arylpropanoids, aldehydes, ketones and others. Essential oils (EO) comprise a complex mixture of volatile products of low molecular weight, lipophilic, usually odoriferous liquid and extracted from plants. This work is the first chemical profile investigation of the *P. tectoniifolium* volatiles from its different organs (leaves, stems and inflorescences) obtained by hydrodistillation (HD) and SPME. The fresh plant materials were fragmented and submitted separately to HD in a Clevenger-type apparatus for 2 h. The essential oil obtained was collected, stored at low temperature, and finally analyzed by (GC-MS) using a chromatograph Shimadzu QP 5000. Identification of the components was achieved through the retention index (RI) for the constituents and comparison with literature and spectrometer database. At the same time, leaves, stems and inflorescences were reduced to small pieces and extracted by HS-SPME, with CAR-DVB and PDMS fibers, at 60 °C, and sample/headspace equilibration time of 15 min. The extracted materials were also analyzed by GC-MS. The EO obtained from the leaves and stems *P. tectoniifolium* presented chromatographic profiles with their respective components at different concentrations. The EO from leaves presented as major component germacrene D (15.7 %), followed by β-elemene (8.3 %) and α-caryophyllene (7.3 %), and from stems, more complex than the leaves, a greater number of substances was found, such as the (E)-asarone (10.1 %) followed by α-muurolol (5.6 %) and hinesol (4.9 %). The EO, despite having a large number of substances, consists primarily of sesquiterpenes, highlighting the germacrene D as major constituent. The SPME technique exhibited the patterns: PDMS fiber for leaves: germacrene D (18.8 %), trans-β-guaiene (16.0 %) and β-elemene (6.6 %); for stems: germacrene D (18.1 %), α-humulene (8.6 %) and β-bisabolene (7.9 %); and for inflorescences: β-pinene (17.2 %), germacrene D (14.7 %) and α-pinene (13.8 %). CAR-DVB fiber for leaves: trans-β-guaiene (16.2 %), germacrene D (14.3 %) and α-humulene (5.7 %); for stems: germacrene D (10.0 %), α-humulene (8.4 %) and α-copaene (7.4 %); and for inflorescences: β-pinene (13.8 %), germacrene D (12.6 %) and α-pinene (11.1 %). The results obtained from SPME revealed the occurrence, among the three analyzed organs (leaves, stems and flowers), of relevant amounts of the sesquiterpene germacrene D (>10 %) for both fibers (CAR-DVB and PDMS).

Acknowledgements: Faperj, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Obtaining of Spathulenol from *Baccharis dracunculifolia* (Asteraceae) Essential Oil

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Keywords: *Baccharis dracunculifolia;* essential oil; spathulenol.

The search for new therapies based on medicinal plants for the treatment of schistosomiasis, a tropical neglected disease with high mortality rate, has been focused on our research group, especially essential oils. *Baccharis dracunculifolia*, Asteraceae, is a shrub plant that occurs in Brazil, from Minas Gerais to Rio Grande do Sul states. It has antimicrobial cytotoxic, hypoglycaemic and anti-inflammatory activities, and its Essential oils is rich in sesquiterpenes, mainly nerolidol and spathulenol. This study aimed to obtain an enriched fraction in spathulenol to evaluate schistosomicidal activity. OE's of 105 natural *B. dracunculifolia* specimes were obtained from fresh leaves collected at the winter season in 2012, on different locations like, Campos do Jordão/SP, Campinas/SP and Ubatuba/SP, were subjected to hydrodistillation separately in a Clevenger-type apparatus for 1 h and 30 min each and analyzed by gas chromatography coupled with mass spectrometry detection (GC-MS). The richest samples on spathulenol were clustered (nine EO's samples, 1.08 g) and fractionated on dry chromatography column (DCC) with silica gel 60 (0.063 to 0.200 mm) and dichloromethane, resulting 10 fractions (F1 to F10) which were analyzed by GC-MS. All plants showed OE yield greater than 0.5 %, especially Campinas and Ubatuba (1.11 and 1.09 %, respectively). Regarding spathulenol content, plants collected in Ubatuba had on average 22.6 % of this compound, those from Campos do Jordão 19.6 % and those from Campinas 10.8 %. The fractionation of the mixture containing nine OE's provided three fractions enriched in spathulenol: F6 (29.4 %), F7 (59.6 %) and F8 (30.6 %), with yields of 17.4, 19.4 and 12.5 % (w/w), respectively. The fractionation of *Baccharis dracunculifolia* essential oil by this method proved to be a fast and feasible alternative to getting rich fractions of spathulenol.

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Acknowledgements: FAPESP (2012/14136-9; 2013/05052-9).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Culture of Japana Branca (*Ayapana triplinervis* (Vahl) RM King & H. Rob.) in Different Levels of Fertilization and Shadding.

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Keyword: production; biomass; essential oil.

This species is an herb that occurs in the Amazon belonging to Asteraceae family, which use is in the traditional medicine of the region. [1] It has also been indicated for essential oil extraction aimed at the pharmaceutical industry, perfumery and cosmetics. [2,3] The objective of this study was to evaluate the culture Japana Branca under different shading levels and fertilization types, to increase the biomass production efficiency and essential oil. The assay was conducted on the experimental field of the Institute of Agricultural Sciences at the Federal Rural University of Amazonia, campus Belém– Pará-Brazil. To install the assay was adopted a factorial 4 x 4 in a completely randomized design with two repetitions, where the shading levels were 0 %, 30 %, 50 % and 70 % and the fertilization types (organic, organic + mineral, mineral and without fertilization), with 15 plants per plot. The following amounts of fertilizer per treatment were used: 1500 g of bovine manure; 200 g of NPK (10-28-20); and 1500 g of bovine manure + 200 g of NPK (10-28-20). The following parameters were evaluated: survival rate, weight of fresh mass, essential moisture-free oil content and oil yield / ha. The means were evaluated by Tukey ($P < 0.05$). From the results obtained it was concluded that to culture of Japana Branca: a mineral fertilizer in the amount and formulation used (200 g NPK 10-28-20), influenced negatively on survival and essential oil content of plants; the organic fertilization influenced positively in biomass production and essential oil yield/ha; the shading level did not significantly affect the survival of plants and oil content, however, it had a tendency to obtain higher yields of biomass and yield of essential oil/h with increasing shading level; a significant interaction between the shading levels and types of fertilization was founded, where: the higher biomass yields were achieved with organic fertilization and shading levels of 30 % and 50 %; and higher yields of oil / ha with organic manure (62.25 kg ha^{-1}), organic + mineral (82.81 kg ha^{-1}) or no fertilizer (79.03 kg ha^{-1}) at the level of 70 % shading.

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Acknowledgements: UFRA, MPEG, UFPA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Propagation of Japonica Branca (*Ayapana triplinervis* (Vahl) R. M. King & H. Rob.) by Cutting.

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Keyword: aromatic; asexual propagation.

The objective of this study was to develop a propagation system by cutting for cultive of Japana branca. This species is an herb that occurs in the Amazon belonging to Asteraceae family, which use is in the traditional medicine of the region. [1] It has also been indicated for essential oil extraction aimed at the pharmaceutical industry, perfumery and cosmetics. [2,3] The trials were conducted in the experimental area of the Institute of Agricultural Sciences of the Federal Rural University of Amazonia, campus Belem – Para - Brazil. Cuttings with 20 cm in length were obtained from mother plants grown in full sun. The experimental design was a randomized blocks design with 3 types of cuttings (apical, median and basal branch), 9 replications and 5 cuttings per treatment. The cuttings were planted in plastic tubes with dimensions of approximately 20 cm long and 5 cm in diameter, containing vermiculite as substrate and placed in a greenhouse at 50 % brightness and intermittent mist, where they remained for a period of 60 days. The following parameters were evaluated: rooting percentage, root number and length of roots. Data were submitted to analysis of variance by SISVAR program and averages submitted to Tukey test at 5 % probability. The assay produced on average 90 % of rooted cuttings and statistically significant difference only for the number of roots, where the average of the number of roots in the apical stakes were higher than other types of cuttings. By results obtained the Japana branca can be propagated, normally, by cuttings taken from the plant apical, median and basal part, however, the apical cuttings was more efficient.

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Acknowledgements: UFRA, MPEG, UFPA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development and Optimization of Gas Chromatography Method for the Determination of Selina-1,3,7(11)-trien-8-one and Selina-1,3,7(11)-trien-8-one epoxide in *Eugenia uniflora* Essential Oil by Central Composite Design.

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Keywords: *Eugenia uniflora*; essential oil; gas chromatography.

Eugenia uniflora is a perennial tree, named in Brazil as Pitanga, belonging to the *Myrtaceae* family and native from the central part of Brazil to the north of Argentina. The essential oil of *E. uniflora* has been largely studied in several reports, as Rodrigues *et al.*[1] that have studied the *in vitro* anti-leishmania activity of this oil with promising results. [1] The main constituents of *E. uniflora* are selina-1,3,7 (11)-trien-8-one and selina-1,3,7(11)-trien-8-one epoxide.[2] The isolation of these sesquiterpenoids by countercurrent chromatography is currently the subject of a project conducted by our research group, which analyses are optimized by a fast gas chromatography method. Central composite design was applied, based in an original method used in our laboratory, to three different columns in order to determine the best suited. The chosen columns were the DB-5, DB-35 and DB-17ht. Phenyl groups in this phase interact with the more polar oxygenated terpenes resulting in better shaped peaks and faster and reliable analysis. The three columns were tested with 15 different methods generated by the central composite design using initial oven temperature, heating rate and flow as factors. Evaluation of the chromatograms indicated the DB-35 column as the most suited for the analysis. Method optimization for this column was carried out using initial oven temperature, heating rate and carrier gas flow as factors. Two conditions were selected for validating the model generated: (1) 72.9 °C initial temperature, 4.7 °C min^{-1} heating rate and 1.3 mL min^{-1} of carrier gas flow; (2) 70 °C initial temperature, 6.5 °C min^{-1} heating rate and 1.46 mL min^{-1} carrier gas flow. Results fell within the predicted values with the exception of selina-1,3,7(11)-trien-8-one in the second condition which have a higher than expected resolution and was then considered suitable for analysis. These conditions were selected because they have a lower analysis time. This method was validated for the determination of selina-1,3,7(11)-trien-8-one and selina-1,3,7(11)-trien-8-one epoxide.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antimicrobial Activity of the Essential Oil of Oregano (*Origanum vulgare* L.)

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Keywords: *Origanum vulgare*; antimicrobial activity; carvacrol.

Origanum species have been used for thousands of years as spices and in ethnomedicine. *Origanum vulgare* (L.) is a medicinal perennial plant. Many herbs are commonly used in home-type cure therapies, complementary medicine and modern medicine because of their perceived antioxidant, antimicrobial and anticancer properties. [1] As they possess such biological activities, their potential must be revealed by scientific studies and it explained to the public, since these herbs are available in almost all public markets. The aim of this study was evaluate the antimicrobial activity of the essential oil (EO) from *O. vulgare*. The antimicrobial activity was tested against 8 bacterial; *Escherichia coli*, resistant and sensitive *Staphylococcus aureus*, two *Enterobacter* species, carbepenemase positive and negative *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and ten fungi strains; *Candida albicans*, *Cryptococcus neoformans*, *Fonsecaea pedrosoi*, *Rhizopus oryzae*, *Trichophyton rubrum*, *T. tonsurans*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* and *Epidermophyton floccosum*. The antimicrobial activity was evaluated as M7-A6 protocols, M27-A2 and M38-A2 for bacteria, yeast and filamentous fungi, respectively. [2] The identification of EO was performed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70eV. The results of chemical analysis showed twenty five constituents, being carvacrol (59.0 %), γ-terpinene (16.5 %) and *o*-cimene 6.5 % the major components. The EO exhibited antimicrobial activity against all microorganisms tested and the minimum inhibitory concentration (MIC) values ranged from 156 to 1250 µg mL^{-1}, and the best result was for *P. aeruginosa*. In conclusion, the EO from *O. vulgare* can be used as a potential natural antimicrobial agent to the bacteria above cited.

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Acknowledgements: CNPq, CAPES, FAPERJ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Hormetic Effect Caused by Essential Oil of *Cymbopogon citratus* on *Staphylococcus aureus* Biofilm

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Keywords: Essential oil; hormesis; *Cymbopogon citratus*; *S. aureus*.

Hormesis has been classified as a biphasic dose-response phenomenon, characterized by high-dose inhibition and low-dose stimulation. Many compounds are capable of causing the phenomenon, including physical and chemical compounds. The hormesis occurrence in pathogenic strains can be a serious problem, particularly in the treatment of microbial diseases. Calabrese [1] recognized that low doses of antiviral, antibacterial and antitumor drug can improve the growth of these potentially harmful agents (microorganisms and cells) hurting the treatment of patient. During our study about the use of essential oils to control *Staphylococcus aureus* biofilm we observed, unexpectedly, the occurrence of hormesis caused by *Cymbopogon citratus* oil. We studied eleven clinical isolates of bovine mastitis, reported by Castelani *et al.* [2] The oil concentration was assessed in the range of 1.0 to 0.015 $mg.mL^{-1}$ and the minimum inhibitory concentration (MIC) was calculated using microdilution method (CLSI, 2005). The quantification of the biofilm was determined by Hess *et al.,* [3] as by other methodologies. The results showed that the essential oil of *C. citratus* inhibited biofilm formation at various concentrations tested (0.06 – 0.5 mg mL^{-1}). However, the sub-MIC concentrations increase biofilm formation in more than 50 % relative to control. We believe this is the first time that essential oils are associated with hormesis phenomenon in *S. aureus* biofilm. This observation is very important and should incite the attention of researchers to the modulatory effects of essential oils in sub-MIC concentrations.

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Acknowledgements: CNPq, UNICAMP, CPQBA.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Preliminary Study of Bioactivity of Essential Oils from Flowers of *Nectandra megapotamica* (Spreng.) Mez (Lauraceae) from Mato Grosso do Sul

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Keywords: *Nectandra megapotâmica*; Mato Grosso do Sul; essential oil.

The Lauraceae family is rich in secondary metabolites belonging to different classes, including bioactive terpenes.[1] In order to contribute to the discovery of new constituents, studies were performed with volatile oils extracted from flowers of *Nectandra megapotamica* (Spreng.) Mez. The bioassay conducted with the oil, aiming to know the biological potential to inhibit bacterial growth, presented significant values. The test was positive in three strains of bacteria *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*,. These results can be considered as positive values greater than the 9 mm halo. [2] The chromatographic profile of the oil was evaluated by gas chromatography with detection by flame ionization and mass spectrometry. The chromatograms obtained for the oil by GC-MS and GC-FID are equivalent in their chromatographic profiles. A total of 16 compounds were identified by comparison to the mass spectra in NIST MS Search 2.0 library with a probability between 60 and 90 %. The retention indices were calculated by the use of a homologous series of hydrocarbons C7-C30 (Sigma-Aldrich) and compared with literature values according to Adams library.[3] Major compounds found were spathulenol (20.3 %), *α-cadinol* (5.0 %), *copaene* (4.6 %), globulol (4.3 %), *allo*-aromadendrene (4.1 %) and caryophyllene oxide (2.3 %). The oil yield was 0.34 %; however, it still necessary more studies on the chemical composition and biological action of the essential oil of flower specimens of this family. In this sense, this work aims to contribute to the literature on it.

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Acknowledgements: Fundect, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition and Preliminary Study of Biological Effects of the Essential Oil of *Cunila fasciculata* (Lamiaceae)

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Keywords: Cunila; cytotoxic activity; *Acinetobacter baumannii*.

The genus *Cunila* (Lamiaceae: Nepetoideae) occurs in both North and South America, and comprises 20 species with two different centers of distribution. Many studies on the chemical composition of essential oils of *Cunila* species have also reported bactericidal, fungicidal, and insecticidal activities.[1] *Cunila fasciculata* is a native plant of Southern of Brazil, Argentine and Uruguay, used in the Brazilian folk medicine as a stimulant, emmenagogue, antispasmodic and in the treatment of respiratory infections. In this work the chemical composition of the essential oil of aerial parts of *C. fasciculata* obtained by hydrodistillation was analyzed by CG/FID and CG/MS. Samples of aerial parts of *C. fasciculata* plants were collected in the municipality of Ibarama, central region of the Rio Grande do Sul State, Brazil. This oil was tested *in vitro* against nine human cancer cell lines by Sulforhodamine B assay to determine the concentration of total growth inhibition (TGI) in $\mu g.mL^{-1}$. Cancer cells lines tested were U251 (glioma) MCF-7 (breast), NCI-ADR/RES (drug resistant ovarian), 786-0 (kidney), NCI-460 (lung), OVCAR-3 (ovarian), HT-29 (colon), K562 (leukemia) and PC-3 (prostate). Additionally, we report the antimicrobial activity against *Acinetobacter baumannii,* a tough bacterium isolated from human clinical process (University hospital, UFGD, Dourados, Brazil). For this experiment, the *Cunila* oil was subjected to an initial screening using the disk diffusion method on agar according to the Clinical and Laboratory Standards Institute with adaptations. The oil was obtained in a yield of 0.6 %. Among the major compounds identified in the oil were found the monoterpenoids menthone (15.5 %), piperitone (8 %), isomenthone (7.3 %), and linalol (4.8 %), and the sesquiterpenes caryophylene oxide (16 %), caryophylene (7.2 %), spathulenol (6.8 %), humulene (5.4 %) and germacrene D (5.0 %). The *Cunila* oil was more effective in inhibiting the growth of cells OVCAR-03 (ovarian) (TGI 49.7 $\mu g\ mL^{-1}$). In this previous screening was considered the *Cunila* oil as active against bacteria *A. baumannii*, showing a zone of inhibition of 10 mm. This finding is highlighted because this bacterium is a resistant strain to antibiotics.

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Acknowledgements: Fundect, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comprehensive 2D GC Coupled to Rapid Triple Quadrupole Mass Spectrometry for the Simultaneous Untargeted (MS Database Matching &LRI-Filtering) and Targeted (MRM Transitions) Analysis of Essential Oils.

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Keywords: GC×GC-QqQ MS; flow modulation; essential oil.

Comprehensive two-dimensional gas chromatography (GC×GC) has been widely used in combination with mass spectrometry (MS), only throughout the last decade. With regards tothe mass-spectrometric instrumentation type used, low-resolution high-speed time-of-flight (ToF) systems have been the most popular choice, followed by rapid-scanning low resolution single quadrupole instruments. The main objectives of most such GC×GC-MS applications have been the quantitative and/or qualitative determination of untargeted analytes. There has been only a low number of publications describing the use of other MStypes, a fact related to slow spectral production rates. The requirement for high-speed MS systems is related to the very fast second-dimension separations. The present investigation is focused on the exploitation of a novel high-speed triple quadrupole (QqQ) mass spectrometer, in flow-modulated GC×GC applications. The QqQMS is capable of operation in the simultaneous full-scan/MRM (multiple reaction monitoring) mode, under the high-speed spectral production conditions required for GC×GC identification and quantification. It will be shown that, in the same GC×GC-QqQ MS analysis, both full-scan spectra relative to untargeted compounds, and MS-MS spectra relative to targeted ones, can be attained. Obviously, the use of the MRM mode enables a great increase both in sensitivity and selectivity. An untargeted/targeted GC×GC-QqQ MS application on an essential oil, containing trace-amount contaminants, will be illustrated.

Acknowledgements: University of Messina.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Content and Composition From *Lippia alba* Accessions of UFRRJ Germplasm Collection

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Keywords: *Lippia alba*; essential oil; biodiversity.

Genetic knowledge concerning to essential oils production and quality is important to development of selection strategies looking for commercial varieties. Essential oil yield depends of vegetative characteristic such as leaves production, and its quality is controlled by biochemistry and physiologic aspects. [1] Oil yield is impacted mainly by combination of genetic and edaphoclimatic conditions, but oil quality is strongly controlled by genetics. [2] Thus with this aim, essential oils from *Lippia alba* (Mill.) N.E. Brown accessions extracted, as well as, their chemical composition and content were evaluated. For this proposal, 20 accessions, 3 blocks, 5 plants/ block, totaling 300 plants were planted and grown at experimental area of Department of Plant Science (UFRRJ). Leaves and flowers samples were collected (harvested in November 2013), homogenized and separated into replicates (n=3). The essential oil was extracted by hydrodistillation (50 g dried weight at 2 h), then, 15 mL of distilled water plus essential oil samples were collected and partitioned with 3 X 5 mL of dichloromethane. The less polar phase was dried over anhydrous sodium sulfate, filtered and concentrated with nitrogen gas at room temperature until constant weight. Gravimetric measurements were performed base on the dry weight of leaves and fruits and converted to essential oil percentage (w/w). The oils were analyzed by GC/FID (5890 Series II, Hewlett-Packard, USA) and GC/MS (QP-2010 Plus, Shimadzu, JPN), both with Factor Four-VF-5ms fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL min^{-1}. . The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature. Concerning to essential oil content variations from 0.26 to 1.10 % were observed, highest levels were found on accessions 3, 4 and 8 (1.02, 1.06 and 1.10 %, respectively). Also, 6 chemotypes were discriminated: Citral (56-75 %), accessions 1, 2, 5, 11, 12, 15, 18, 19 and 20; β-myrcene/ citral (12-16% and 44-52 %), accessions 6 , 9, 14, 16 and 17; limonene/ carvone (19-27 % and 46-58 %), accessions 3, 7 and 8; linalool (67 %), accession 4; β-caryophyllene/ citral (19 and 54 %), accession 10 and myrcene/ β-caryophyllene (16-29 %), accession 13.

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Acknowledgements: Faperj, PPGQ/UFRRJ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Investigation of the Tocolytic Effect of Essential Oil from *Hyptis suaveolens* and *Hyptis martiusii* Leaves (Lamiaceae): a Comparative Study

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Keywords: *Hyptis*; rat uterus; tocolytic effect.

Hyptis genus (Lamiaceae) is popularly used as tocolytic agent, [1] however there is no scientific data showing that activity. Therefore, the aim of this study was to investigate a possible tocolytic effect of the essential oil from leaves of *H. suavelons* (HS-EO) and *H. martiusii* (HM-EO) on female rats, and compare their relative efficacy and potency. Adults female rats (*Rattus norvegicus*) were estrogenized (diethylstilbestrol 1.0 mg kg^{-1}, s.c.) 24 h before experiments. The uterus was removed, cleaned, suspended in organ baths on appropriated conditions and the isotonic and isometric contractions were monitored. All experimental protocols were previously approved by Ethical Committee in Animal Use of UFPB (nº 048/2015). HS-EO (1-243 µg mL^{-1}, n = 5) and HM-EO (1-729 µg mL^{-1}, n = 5) antagonized the phasic contractions induced by oxytocin 10^{-2} UI.mL^{-1} (IC_{50} = 38 ± 2.1 and 71.6 ± 4 µg mL^{-1}, respectively) and carbachol (CCh) 10^{-5} M (IC_{50} = 18.9 ± 0.9 and 44.8 ± 1.8 µg mL^{-1}, respectively) in a significant and concentration-dependent manner. Both oils induced maximum efficacy (E_{max}) of 100% compared to the tested agonists. As both oils showed higher potency front contractions induced by carbachol, suggesting the involvement of muscarinic receptors in this tocolytic effect. In other experiments, HS-EO and HM-EO (1-729 µg mL^{-1}) relaxed rat uterus (n = 5) pre-contracted with KCl 60 mM (EC_{50} = 13.9 ± 2.3 and 33.8 ± 4.5 µg mL^{-1}, respectively) or oxytocin 10-2 UI.mL^{-1} (EC_{50} = 19.2 ± 0.6 and 71.2 ± 10 µg mL^{-1}, respectively). The essential oils showed 100 % of tocolytic efficacy and higher potency when the organ was pre-contracted by CCh compared to KCl. The data showed that HS-OE was equipotent and HM-EO was more potent in relaxing pre-contracted uterus with KCl, suggesting the involvement of voltage-gated calcium channels (Ca_V) in the spasmolytic effect of oils, since activation this channels is a common step in these contractile agents pathway. Finally, in this study, it was confirmed the folk medicinal indications of *H. suavelons* and *H. martiusii*, showed tocolytic activity on rat, being HS-EO more potent than HM-EO all protocols tested.

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Acknowledgements: CNPq, CAPES, PgPNSB/CCS/UFPB.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Variability and Antiphytopathogenic Activity of Essential Oils from *Hymenaea courbaril* L. var. *courbaril* Leaves (Fabaceae: Caesalpinioideae).

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Keywords: Phytopathogens; volatile compounds; Caatinga.

The use of pesticides in agriculture is risky to human health and frequent damages the environment. Thus, the search for alternative methods on the control of plant pathogens is aimed in this study to evaluate the chemical variability and the antimicrobial activity of essential oil from leaves of *Hymenaea courbaril* L. var. *courbaril*, collected in four different phytophysiognomy of the Catimbau National Park, Pernambuco, Brazil. To obtain oils (I, II, III and IV), the hydrodistillation method was used in a Clevenger apparatus, and constituents were identified by GC/MS. The evaluation of the antimicrobial activity was performed using the microdilution broth method for determining the minimum inhibitory concentration (MIC) and bactericidal/ minimum fungicidal concentration (MBC/MFC). The microorganisms used were six phytopathogen bacteria: *Acidovorax citrulli* (DEPA 1.12), *Pectobacterium carotovorum* subsp. *carotovorum* (31 DEPA), *Ralstonia solanacearum* (DEPA CRM 10), *Xanthomonas campestris* pv. *campestris* (53 DEPA), *X. campestris* pv. *malvacearum* (DEPA 11.2.1), *X. campestris* pv. *viticola* (DEPA 137) and six phytopathogenic fungi: *Fusarium oxysporum, F. moniliforme, F. solani, Verticillium lecanii, Rhizopus stolonifer* and *Aspergillus flavus*. Temperature, relative humidity, amount of water and nutrients in the soil were analyzed in order to verify their influence on the collected samples. The oils showed yields of 0.86, 0.87, 1.32 and 1.45 % for samples I, II, III and IV, respectively. In GC/MS analyses, caryophyllene oxide, β-caryophyllene and junipene were the major compounds. All oils were effective for all tested microorganisms, being most active against bacteria *X. campestris* subsp. *malvacearum*, presenting MIC of 1.56 µl mL^{-1} and MBC of 1.56 µl mL^{-1}. For the fungi tested, the sample IV presented CIM of 12.5 µl mL^{-1} and CFM of 12.5 µl mL^{-1} against the *F. moniliforme*. The essential oil of *H. courbaril* is a potential source of biopesticide against the assessed pathogens. Other studies are needed to assess the safety of using this essential oil as biopesticide.

Acknowledgements: UFPE, Facepe, INSA, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Seasonal Yield and Chemical Composition of the Essential Oil from *Lippia origanoides* Kunth and its Relationship with Meteorological Elements in the Cerrado, Federal District, Brazil

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Keywords: *Lippia origanoides*; seasonality; Cerrado.

Lippia origanoides (alecrim pimenta) is a wild shrub of the family Verbenaceae, native to Brazil. Its main product is the essential oil rich in thymol, produced in specialized glands of leaves and flowers and has potential application in the pharmaceutical and cosmetic industries. The main objective of this study was to evaluate the variation in the yield and essential oil composition of of *Lippia origanoides* harvested three times in one year and its relation to the air temperature, relative humidity and solar radiation in the Cerrado, DF. The experiment was conducted at Embrapa Cerrados, Planaltina, DF, in irrigated pots (~70L) placed in the field. Plant material was harvested in the morning and during flowering in the three seasons (autumn-May / 2013; winter-August / 2013; summer-February / 2014). After drying in an oven at 38 ^{o}C for 48 h, the essential oil (EO) was extracted by hydrodistillation in a Clevenger modified apparatus, dried with Na_2SO_4, and stored away from moisture and light. The constituents of the essential oil were identified by gas chromatography (GC-FID and GC-MS). The values of the areas and their relative percentages were obtained in triplicate. For thymol quantitation, an analytical curve was built using an authentic standard (external standard method). The air temperature values (T), relative humidity (RH) and solar radiation (RS) were recorded for the harvesting date and time, at 10 am, in three seasons. The design was completely randomized, a total of 3 treatments x 5 replications. The EO yield ranged from 2.45 % to 3.21 %, and 43 constituents were detected, being thymol the highest (71-75 %). The only significant correlation between the yield of OE and meteorological elements was with solar radiation (Pearson correlation at 5 % = - 0.929). Thymol did not correlate significantly with T, RH and RS. The nonparametric Kruskal-Wallis to the level of 5 % probability was significant for EO income in the comparison between August / 2013 and February / 2014, with the highest average for August (3.21 %). The results showed that of the 43 constituents, thymol is the major primary (> 71 %) and does not suffer apparent variation of T, RH and RS; the essential oil yield is higher under lower solar radiation conditions especially in the winter season in the Cerrado, Federal District, Brazil.

Acknowledgements: Embrapa.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Coffee Leaves Essential Oil from Natural and Commercial Sources.

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Keywords: coffee leaves; essential oil; hydrodistillation.

Coffee is an important commodity throughout the world and Brazil is currently the largest producer and exporter of coffee beans[1]. Worldwide, hot tea is the leading beverage besides hot coffee, with different patterns in countries due to the legacy of colonial expansion, geopolitics and trade market. But what about making tea with coffee? The aim of this study was to perform a hydrodistillation of *Coffea arabica* leaves from two origins: one directly picked from *C. arabica* trees (NL) from Morro Azul do Tinguá, Rio de Janeiro, RJ and the other from a commercial source (CL), in order to obtain the essential oils and compare the volatiles profile by GC/MS. Fresh natural leaves (NL) dried in a hot oven at 50 °C for 24 h (51 g) and commercial leaves (CL) (49 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 5 h. The water content from both leaves was monitored by thermogravimetry. The oils were analyzed by GC/MS in an Agilent 5975C system, with a DB-Wax column (30 m X 0.25 mm X 0.25 µm). Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature[2]. For NL, the water content was 1.5 %, while for commercial leaves it was of 10.9 %. In both oils, 47 peaks were integrated, from which 27 compounds were identified for NL, and 18 for CL. NL oil presented compounds previously reported in roasted coffee such as pyrazines and furans, besides some terpenoids. Major compounds in NL were 5-methylfurfural (14 %), furfural (11 %) and methyl salicylate (8 %). CL essential oil presented α-terpineol (42 %), followed by octanoic acid (18 %). Three compounds were found in both samples, which were furfural, α-terpineol and hexahydro-farnesyl acetone. Differences between the samples can be due to use of different species, age of leaves, geographic origin or even adulteration. There are few reports in the literature on the composition of coffee leaves essential oil, most of them reported the utilization of the coffee leaves for medical purposes or in combating coffee pests. Regarding coffee-leaf tea advantages, the claim is that it has a lower content in caffeine and higher in antioxidants, mainly compared to green tea. However, more studies are needed to clarify and establish the various benefits of this matrix and its volatile compounds.

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Acknowledgements: CNPq, Embrapa Café, Faperj and CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Antimicrobial Activity of *Cordia verbenacea* Essential Oil Against Pathogenic Bacteria

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Keywords: *Cordia verbenacea*; *Staphylococcus aureus*; *Escherichia coli*.

Cordia verbenacea is a plant native throughout most of Brazil mainly on the coast, also in common Atlantic rain forest. The medicinal activities of this plant are scientifically validated and assigned to its essential oil constituents. *Staphylococcus aureus* and *Escherichia coli* are bacterial pathogens causing foodborne illnesses and infections. Research of alternative methods to control pathogenic bacteria through essential oils has increased because the microorganism resistance prevention. [1,2] This work aimed to evaluate the antimicrobial activity of the essential oil of *C. verbenacea* against Gram positive and gram negative pathogenic bacteria. *C. verbenacea* was cultivated according in a organic production system. Oils from the leaves were obtained by hydrodistillation using a Clevenger-type system and their minimal inhibitory concentrations (MIC) were determined by microdilution method. [3] The antimicrobial activity from essential oil was tested against *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739) in seven concentrations (4000, 2000, 1000, 500, 250, 125, 62.5 µg mL^{-1}). The experimental design had four replicates per treatment. The MIC of *C. verbenacea* essential oil for *S. aureus* was 4000 µg mL^{-1} and *E. coli* was not inhibited at the concentrations tested. According with Aligianis' classification [4] this essential oil showed low antibacterial activity against *S. aureus*.

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Acknowledgements: FAPEMIG and CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Fractioning of Green Mandarin Essential Oil by Vacuum Fractional Distillation.

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Keywords: Citriculture; vacuum distillation; GC-MS.

The harvest of citrus fruits presents great importance worldwide, as its production, in 2013, was approximately 111.5 millions of tons. The main citrus fruits are orange, lemon, mandarin and lime, which have a high commercial value as sell 'in nature' or being processed in food industry.[1] The citrus essential oil is a mixture of terpenes, many of them with important industrial applications in medicine, pharmacology, cosmetics and food industry. Though, to have a practical application for the minority compounds (which, in general, have more value), it is necessary to properly separate the mixture of substances in the raw oil. [1,2] Aldehydes, alcohols and ketones that generally appear as minority compounds or as trace, are important in the composition of the oil flavor, being products of high value when compared to other terpenes present in the mixture. The green mandarin essential oil was obtained by pressing, being stored away from sunlight and at room temperature. The oil was distillated in a glass column, filled with Raschig rings with 8 mm of diameter. The column had four stages, including the bottoms. A vacuum pump was coupled to the condenser, to reduce the pressure inside the column. It was utilized 120 mL of raw essential oil in each batch. The vapor was collected for 10 min, from the time the vapor reached each of the stages. The distillation in the last stage was kept until the volume in the distillation flask (bottoms), and less than 10 mL. The collected samples and the raw oil were analyzed by an Agilent GC model 6890 Series, and using a HP Innowax column (30 m X 320 µm i.d.) with film thickness of 0.5 µm and by an Agilent mass spectrometer model MSD5973 with a fused silica HP-Innowax column (30 m X 250 µm) with film thickness of 0.5 µm. Volume of distillates were near to 30 mL, and the remaining bottoms were less than 5 mL. GC/MS analysis showed that only hydrocarbon terpenes were distillated. Although the raw oil was mainly composed by limonene (80.3 %) and γ-terpinene (19.1 %), the distillation patterns were different in each stage. The terpenes with other chemical functions (alcohols, aldehydes, amines) remained in the bottoms. For some compounds, the bottoms concentration was more than 20 times the concentration in the raw oil, as for dimethyl anthanilate and α-sinensal.

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Acknowledgements: SDECT; EcoCitrus.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Physicochemical Characterization and Evaluation of the Antioxidant Activity of Essential Oil from *Myrcia amazonica* DC. (Myrtaceae) from the Region of Santarem, Pará, Brazil

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Keywords: *Myrcia amazonica* DC.; antioxidant activity; essential oil.

In the Amazon region there is a big variety of aromatic and medicinal plants that present a high economic potential, but there are few that have been explored commercially. The physicochemical and pharmacological study obtained from vegetal species can drive us to the finding of substances of interest for men. In this research, a physicochemical study and antioxidant activity of essential oil from leaves of native *Myrcia amazonica* DC. were conducted. Essential oil was obtained by hydrodistillation (HD). The essential oil chemical composition and the volatile compounds found in the flowers were identified by GC-MS. The antioxidant activity was calculated by the $ABTS^{+\cdot}$ and the ORAC methods.[2] There were found majority compounds of interest in the composition of the essential oil from *M. amazonica* like germacrene D (10.1-16.6 %), germacrene B (10-11.1 %) and 1-epi-cubenol (14.7-20.2 %). The yield of the essential oils varied between 0.65 to 0.96 % for fresh and dry leaves respectively. This difference in essential oil yield from was not revelant. The highest value for the antioxidant activity of essential oil was recorded with the ORAC (1310 ± 11 µmol Trolox g $substance^{-1}$), compared to the $ABTS^{+\cdot}$ (290 ± 7 µmol Trolox g $substance^{-1}$) method.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Tagetes osteni Hicken: Chemical Analysis and Potential Antineoplasic Activity of the Essential Oil from Leaves and Flowers

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Keywords: *Tagetes osteni*; essential oil; antineoplasic activity.

Asteraceae family has several plants producing essential oils with commercial importance, being *Tagetes* one of the occurrences of genus in Brazil with distribution in almost all the territory and four species are described: *T. minuta, T. erecta, T. patula* and *T. osteni* [1]. *Tagetes osteni* Hicken is native to southeast and south of the country and has not studies related to essential oil. Therefore, this work aims to investigate the chemical composition and biological activities, such as antioxidant and cytotoxic, of the essential oil of this species. For this purpose, leaves and flowers of *T. osteni* were collected in April 2015 in Santo Antônio da Patrulha, Rio Grande do Sul, Brazil. A voucher specimen was deposited in the herbarium of UFRGS (registry: ICN 181985). Fresh leaves and flowers were submitted to hydrodistillation separately in Clevenger-type apparatus for 3 h. The oils were analyzed by GC/MS equipped with DB-5 fused silica capillary column (30 m X 0.25 mm X 0.25 µm) and the oil components were identified by comparison of its index retention and their mass spectra, with data taken from the literature and database NIST 62. Regular monitoring of the chemical composition was carried out by CG considering the analysis of the oil after extraction, after 24 h/ and after one week. Human cervical cancer cells (SiHa) were seeded in culture plates in (DMEM)/10 % FBS and non-neoplasic cells (human lymphocytes) were obtained from peripheric blood using Histopaque® and culture medium RPMI. The cells were maintained at 5 % of CO_2 and 37 °C and treated with the flower and leaves oils in concentrations from 0.5 to 20 µg mL^{-1} for 24 and 48 hours using propylene glycol as a carrier. MTT assay was performed (0.5 mg mL^{-1}) with treated cells oil, control medium (DMEM) or vehicle control in both lines, SiHa and Hacat. Oil yields were 1.8 % and 1.0 % for flowers and leaves, respectively. The leaves oil presented as characterized by dihydrotagetone (64.2 %) and (*Z*)-tagetone (15.9 %), while for the flowers (*Z*)-β-ocimene (26.1 %), (*Z*)-ocimenone (17.6 %) and (*E*)-ocimenone (40.0 %) were the major components. In relation to antineoplasic effect, the treatment with different concentrations of flower oil significantly inhibited the viability of cervical cancer cells in different times (95 %). Meanwhile, oil concentrations between 10 and 20 µg mL^{-1} did not induce significant effects on the viability of non-tumor cells Hacat. These results emphasize the relevance of studies involving this native plant as a new potential therapeutic alternative for human cervical cancer.

[1] Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available in: <http://floradobrasil.jbrj.gov.br/>. Accessed in: 14 Ago. 2015.

Acknowledgements: CNPq, FAPERGS, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Composition of Five *Piper* Species from the Brazilian Atlantic Forest

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Keywords: *Piper*; GC-MS; *E*-nerolidol.

Species of Piperaceae are well known for their essential oil composition based on monoterpenes, sesquiterpenes and arylpropanoids that non rare are the main fraction of the oil. [1] Piperaceae species are distributed from North to South of Brazil, including the Brazilian Atlantic Forest that is highly threatened by the human action. Atlantic Forest is one of hotspot of biodiversity and by this mean it is very important to study plants from this biome. Five *Piper* species were collected in a private area in March of 2015, in the city of Teresópolis/RJ, and were identified as *P. aduncum* L., *P. gaudichaudianum* Kunth, *P. arboreum* Aubl. var. *arboreum*, *P. bennetianum* C. DC. and *P. lucaeanum* var. *grandifolium* Yunck. Voucher of the botanical materials were deposited at the herbarium of the Research Institute of Botanical Garden of Rio de Janeiro. Fresh leaves (150 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 2 h. The essential oils were diluted in dichloromethane and then analyzed by GC/MS and GC/FID (GC Agilent 6890N coupled to an Agilent 5973N MS, equipped with DB-5MS fused silica capillary columns (30 m X 0.25 mm i.d. X 0.25 µm film thickness); helium was used as carrier gas for GC-MS and CG-FID, respectively, with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 240°C at 3°C min^{-1}. Mass detector was operated in electronic ionization mode at 70 eV. The essential oil compounds were identified by comparison of both mass spectra (NIST and WILEY) and linear retention indices with literature records. [2] The main compounds identified were: *P. aduncum* (10-*epi*-γ-eudesmol, 3.0 %; *trans*-dihydrooccidentalol, 6.7 %; (*E*)-nerolidol, 58.7 %); *P. gaudichaudianum* (*trans*-dihydrooccidentalol, 6.7 %; elemol, 8.9 %; (*E*)-nerolidol, 41.6 %); *P. arboreum* var. *arboreum* (elemol, 9.0 %; cubenol, 11.2 %; (*E*)-nerolidol, 21.3 %); *P. bennetianum* (curcumene, 10.5 %; β-elemene, 12.6 %; (*E*)-nerolidol, 30.9 %); *P. lucaeanum* var. *grandifolium* (cubenol, 7.0 %; linalool, 8.4 %; (*E*)-nerolidol, 66.4 %). It interesting to note that all essential oils are composed mainly by sesquiterpenes. The presence of the (*E*)-nerolidol identified as the major sesquiterpene can be considered a chemical marker for these five species in the Atlantic biome.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Bioactivity Evaluation of Citrus Essential Oils Against Dermatophytes

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Keywords: *Citrus;* essential oi; dermatophytes.

Citrus are the most produced and consumed fruits around the world, and their peels produce several classes of metabolites. [1] Among these classes, the essential oils are of great interest due to their antimicrobial and antiviral activities. Antimicrobials from natural sources are economical alternatives to combat pathogenic microorganism. [2] In this context, the objective of the work was to evaluate, *in vitro*, the antifungal activity of essential oils from *Citrus limonia*, *Citrus latifolia*, *Citrus limon* and *Citrus aurantifolia* against *Trichophyton tonsurans, Epidermophyton floccosum, Microsporum canis, Microsporum gypseum, Trichophyton mentagrophytes.* Essential oil components were characterized by GC/FID and GC-MS analyzes. Minimum Inhibitory Concentration (MIC) was determined by serial dilution of the essential oils in RPMI medium and inoculation with fungus, incubated for 48 h at 37 °C. Resazurin, a cellular viability indicator, was used and the results revealed *C. aurantifolia* as the most active among the essential oils tested, inhibiting all dermathophytes of the study. The most sensitive fungus was *E. floccosum*, with MIC values of 78 µg mL^{-1}, 312 µg mL^{-1}, 312 µg mL^{-1} and 625 µg mL^{-1}, to essential oils of *C. aurantifolia, C. limonia, C. latifola* and *C. limon* respectively. MIC values ranging from 625 µg mL^{-1} to 1250 µg mL^{-1} were detected against *M. canis* and *T. tonsurans*. The fungi *M. gypseum* and *T. mentagrophytes* were the most resistant among the dermathophytes tested, with MIC values higher than 1250 µg mL^{-1}. Chemical composition analyzes of these essential oils showed limonene as the major substance, in different concentrations. Nevertheless, essential oils are complex mixtures including other antimicrobial compounds such as β-pinene, γ-terpinene, neral and geranial. On this sense, future assays will be necessary to identify the components responsible for the biggest antifungal activity of *C. aurantifolia* when compared with the other essential oils studied.

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Acknowledgements: CAPES, CNPq, FAPERJ e UFRJ.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Phytotoxic effects of essential oils of *Eugenia brejoensis* (Myrtaceae) leaves.

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Keywords: Phytotoxicity; volatile compounds; allelopathy.

Eugenia brejoensis Mazine is a shrub-tree of 1.6–11 m, endemic of hinterland highland forest enclaves, locally called "brejo", in the Caatinga Domain, in "restinga" and "floresta de Tabuleiro" (Sergipe and Espírito Santo states).[1] Here, we studied a possible phytotoxicity of EOs of *E. brejoensis* on the seed germination and the shoot and root elongation of the sensitive indicator lettuce (*Lactuca sativa* L.). Leaves from three individuals of a population were harvested in May 2015, at the Catimbau National Park (Caatinga Domain), Pernambuco, Brazil (voucher at herbarium of Angronomic Institute of Pernambuco State no. IPA84480). Leaves (300 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The EOs were analyzed by GC/FID and GC/MS, and composition normalized from FID. EOs components were identified by comparison of both mass spectra and LRI. Lettuce seeds were purchased from a local seed shop, sterilized for 5 min with NaClO (2 %), and then rinsed with distilled H_2O. Four replicates (each 50 seeds) were prepared for the contact tests with EOs (dispersed as an emulsion in dist. H_2O using Tween 20 [0.1 %]), using sterile Petri dishes lined with double-sterile filter paper. Four doses of the EOs (0.125, 0.25, 0.5 and 1 µL mL^{-1}) were obtained by dilution of the emulsion in deionized H_2O. The dishes were then moistened with 5 mL of oils at different concentrations or controls (H_2O, used as a negative control and glyphosate water solution (100 µL mL^{-1}), positive control). Oil yields were 1.56 %. A total of 36 compounds were identified (91.3 %). The major components were hydrocarbons (61.4 %) and oxygenated sesquiterpenes (27.3 %). The major constituents were δ-cadinene (21.3 %), β-caryophyllene (15.7 %), α-muurolol (9.0 %), α-cadinol (8.2 %) and bicyclogermacrene (8.0 %). Seed germination and seedling early growth varied according to the concentration of the oil. Germination of lettuce seeds was totally inhibited by the highest dose of 0.5 and 1 µg mL^{-1}. The oil was not effective when tested at the lowest concentration of 0.125 µg mL^{-1}. The oil inhibited the early growth of lettuce. The dose-dependent inhibitory effect was observed on the elongation of the lettuce roots and shoots. Hence, the root and shoot elongation seemed to be more affected by the oils than the seed germination.

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Acknowledgements: INSA, Facepe, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparison of Essential Oil Profile of Spontaneous and Cultivated Individuals of *Bidens pilosa* L. (Asteraceae)

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Keywords: *Bidens pilosa*; essential oil; Asteraceae.

Bidens pilosa (Asteraceae) is a shrub distributed throughout the pantropical regions of the globe and commonly used in Brazil in the treatment of malaria and inflammations in general. This plant is also known to be a producer of polyacetylenes and phenolic compounds (mainly flavonoids and phenylpropanoids). The completely cultivated and spontaneous individuals (about 500 g of fresh plant) were submitted to simultaneous hydrodistillation in modified Clevenger apparatus for 6 h. The extracts were then partitioned each with 50 mL of methylene chloride (HPLC grade), further dried in a rotational evaporator at 40 °C, and then kept in freezer. The analyses of the volatile fraction were performed in a QP-2010 Shimadzu GC-MS equipment. The DB-5MS column was 30 m X 0.25 mm X 0.25 µm (Agilent Technologies, J&W Scientific Products). Helium was used as carrier gas and the operating temperature conditions ranged from 50 ^{o}C (5 min.) to 280 ^{o}C (5 min.) 5^{o} C min.$^{-1}$ The injector and detector temperatures were both set at 250 ^{o}C. The compounds were identified by comparison of their retention times and mass fragmentation patterns to the standards and MS spectra library (Shimadzu Solution Software v. 2.4). Identification of compounds was done by comparison of mass spectra to the database Wiley Library Software 59943B and by Kovats Indexes (a mixture of linear alkanes from C_7 to C_{26}, C_{28} and C_{30} were injected in the chromatograph in the same conditions of the samples). [1] Quantitation was made by peak area measurement. Oil yields were 0.3 and 0.2 % w/w for individual cultivated and spontaneous respectively. In the oil from aerial parts of from cultivated *B. pilosa* 39 compounds were identified (78 %), whereas in the oil from the spontaneous plant 23 were detected, corresponding to 90.5 % of the oil. Both cultivated and spontaneous *B. pilosa* showed as major constituents the sesquiterpenes germacrene D (45.0 % and 56.4 %), bicyclogermacrene (10.0 % and 7.8 %), β-caryophyllene (14.2 and 7.7 %) and α-humulene (3.1 and 2.1 %) respectively. These results, obtained for both individuals harvested in the winter 2014 and from the same site (with identical geographical coordinates), shows a clear different production of monoterpenes: the oil from individual spontaneous was rich in α-pinene (6.4 %) and β-phellandrene (3.6 %), while the cultivated plant showed traces of this substance. This study generated data that must always be taken into consideration when studies on biological activity or production/validation of essential oil medicinal plants are concerned.

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Acknowledgements: Farmanguinhos/Fiocruz, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil Yield and Composition of *Baccharis* Species from Araucaria Forest of Parana State, Brazil.

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Keywords: essential oil; limonene; aromatic plants; *Baccharis*.

The *Baccharis* genus presents great diversity of essential oils. The chemical composition has been studied due the economic importance for the fragrance and flavoring industries. This study aimed to evaluate the essential oil yield and composition of 10 species of *Baccharis* of Araucaria Forest, Piraquara - PR. Essential oil samples from fresh leaves of *Baccharis articulata* Pers., *B. trimera* (Less) DC., *B. milleflora* DC., *B. oblongifolia* Pers., *B. anomala* DC., *B. calvescens* DC., *B. uncinella* DC., *B. axillaris* DC., *B. mesoneura* DC., and *B. myriocephala* DC were obtained by hydrodistillation in a Clevenger apparatus during 4.5 h. The chemical constituents were analyzed by GC/MS. In both analyzes fused silica capillary column was applied, HP-5MS (30 m X 0.25 mm X 0.25 µm), using helium as the carrier gas (1.0 mL min^{-1}) at a temperature of 120 °C. The oven temperature ranged from 60 °C to 240 °C at a heating rate of 3 °C min^{-1}. Identification of essential oil component was done by comparison of both mass spectra and retention indices values with compounds described in the stored data and literature. [1,2] The essential oil content of *B. articulata, B. trimera*, *B. milleflora*, *B. oblongifolia*, *B. anomala*, *B. calvescens*, *B. uncinella*, *B. axillaris*, *B. mesoneura* e *B. myriocephala* were 0.07; 0.76; 0.90; 0.69; 0.72; 0.70; 1.89; 0.03; 0.97; e 0.02 %, respectively. It were identified 32 constituents (90.1 %) in the essential samples of *B.articulata,* 30 (91.9 %) in *B. milleflora*, 20 (96.2 %) in *B. oblongifolia*, 24 (81.3 %) in *B. anomala*, 29 (96.0 %) in *B. calvescens*, 29 (96.7 %) in *B. uncinella*, 23 (94.3 %) in *B. axillaris*, 25 (70.3 %) in *B. trimera*, 23 (82.0 %) in *B. mesoneura* and 28 (91.6 %) in *B. myriocephala*. The major constituents of *B. articulata* were limonene (38.4 %) and β-pinene (30.2 %); of *B. trimera* carquejila acetate (52.7 %) and limonene (18.6 %); of *B. milleflora* viridiflorol (24.1 %) and limonene (22.6 %); of *B. oblongifolia* limonene (32.7 %) and germacrene D (6.3 %); of *B. anomala* limonene (23.9 %), germacrene D (19.7 %) and δ-cadineno (8.0 %); of *B. calvescens* limonene (39.5%) and β-pinene (13.7 %); of *B. uncinella* limonene (24.1 %) and spathulenol (17.2 %); of *B. axillaris* α-pinene (41.9 %) and limonene (31.9%); of *B. mesoneura* limonene (32.2 %), α-pinene (15.5 %) and α-thujene (14.4 %); of *B. myriocephala* limonene (41.9 %) and β-pinene (16.7 %).

[1] Wiley Registry of Mass Spectral Data, 6th edn. Wiley Interscience, New York, 1994.
[2] Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Carol Stream: Allured Publ. Corp., 2007.

Acknowledgements: Embrapa, UFPR/PGAPV, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oil of *Piptocarpha angustifolia* Dusén *ex.* Malme

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Keywords: Vassourão-branco; essential oil; Brazilian native species.

The *Piptocarpha* genus (Asteraceae) is represented by 9 woody species belonging to Mixed Ombrophilous Forest (MOF) from Paraná State, being 4 climbing plants [1] and 5 trees. *Piptocarpha angustifolia* is a native tree and its forestry use includes production of softwood shading of crops and ecosystem recovery. In the present work, the chemical composition of the essential oil from leaves and inflorescences of *P. angustifolia* collected was investigated. This is the first study of essential oil of this species. The leaves and inflorescences were collected from adult tree (30 cm DAP) in winter 2014 in Piraquara city – Paraná State and a voucher specimen was deposited in the Herbarium of Museu Botânico Municipal de Curitiba. The plant materials (70 g) were submitted to hydrodistillation separately for 4 h in a Clevenger-type apparatus in triplicate. The oils were analyzed by GC/MS in Shimadzu GC-2010 systems coupled with a mass spectrometer detector Shimadzu GCMS-QP2010 Plus. The GC/MS measurements were performed using a non-polar capillary column Rtx-5MS (5 % diphenyl and 95 % dimethyl polysiloxane, 30 m X 0.25 mm X 0.25 µm) operated under a temperature-programmed condition from 60 °C to 250 °C at 3 °C min^{-1}. The carrier gas was helium with a flow rate of 1.02 mL min^{-1}, injection volume of 1.0 µL in split mode (ratio 1:10). Oil components were identified by comparison of both arithmetical index (based on a homologous series of hydrocarbons from 9 to 22 carbons analyzed in the same conditions) and mass spectra with literature and spectral library. It was possible to identify 58 and 22 compounds in the essential oil of the leaves and inflorescences, respectively. The chemical composition of the essential oil of leaves showed mainly oxygenated sesquiterpenes (44.4 %), monoterpenes (17.9 %) and sesquiterpenes (10.7 %) comprised to monoterpene limonene (13.4 %) and oxygenated sesquiterpene helifolen-12-al syn-syn-syn (13.3 %) with smaller abundances of sesquiterpenes. The essential oil of inflorescence is rich in oxygenated sesquiterpenes (66.3 %), mainly comprising thujopsan-2-α-ol (17.8 %), spathulenol (13.4 %) and α-cadinol (11.3 %). The yields of essentials oils of leaves and inflorescence were not determined because of small quantities. The antimicrobial activity and pest control will be evaluated.

[1] Grokoviski, L.; Cervi, A. C.; Tardivo, R. C. The genus *Piptocarpha* R.Br. (Asteraceae: Vernonieae) in the Paraná state, Brazil. *Acta Botanica Brasilica* **2009***, 23,* 486. [CrossRef]

Acknowledgements: UFPR, Herbário do Museu Botânico Municipal de Curitiba.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Comparative Analysis of the Essential Oil yield and Chemical Composition of Leaves and Fruits of *Campomanesia xanthocarpa* Berg and *C. guaviroba* (DC.) Kiaersk. (Myrtaceae).

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Keywords: hydrodistillation; aromatic plants; Atlantic Forest.

Native to the Atlantic Forest biome, the *Campomanesia* genus is represented by trees and shrubs, which fruits are consumed fresh and processed in the form of candy, ice cream, soft drinks and flavoring in alcoholic distillates. This research aimed to evaluate the essential oil yield and chemical composition of leaves and fresh fruits of *Campomanesia xanthocarpa* and *C. guaviroba* in a segment of Araucaria Forest, in the state of Paraná. Fresh leaves (100 g), and fresh fruit (200 g) of *C. xanthocarpa* and *C. guaviroba* were collected in the municipality of Piraquara - PR. The essential oil extraction was performed by hydrodistillation in a Clevenger apparatus for 4.5 h, in triplicate. To calculate the essential oil yield, 20 g of samples of leaves and fruits were dried in an oven with air circulation at 65 °C for 24 h. Quantitative and qualitative analyses of essential oil were performed by GC and GC/MS in an Agilent 7890-A and Agilent 5973-N, respectively. A capillary column of fused silica was applied, coated with HP-5MS (30 m X 0.25 mm X 0.25 µm). Injector and flame ionization detector (FID) were kept at 250 °C and 260 °C, respectively; the oven temperature ranged from 60 °C to 240 °C for 3 °C.min^{-1}; helium gas as the carrier gas (1.0 mL min^{-1}).The percentage compositions were obtained from electronic integration measurements using FID. The analysis of GC-MS was performed in the same conditions of GC. The transfer line was maintained at 260 °C, the ion source at 230 °C and the analyzer (quadrupole) at 150 °C. Identification of essential oil component was done by comparison of their fragmentation spectrum patterns to the reference spectrum[1] and comparing the KIs based on bibliographical references. The essential oil yield in the leaves of *C. xanthocarpa* and *C. guaviroba* were 0.03 and 1.36%, respectively. In fruits, the essential oil yields were 0.02 to 0.08% for *C. xanthocarpa* and *C. guaviroba,* respectively. The fruits of *C. xanthocarpa* and *C. guaviroba* showed 41 (92.5%) and 16 (100% of the oil) components, respectively. In leaves, the major components of *C. xanthocarpa* were limonene (41.5%) and spathulenol (5.8%); and the species *C. guaviroba* were β-eudesmol (16.8%) and α-eudesmol (10.3%). In fruits, the major compounds were spathulenol (19.1%) and α-cadinol (13.3%), and for *C. xanthocarpa* were limonene (30.0%), sabinene (16.8%), α-pinene (13.4%) and spathulenol (7.2%).

[1] Wiley Registry of Mass Spectral Data, 6th edn. Wiley Interscience, New York (1994).

Acknowledgements: Embrapa, UFPR/PGAPV, IFC, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Seasonal Analysis of the Volatile Constituents Present in Fresh (recent) and Solid (aged) Oil-resin Formed in *Protium heptaphyllum*

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Keywords: *Protium heptaphyllum*; oilresin; seasonal analysis.

Protium heptaphyllum (Burseraceae) produce an aromatic Oil-resin rich in volatile and non-volatile terpenes. The Oil-resin has pasty or soft consistent when its exudation is recent, and is rich in monoterpenes. Over time the oil-resin becomes solid, and loses significant amounts of volatile compounds. In a previous study from our group by comparing the essential oil of fresh and dried oleoresin it was observed that the fresh one was rich in terpinolene and the dried one in *p*-cymene, suggesting that this last could be a degradation product. In the present work, we carry out a comparative study of seasonal samples of dried, fresh and intermediate oilresin of *P. heptaphyllum* to see how the natural aging process affects the volatile constituents. Oilresins were collected seasonally in São João da Barra, State of Rio de Janeiro, during the years 2014 and 2015. The samples were characterized by their texture in: Softened oilresin – a recent exudate; Semisolid oilresin – an intermediate exudate; and Solid oilresin – an old exudate. The oilresins were hydrodistillated with a Clevenger apparatus for 4 h. The essential oils (EOs) obtained were analyzed by GC/FID and GC/MS, using Agilent system 5973N, with a capillary column HP-5 (30 m x 0.25 mm X 0.25 µm). The temperature was programmed from 60 to 240 °C (3 °C min^{-1}). The identification was made by comparison of the mass spectra (Wiley database) and retention indices calculated from the injection a series of *n*-alkanes. The EOs obtained from recent exudate (11.3-24.9 % v/p) were mainly constituted of terpinolene (49.8-62.2 %) and presented low content of *p*-cymene (4.6-13.5 %) and *p*-cymen-8-ol (2.7-7.4 %), whereas the EOs obtained from old exudate (2.7-4.5 % v/p) had a decrease of terpinolene (21.4-26.3 %) and an increase of *p*-cymene (16.1-18.8 %) and *p*-cymen-8-ol (16.5-26.4 %). The EOs of intermediate exudate (10.1-14.2 % v/p) presented a reduction of terpinolene content (44.5-47.6 %) and an increase in *p*-cymen-8-ol (7.5-9.2 %) when compared to the EOs from recent exudate. With natural aging process the yield of EOs can be reduced up to 10 times, varying from 24.9 % to 2.7 %. The volatile composition of the 0ilresin also change during the natural aging. The increase of *p*-cymene and *p*-cymen-8-ol could be influenced by abiotic factors (light, temperature, oxygenation, humidity) that induce the conversion of the majority non-aromatic monoterpene (terpinolene) in these aromatic monoterpenes.

Acknowledgements: Embrapa, Faperj, CNPq.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development of Larvicidal Nanoemulsions Containing *Baccharis arctostaphyloides* Baker Essential Oil.

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Keywords: *Baccharis arctostaphyloides*; nanoemulsion; larvicidal activity.

Nanotechnology emerged as a promising area for development of innovative phytoproducts, including nanoemulsions.[1] Here, we developed a nanoemulsion with *Baccharis arctostaphyloides* essential oil and evaluate its larvicidal activity against *Aedes aegypti.* Plant material was colleted at Restinga de Jurubatiba National Park (Carapebus, RJ, Brazil). Essential oil from the leaves was extracted by hydrodistillation using a Clevenger apparatus and analyzed by GC/MS and GC/FID. Hydrophilic-lipophilic balance (HLB) of the essential oil was determined by blending polysorbate 80 and sorbitan monooleate at different ratios. Low energy non-heating emulsification method was employed by adding water through organic phase (surfactants mixed with essential oil) under constant magnetic stirring. Nanoemulsions was stored under room temperature (20 ± 2 °C) and evaluated after 1, 7 and 21 days of preparation. Droplet size and polydisersity of the nanoemulsions was determined by photon correlation spectroscopy. Results were expressed as the mean diameter. Larvicidal activity was evaluated according to WHO protocol. Twenty-one compounds were identified, being limonene, β-myrcene, bicyclogermacrene, β-caryophyllene and β-eudesmol the major constituents. Manoyl oxide, 13-*epi*-manoyl oxide and kaurene were first identified in the essential oil of the genus *Baccharis*. Nanoemulsions at HLB 15 presented the smallest mean droplet size and low polidispersity index. Most stable formulation occurs when HLB of the surfactant (s) coincides with HLB of the oil. Thus, required HLB of *B. arctostaphyloides* may be considered 15. Low variation at mean droplet size was observed for nanoemulsion at HLB 15 (Day 0 – 88.96 ± 0.210 nm; Day 21 – 83.90 ± 2.401 nm) suggesting stability of this system. DL50 (48 h) against *A. aegypti* was 118.94 ppm expressed as essential oil content. The present study allowed determination of HLB and development of a potential natural product-based nanoemulsion with larvicidal activity.

[1] Fernandes, C. P.; Mascarenhas, M. P.; Zibetti, F. M.; Lima, B. G.; Oliveira, R. P. R. F.; Rocha, L.; Falcão, D. Q. HLB value, an important parameter for the development of essential oil phytopharmaceuticals. *Brazilian Journal of Pharmacognosy* **2013**, *23*, 108. [CrossRef].

Acknowledgements: FAPEAP, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Essential Oils from Male and Female Flowers of *Clusia hilariana* Schlecht (Clusiaceae)

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Keywords: *Clusia hilariana*; essential oil; flowers; Restinga de Jurubatiba.

The large Neotropical genus *Clusia* comprises more than 300 species and is distributed from southern Florida (Key West) to southern Brazil. Most of *Clusia* species have dioecious flowers[1]. *Clusia hilariana* Schlecht occurs in the state of Rio de Janeiro. Here, we evaluated the chemical composition of volatile substances of essential oil obtained from male and female flowers of *C. hilariana*. Plant material was collected at Carapebus (RJ). Extraction was performed using a Clevenger-type apparatus. Chemical analysis was performed by GC/MS and GC/FID. The essential oils from male and female flowers yielded, respectively, 0.04 % and 0.06 %. In all, 40 components were identified. The major constituent found in both essential oils was (*E*)-caryophyllene, corresponding to 49.7 % and 37.1 % of total composition of male and female flowers essential oils, respectively. α-Cubebene (0.5 %), α-ylangene (0.2 %), aromadendrene (0.2 %), *cis*-muurola-4(14),5-diene (0.3 %), δ-selinene (0.3 %), *trans*-β-guaiene (1.4 %), β-bisabolene (2.2 %), γ-(*E*)-bisabolene (4.2 %), selina-3,7(11)-diene (0.2 %), α-calacorene (0.7 %), gemacrene B (0.3 %) and caryophyllenyl alcohol (0.2 %) were found only in the essential oil from male flowers. The chemical composition of essential oil from female flowers of *C. hilariana* has not been previously described. β-elemene (0.7 %), α-guaiene (2.3 %), 4,5-di-*epi*-aristolochene (0.2 %), α-selinene (8.5 %), α-bulnesene (4.1 %), 7-*epi*-α-selinene (0.3 %), spathulenol (0.3 %), globulol (0.6 %), rosifoliol (0.5 %), humulene epoxide II (0.7 %), α-bisabolol (0.3 %) and aristolone (0.3 %) were identified as chemical constituents of this essential oil, but were not observed on the essential oil from male flowers. The high sesquiterpene content of both male and female essential oil from flowers may suggest that they may play an important role as attractor for pollinators, and contributes to attract the pollinators on the same level, showing there is not a preference for male or female flowers by pollinators.

[1]Kelecom, A.; Reis, G. L.; Fevereiro, P. C. A.; Silva, J. G.; Santos, M. G.; Mello Neto, C. B.; Gonzalez, M. S.; Gouvea, R. C. S.; Almeida, G. S. S. A multidisciplinary approach to the study of the fluminense vegetation. *Annals of the Brazilian Academy of Sciences*, **2002**, *74*, 171. [CrossRef] [Pubmed]

Acknowledgements: CNPq, CAPES and FAPEAP

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Parsley Essential Oil under Drought Stress

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Keywords: *Petroselinum crispum*; water; terpenoids.

Biotic factors can affect content and chemical composition of plant essential oil. The effects differ according to environment, duration and intensity of stress. Drought alters physiological and biochemical processes, and in turns interferes with essential oil biosynthesis[1]. This research aims to evaluate drought stress effect on parsley essential oil production. Plants were grown in 5.5 L pots under protected environment. Treatments were composed by 20, 40, 60, 80 and 100 % of pot water retention capability. At 10 days before harvest the treatments were applied. To monitor water percentage it was used tensiometer. It was used randomized block design with six repetitions and the experimental unit was composed by one pot with three plants. At 99 days after sowing plants were harvested and submitted to drying (4 °C). It was used 2 g of plant material for essential oil extraction. The extraction was carried out in a Clevenger apparatus (hydrodistillation) for 2 h after the beginning of condensation. For essential oil collection 5 mL of hexane was added to collector tube. The volume of collected organic phase was weight and the difference computed as essential oil yield. Essential oil chemical composition was evaluated by gas chromatography (DB-5 column, 30 m X 0.25 mm X 0.25 µm) and analytical conditions: injection temperature 220 °C, starting temperature 50 °C, hold time 5 °C, temperature variation 4 °C, final temperature 240 °C, pressure 67,4 KPa, column flux 1.2 mL min^{-1}, linear velocity 39.7 cm sec^{-1}). It was injected 1 µL of sample. For identification of compounds it was used equipment library. It was not observed effect of drought stress for a period of 10 days before harvest on essential oil content and chemical composition. The average content was 0.67% ($T_{20\%}$ 0,75 %; $T_{40\%}$ 0,80 %; $T_{60\%}$ 0,6 %; $T_{80\%}$ 0,48%; $T_{100\%}$ 0,70 %). The following compounds were observed α-pinene (11.2 to 25.0 %), β-mircene (4.8 to 7.0 %), β-phellandrene (7.2 to 22.8 %), carotol (6.5 to 21.5 %), menthatriene (12.3 to 33.5 %) and apiole (42.4 to 69.9 %).

[1]Kleinwächter, M.; Selmar, D. New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. *Agronomy for Sustainable Development* **2015**, *35,* 121. [CrossRef]

Acknowledgements: CNPq, UENF.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development of a Larvicidal Nanoemulsion Containing *Siparuna guianensis* Aublet Essential Oil

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Keywords: *Siparuna guianensis;* nanoemulsions; larvicidal activity.

Siparuna guianensis Aublet (Siparunaceae) is popularly known as capitiú and negramina, and is a potential larvicidal agent against *Aedes aegypti* and *Culex quinquefasciatus*[1]. However, poor water solubility of essential oils (EO) is considered a technological problem for their application as effective larvicidal products. On this context, O/W nanoemulsions have been considered potential innovative products for this purpose. Here, we evaluated the larvicidal activity of nanoemulsions containing EOs from *S. guianensis* against *A. aegypti* and *C. quinquefasciatus* larvae. Leaves of *S. guianensis* were collected in June 2014 at the Macapá, AP (voucher at IEPA, no. 12819). EO was extracted from the fresh leaves (1 kg) by hydrodistillation, using a Clevenger apparatus. Chemical analysis was performed by GC/MS. Nanoemulsion was obtained by a low energy method using 95.5 % (w/w) of water, 0.25 % (w/w) of essential oil and 0.25 % (w/w) of polysorbate 80. The EO and polysorbate 80 were mixed and stirred at 500 rpm using a magnetic stirrer for 30 min. Then, water was added and the mixture was stirred at 500 rpm for 60 min. Nanoemulsion was stored at 20 ± 2 °C and analyzed after 1, 7, 21 and 30 days of preparation. Droplet size and polydispersity of the nanoemulsion was determined by photon correlation spectroscopy. Nanoemulsion was diluted with water for injection (1:25) and measurements were performed in triplicate. Results were expressed as the mean diameter and standard deviation. The EO has monoterpenes, including α-pinene and myrcene, and sesquiterpenes like γ-cadinene, δ-cadinene and *epi*-α-cadinol. Aliphatic ketones were also found. Nanoemulsion presented fine translucent aspect and bluish reflect, which is characteristic of this disperse system. Small mean droplet size was observed, ranging from 176.0 ± 12.26 at day 0 to 152.6 ± 1.562 after 30 days of storage. Moreover, low polidispersity index was observed (< 0.500) suggesting stability of the nanoemulsion. DL50 (24 h) for *A. aegypti* and *C. quinquefasciatus* were respectively 13.28 ppm and 24.93 ppm (expressed as essential oil content). This study contributes to nanobiotechnology of natural products, presenting a potential larvicidal nanoemulsion prepared with *S. guianensis* essential oil.

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Acknowledgements: Fapeap, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Composition of Essential Oils from the Species *Ardisia humilis a*nd *Myrsine lineata* (Primulaceae)

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Keywords: Myrsinoideae; *Ardisia humilis*; *Myrsine lineata*.

Atlantic Forest is considered one of the most important tropical forests in the world due to its high level of diversity and endemism. This biome consists of a variety of formations associated with the climatic characteristics of each region. Primulaceae (order Ericales) comprises 2590 species grouped in 58 genera. The genus *Ardisia* is commonly used as ornamentation although it is used in Chinese medicine for different illnesses. The genus *Myrsine* is endemic of Brazil. The species belonging to the genus *Myrsine* have been investigated concerning their chemical compounds and activity on human health. Leaves of the species *A. humilis* Vahl. and *M. lineata* (Mez) Imkhan. were collected in the Rain Forest Submontane in the state of Rio de Janeiro. A voucher specimen was deposited in the herbarium of Rio de Janeiro Botanic Garden (RB) RBv2091 and RB 605196. About 20 g of dried leaves of each species were subjected to hydrodistillation separately in a Clevenger-type apparatus for 3 h. The oils were analyzed by GC/FID in an Perkin-Elmer Autosystem and GC/MS in an Agilent 6890N and an Agilent 5973N systems, both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of mass spectra and linear retention indices with spectral library. About 12 compounds were identified from the essential oils of *A. humilis*, representing 95.6 % of the sample. The essential oil from *A. humilis* was characterized by major compounds β-caryophyllene (40.6 %), limonene (21.6 %) and *trans*-nerolidol (14.2 %). The monoterpenes comprised 23.8 % of the total sample, all belonging to the hydrocarbons class. The sesquiterpenes compounds accounted for 67.9 % of the total sample, 49.3 % of which belong to the hydrocarbons class and 18.6 % to the alcohol class. *M. lineata* had 99.7 % of the sample indentified, represented by 26 compounds. The major compounds in *M. lineata* were α-pinene (35.5 %), β-pinene (20.3 %) and silvestrene (12.8 %). The monoterpenes accounted for 90.6 % of the sample total, of which 89.2% belong to hydrocarbons class and 1.4 % to the alcohol class. The sesquiterpenes comprised 7.5 % of the total sample, of which 7.1 % belong to the hydrocarbons class and 0.4 % to the alcohol class. Thereby the extraction and analyses of the essential oil from *A. humilis* and *M. lineata* was succeeded once the identification was possible of over than 95 % of the samples.

Acknowledgements: Embrapa, Faperj, CNPq, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Rapanea leuconeura (Mart.) Mez: The Essential Oil from the Specie Primulaceae

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Keywords: Pororoca; bicyclogermacrene; Clevenger.

Rapanea leuconeura a tree species that occurs in Brazil and is adapted to the states of Goiás, Bahia, Minas Gerais, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, blooms from July through August and fruits from September through January[1]. Leaves of plants grown in the region of Sacramento - MG and Brodowski – SP were collected for the chemical identification of compounds present in the *R. leuconeura* essential oil. Plant exsiccates were deposited (HPMU 3077and 3079) in the Herbarium of the Biotechnology Division at UNAERP - University of Ribeirão Preto. Dry leaves from species collected in both locations were subjected to hydrodistillation separately for 3 h each using a Clevenger-type apparatus. The extracted oils were then analyzed using a Shimadzu (QP-2010) Gas Chromatography/Mass Spectrometry system, with DB-5MS (30 m X 0.25 mm X 0.25 µm) column, Helium was used as carrier gas with a flow rate of 1.0 mL min^{-1}, oven temperature from 60 to 240 °C at 3 °C min^{-1}. The mass spectrometer was operated in the electronic ionization mode (70 eV). Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature[2]. The yields of essential oils obtained from leaves collected in Sacramento and Brodowski were 0.042 % (w/w) and 0.024 % (w/w) respectively. The substances identified in the leaf oil of plants collected in either Minas Gerais or São Paulo were the same varying only in concentrations. Fifty-three components were identified in the oil extracted from Sacramento plants, representing 96.8 % of the total volatiles. The leaf oil was rich in sesquiterpenes (96.0 %) and the major components were bicyclogermacrene (20.1 %), spathulenol (10.4 %), α-guaiene (8.4 %), δ-cadinene (15.6 %), maaliol (4.7 %), epi-α-muurolol (4.6 %) and dauca-5,8-diene (3.7 %). The essential oil extracted from plants grown in Minas Gerais and São Paulo was identical, however, concentration levels varied. The chemical composition of the essential oil from *R. leuconeura* has never been reported before.

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Acknowledgements: UNAERP, CAPES.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Volatile Constituents of *Zingiber officinale* Roscoe (Zinziberaceae) from Rio de Janeiro - Brazil Obtained by Solid Phase Micro-Extraction (SPME) - GC/MS

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Keywords: *Zingiber officinale* Roscoe; essential oil; solid phase micro-extraction.

The Zinziberaceae family has 53 genera and over 1200 species of plants. The genus *Zinziber* consists of a set of medicinal and food plants, and the species *Zingiber officinale* Roscoe has a high therapeutic potential in several diseases. The rhizome is the most important part of the plant as it is rich in carbohydrates, lipids, volatile oil and oleoresin. This part of the plant has been well studied for medicinal purposes due to anti-inflammatory activity, anti-emetic, antinausea, anti-ulcer, hypoglycaemic, antibacterial and others. The essential oil of ginger oleoresin contains considerable quantities of phenolic compounds, which are responsible for the antimicrobial potency. The rhizome and fresh leaf were acquired in Rio de Janeiro, Brazil and subjected to head space solid-phase microextraction (HS-SPME) using a 100 µm polydimethyl- siloxane (PDMS) fiber, exposed for fifteen minutes to the headspace of fresh plant, in bath with boiling water. Desorption time of 20 min. The volatile constituents were analyzed by GC/MS on a Shimadzu QP 2010 system, both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Helium was used as carrier gas for GC/MS, with a flow rate of 1.0 mL min^{-1}. Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Injector was kept at 260 °C and mass detector was operated in electron ionization mode at 70 eV. Oil components were identified by comparison of mass spectra with spectral library and literature. In the rhizome in a total of ten constituents were identified: α-zingiberene (65.4 %), germacrene D (6.2 %), β-bisabolene (5.3 %), β-sesquiphellandrene (5.0 %), α-copaene (4.1 %), camphene (3.6 %), α-farnesene (3.3 %), α-fenchene (3.0 %), α-pinene (2.3 %) and α-thujene (1.3 %). In the fresh leaf oil only one compound was identified, the sesquiterpene, (*E*)-caryophyllene (98.6 %).

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Acknowledgements: IFRJ, Embrapa.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Chemical Composition of the Essential Oil of *Abies guatemalensis* Rehder, an Endangered Species from Guatemala.

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Keywords: *Abies guatemalensis*; terpenes; essential oil.

Abies guatemalensis Rehder, grows in moist or wet forests of the high mountains and volcanoes of the west of Guatemala, mainly at 2700-3500 m at the provinces of Quiché, Totonicapán, Huehuetenango, Quetzaltenango, San Marcos. It is also found at Chiapas, Oaxaca and Guerrero, in Mexico. *A. guatemalensis*, a tree 45 m high with a trunk almost 1 m in diameter, shows branches dark or grayish brown, leaves appearing 2-ranked, spreading-ascending, linear, 1-4.5 cm long, 1-2 mm wide[1]. The principal use of the tree is for decorative purposes. The small trees have a high demand for this purpose among the foreign and local residents leading almost to its extinction. Thus, since 1979 the tree is protected as endangered species and it is only sold during December at a limited scale with a certificate when cut at controlled forests. Leaves of *A. guatemalensis* were collected in January 2014, from a population found at Mixco, province of Guatemala, at 1600 m, in a site surrounded by pines, at 20 km west from Guatemala City. The oil from 40.0 g of dried material was extracted by hydrodistillation using a Clevenger-type apparatus for 2 h yielding 0.25 % (w/w). The GC/MS analyses were carried out in a Shimadzu 2010 Plus system coupled with a Shimadzu QP-2010 Plus selective detector (MSD), equipped with a DB5-MS capillary fused silica column (60 m X 0.25 mm X 0.25 µm). The oven temperature program initiated at 60 °C, then rose at 3 °C min^{-1} to 246 °C, held for 20 min. The carrier gas was He with a flow rate of 1.03 mL min^{-1}; split ratio of 1:50. Mass spectra were taken at 70 eV. The m/z values were recorded in the range of m/z 40–700 Da. GC/FID analyses were carried out using a Shimadzu 2010 GC apparatus equipped with a DB5 fused-silica capillary column (60 m X 0.22 mm X 0.25 µm, Restek, France). The oven temperature was set from 60 to 246 °C at 3 °C min^{-1} and then held isothermally at 246 °C for 20 min. The carrier gas was N_2 (1.44 mL min^{-1}). The identification of the oil components was done by the evaluation of their mass spectra and retention indices. The compounds found in higher concentration were predominantly terpenes, with β-caryophyllene (30.4 %), limonene (13.8 %) and α-pinene (10.1 %) as the major components. This is the first report on the oil composition of this plant and the results would be useful as basic information for the management of the species and to evaluate the production of the oil from wastes from the pruning of commercial forests.

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Acknowledgements: CAPES, CNPq, IPPN/UFRJ, CONCYT/Guatemala (Proyecto FODECYT 12-2013).

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Development and Evaluation of an Inhalation Chamber for *In Vivo* Tests with Drugs Administered by Pulmonary Route

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Keywords: Inhalation chamber; eugenol; headspace; inhalation.

Fumigation, in which the individual may receive varying doses of volatile active ingredients by inhalation, is a popular practice for medicinal plants. Many models have been described in the literature for assessment of essential oils by inhalation. However, some structural improvements can bring benefits to these works.[1] Bearing this in mind and based on pre-existing models and group discussions, an inhalation chamber was developed to carry out pharmacological and toxicological tests on animals. The animals are inserted in each one of these tubes and nebulized air passes directly and continuously through it, by the animal's snout, following then to an outlet at its distal part. Evaluation of its operation was performed using *Caryophyllus aromaticus* L. essential oil (EO), a nebulizer and a flow meter. The air within the chamber was collected by static headspace and analyzed by GC/FID, employing a fast method of analysis developed for this purpose. It was found that the air flow in each of the five outputs of the chamber was 0.92 LPM. EO showed three major components, eugenol (85.0 %), eugenol acetate (4.8 %) and caryophyllene (10.3 %). Some tests were performed increasing oven initial temperature, increase rate of this temperature and gas flow, and decreasing the maximum temperature to be reached by the oven. Thus, the method with 2.5 min duration, oven temperature at 160 °C increasing at a rate of 20 °C min^{-1} up to 180 °C, and gas flow of 5 mL min^{-1}, was chosen as the method of analysis. It could be shown that between each collecting air ten displacements with the piston should be made to prevent the contaminants accumulation into the syringe during static headspace collecting. The chamber has been evaluated for 25 min nebulization, being air samples collected and analyzed each odd minute. The results show a homogeneous and continuous operation of the chamber without volatile material accumulation inside. In this way, it can be concluded that the inhalation chamber works satisfactorily for *in vivo* tests with medicines designed to be administrated by inhalation. Besides, it does not require a waiting time for saturation and subsequent insertion of animals since, from the first minute, the concentration of volatiles in its internal atmosphere does not vary.

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Acknowledgements: Embrapa Food Technology.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Inhibitory effects of *Lavandula angustifolia* Essential Oil on Selected Metalloproteinases

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Keywords: *Lavandula angustifolia;* essential oil; matrix metalloproteinases (MMPs).

Lavender (*Lavandula angustifolia* Mill.) is promoted as a topically applied essential oil (EO) that can help to relieve the symptoms of psoriasis, dermatitis and eczema, and also has anti-inflammatory, wound healing properties with constituents with antimutagenic and anticancer properties[1]. Matrix metalloproteinases (MMPs) form a group of more than 20 zinc-dependent enzymes, which are involved in the remodeling of several components of the extracellular matrix (ECM). They play a role in many physiological processes such as embryo implantation, bone remodeling and organogenesis, and have additional roles in the reorganization of tissues during pathological conditions such as inflammation, wound healing and invasion of cancer cells[2]. Therefore, MMPs are selected as attractive cancer targets. Here, we evaluated the inhibitory effect of commercial *L. angustifolia* EO on gelatinases (MMP-2 and -9), and collagenases (MMP-1, -8 and -13), respectively. The *in vitro* evaluation of cytotoxic properties of the EO using the MTT assay was performed for the determination of the selectivity and safety of the test materials. EO analysed by GC and GC/MS showed that the major components were linalool 35.8 % and linalyl acetate 36 %. The EO was relatively non-toxic on *3T3* cell lines with the IC_{50}> *500 µg/mL level.* When compared with the standard compound NNGH the inhibition percentage (%) values were; 100% for MMP-2, and 85.4 ± 4.2 % for MMP-9, (at 0.4 mg mL^{-1} concentration), where the EO showed a strong inhibition (66.3 ± 5.3 %) on MMP-9 and a rather weak inhibition (13.21 ± 2.7 %) on MMP-2 at the same concentration, respectively. So, lavender EO can be further evaluated for its MMP inhibitory activities. The inhibition of MMPs plays a role at amelioration of the corrupt pathophysiology of the disease, where gelatinase inhibition might be the underlying mechanism for therapeutic efficacy.

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Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Toxicity of the essential oils from *Piper hispidinervum* C. DC. and *Piper callosum* Ruiz Pav. to cupuassu fruit borer *Conotrachelus* sp. (Coleoptera: Curculionidae)

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Keywords: Piperaceae; insecticide activity; *Theobroma grandiflorum*.

The genus *Piper* has various plants species with insecticide potential, for example *Piper hispidinervum* C. DC. and *Piper callosum* Ruiz & Pav. One way for to comprove the insecticide activity of plants is to test the application of its essential oil and to verify the insect mortality. The target-insect used in experiments was the curculio *Conotrachelus* sp. This insect is considered a pest in the cupuassu (*Theobroma grandiflorum*) tillage. Therefore, the aim with this work was evaluated the toxicity of the essential oils from *P. hispidinervum* and *P. callosum* to the cupuassu fruit borer *Conotrachelus* sp. The essential oils were obtained by hydrodistillation. The experiments were performed in laboratory conditions and the acute toxicity tests (48 h) were performed by contact in contaminated filter paper and contact/ingestion in sugarcane pieces[1]. The essential oils were diluted in acetone and tested in these concentrations: 2, 4, 8 and 16 mg mL^{-1} (test by contact in filter paper) and 4, 8, 16 and 32 mg mL^{-1} (test by contact/ingestion in sugarcane pieces). Acetone was the control group. In the test by contact in filter paper 1 mL of solution was pipetted on the filter-paper and in the test by contact/ingestion, sugarcane pieces were immersed in the solution with essential oil and acetone for thirty seconds. After the solvent evaporation, four adults insects, non-sexed, were placed in the Petri dishes (90 mm) and kept in climatized chamber with 27 ± 2 °C and 12 h photophase. The experimental design used was completed randomized design (CRD) with four replicates. The mortality data were statistically analyzed by dose-response using logistic model and the LC_{50} were estimated using Delta method of the DRC package compiled by R® software[2]. The two essential oils tested showed acute toxicity to the curculio, the test by contact in filter-paper showed that the higher toxicity occurs with essential oil of *P. hispidinervum* with LC_{50} of 3.36 mg mL^{-1}, when compared to essential oil of *P. callosum* with LC_{50} of 4.60 mg mL^{-1}. The test by contact/ingestion in sugarcane pieces showed that the higher toxicity also occurs with essential oil of *P. hispidinervum* with LC_{50} of 12.08 mg mL^{-1}, when compared to essential oil of *P. callosum* with LC_{50} of 15.26 mg mL^{-1}.

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Acknowledgements: Fapeam.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

LRI-Filtered Identification of Essential Oil Constituents by Using a Novel MS Database

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Keywords: essential oils, MS database, LRI.

The linear retention index (LRI) approach can be used in combination with conventional mass spectral searching, with the goal of boosting the identification of "challenging" molecules, occurring in flavours and fragrances. To this end, a GC-MS database (*FFNSC* library), collecting around 3000 spectra derived from pure chemicals, essential oils and perfumes, has been built-up providing each spectrum with mass fragments and structural information, but also with the experimental value of LRI. In the present study such powerfull tools were used to perform the chemical investigation of the essential oils obtained from Iranian plants of *Artemisia kopetdaghensi s*Krasch ex Poljakov, *Artemisia oliveriana* J. Gay ex Besser, *Artemisia austriaca* Jacq. and *Artemisia diffusa* Krasch ex Poljakov. Accurate GC-FID and GC-MS analyses were carried out. Quantitative analysis was based on both the internal standard method and the measurement of FID response factors. LRIs were used to eliminate database matches with high similarity scores but with LRI values far from the target ones.

Acknowledgements: This work has been carried out within the framework of the Research Project "PRIN 2012": assessment of quality and safety of Mediterranean seafoods by "omics" sciences, supported by the Italian Ministry of University and Scientific Research, nr. 2012TLC44W.

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Rhipicephalus microplus: Aguaribay, Lipia and Orange vapours effects on its reproduction

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Keywords: *Rhipicephalus microplus;* essential oils; reproduction.

In the bovine cattle, one of the greatest economic loss occurs because of the diseases caused by the common bovine tick, *Rhipicephalus microplus*. This has motivated the study of natural substances because they are less polluting than the synthetic products that are currently used. The objective of this work is to determine the effect on the reproduction of the tick in the presence of the vapors of the essential oils of aguaribay (*Schinus molle* L.), lipia (*Lippia alba* Mill. N. E. Brown ex Britton & Wilson) and orange (*Citrus aurantium*). The methodology used in the *in vitro* experiments is the immersion proof of adults[1].The technique was to wash and dry the engorged ticks collected from animals with natural infestation, to then select the most vital to carry out the test. They were weighed and placed in Petri dishes and distributed in 4 groups, one for each type of oil and one for witness, with 3 replicates for each group (12 boxes in total). Within the boxes are placed in the essential oil aluminum containers. For the control, it was use distilled water. The top of the box is drilled to allow the entry of air. The boxes were left on the bench at room temperature for 24 h. After this time, the plates were incubated in the oven during 14 days, a period in which it is considered that all engorged ticks laid eggs. The eggs obtained were weighted and incubated in the oven during 25 days, a time that is considered that has finished the oviposition. The percentage of control was 28 % for the essential oil of aguaribay, 70 % for lipia oil and 40 % for the orange oil. It can be concluded that the vapours from these oils in different extent affect the reproduction of ticks, being the lipia essential oil that has the highest percentage of control.

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Acknowledgements: Universidad Nacional del Litoral, CAID PI 2011, "Aceites esenciales provenientes de especies vegetales del Litoral Argentino para el mejoramiento sanitario de la ganadería bovina de Santa Fe".

Revista Virtual de Química
ISSN 1984-6835

8th Brazilian Symposium on Essential Oils

Abstract

Esterification of *Rosmarinus officinalis* L. (Rosemary) essential oil

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Keywords: *Rosmarinus officinalis*; essential oil; esterification.

Essential oils are an important raw material for many industries, where they can be used in the natural or synthetically modified way. One of the most common transformations employed by these industries is the esterification that aims the production of esters from the alcohols presents in the essential oil. This kind of reaction can modify the aroma profile of the essential oil. *Rosmarinus officinalis*, popularly known as "rosemary", is a perennial herb from Lamiaceae family. The essential oil of rosemary, generally isolated by hydrodistillation, is mainly composed by monoterpenes, oxygenated monoterpenes and, in the lesser proportion, by sesquiterpenes. Its aroma is strong and pleasant, remitting to herb, spice or pine wood. The major constituents are camphor, 1,8-cineole, α-pinene and myrcene[2,3]. Fresh samples were bought in a market in Rio de Janeiro - RJ, Brazil. Leaves were separated in three portions (*c.a.* 176 g) and each one was subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The esterification reaction was carried out using essential oil/acetic anhydride/sodium acetate (2:1:0.1) for 2 h at 100 °C. Analyzes were performed by GC/MS (Agilent 6850) and GC/FID (Agilent 6890N) systems both with a HP-5MS (30 m X 0.25 mm X 0.25 µm) capillary column. Helium and hydrogen were used as carrier gases of these systems with a flow rate of 1.0 mL min^{-1} and 1.2 mL min^{-1}, respectively. Oven temperature was raised from 50 to 180 °C at 3 °C min^{-1} and the injection was made at 220 °C for 0.20 min in splitless mode. The percentage composition was obtained by normalization from FID. The identification of the components of the natural and modified essential oil were carried out by comparison of mass spectra data with NIST 14 and Willey 275 libraries, and also, by the calculated and literature linear retention indices. The average yield was 0.5 % and the alcohols correspond to 7.5 % of this total. The major compounds identified were camphor (28.4 %), 1,8-cineole (15.0 %) and α-pinene (12.5 %). Among principal alcohols are α-terpineol (1.9 %), terpinen-4-ol (1.1 %), borneol (1.4 %) and linalool (0.8 %). Only one ester was identified in natural essential oil: bornyl acetate (0.46 %). After the reaction, it were identified the following esters: 1-octen-3-yl acetate (0.6 %), isopulegol acetate (0.3 %), myrtenyl acetate (0.5 %) and, besides the bornylacetate (3.1 %). The aroma profile of the oil changed with the esterification reaction and got a more light and woody odor.

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Acknowledgements: CAPES, CNPq and Fapeal.