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Chemical composition and antibacterial activity of *Ruta graveolens* L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil

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ABSTRACT

This study investigated the chemical composition and antibacterial activities of volatile oil isolated from the fresh leaves of *Ruta graveolens* L., Rutaceae, from São Luís, Maranhão, Brazil. The essential oil was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC-FID and GC–MS. Seven compounds representing 100% of the oil were identified. The main compounds were 2-nonanone (39.17%) and 2-undecanone (47.21%). Antibacterial activity against Gram-positive and Gram-negative bacteria with inhibition zones of 8.30–25.60 mm to MIC values of 0.75–1.40 $\mu g \cdot mL^{-1}$, the most susceptible bacterium was *Bacillus cereus* and *Staphylococcus aureus*. It is concluded from the present study that besides its traditional use, *Ruta graveolens* L. could be used as a natural source for antibacterial compounds and possible applications in the pharmaceutical industry.

1. Introduction

In recent years there has been a great scientific advance regarding chemical and pharmacological studies of medicinal plants aimed at obtaining new compounds with biological properties (Hossain et al., 2012). Among the countless species of medicinal interest, there are plants belonging to the Rutaceae family, which has species of economic, ecological and therapeutic importance (Januário et al., 2009).

The Rutaceae family, also named as Rutaceae, belongs to the order of Sapindales with about 150 genders and over 1600 species. They are hugely distributed throughout the tropical and temperate regions of the globe, being more abundant in tropical America, South Africa and Australia (Albarici et al., 2010).

In Brazil, 33 genders and approximately 192 species are described (Pirani and Groppo, 2010). This family is known for presenting a huge variety secondary metabolites of strongly aromatic due to the presence of essential oils, which has attracted the attention of several research groups regarding its chemical and biological importance of many of these metabolites (De La Cruz, 2008; Bizzo et al., 2009; Costa et al., 2010; Elaissi et al., 2011).

Among the representatives of this essential oil family producers, stand out *Ruta graveolens* L., popularly known as rue, rue-smelly, rude-smell strong, rue and rue-home-of-gardens (Lorenzi and Matos, 2002; Anonymous, 2003), hugely used as a medicinal resource by local people throughout Brazil (Al-Qurainy et al., 2011; Souza et al., 2007).

In folk medicine, there are several therapeutic properties described from *R. graveolens* L., which include the use of this plant in menstrual disorders, skin inflammations, cramps, earache and headache (Mejri et al., 2010; Ratheesh et al., 2011).

Pharmacological trials have their as anthelmintic, abortion, antiparasitic, healing, anti-inflammatory, anti-diarrheic, anti-rheumatic, antifebrile, antiulcer, vermicide repellent, anti-diabetics, anti-rheumatism and antimicrobial properties (Yamashita et al., 2009; Ahmad et al., 2010; Mejri et al., 2010).

In recent years, there has been a growing interest in researches looking for possible uses of plant products as antimicrobial instead of several synthetic antibacterial which can cause several side effects. Historically, natural products and their derivatives have been an invaluable source of therapeutic agents. When in vitro, antimicrobial assays have effectively served as reliable methods to detect several classes of secondary metabolites with high antimicrobial activity (Silver and Bostian, 1990; Koehn and Carter, 2005; Freiesleben and Jäger, 2014).

Antimicrobials from plant sources may exert their activity through different mechanisms from those currently used as synthetic drugs. Thus, they can significantly help in the treatment of resistant microbial strains (Gardete and Tomasz, 2014).

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This paper deals with the possibility of contributing to the pharmacological study of this species in Brazil. Therefore, in this paper we report the chemical composition and the antibacterial activities of the essential oil from the leaves of rue, against standard strains of bacteria.

2. Materials and methods

2.1. Plant material

Aerial parts of *R. graveolens* L. were collected during the flowering stage of plant, around the Maiobão region, from Paço of Lumiar, Maranhão, Northeast of Brazil, in July 2011. The plant was identified in the Laboratory of Botany, Chemistry and Biology Department, Maranhão State University, Brazil. A voucher specimen (no. 107) was deposited at the Laboratory's Herbarium.

2.2. Extraction of essential oil

The essential oil was extracted according to the method described by Viuda-Martos et al. (2011) with few modifications. Briefly, from the fresh plant material was ground and hydrodistilled for 4 h housing a Clevenger-type apparatus. The oily layer obtained on top of the aqueous distillate was separated and dried with a hydrous sodium sulfate. The oil obtained was stored in a tight closed dark vials and covered with aluminum foil at 4 °C until further analysis. Essential oil was obtained with a light green transparent liquid and had specific aroma with a 1.29 ± 0.02% (v/w) yield.

2.3. GC-FID analysis

The essential oil was analyzed using Shimadzu QP-5000 GC equipped with flame ionization detector (FID) and HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) in stationary phase, containing 5% of diphenyl and 95% of dimethyl polysiloxane, whose injector and detector temperatures were maintained at 280 °C. The oven temperature was programmed from 40 °C for 5 min, raised to 240 °C at a rate of 4 °C/min, and isotherm at 240 °C for 7.5 min. Helium was the carrier gas, at a flow rate of 1 mL/min. A sample of 0.3 μL essential oil was injected manually (in split mode 1:10).

2.4. GC–MS analysis

The analysis of essential oil was performed using a Shimadzu QP-5000 GC, equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) in stationary phase containing 5% of diphenyl and 95% of dimethyl polysiloxane. The mass selective detector was operated in electron-impact ionization (EI) mode with a mass scan range at 70 eV. GC conditions were the same as described above. The retention indices were calculated, for all volatile contents using a homologous series of n-alkanes C_8–C_{22}. The essential oil contents were identified by comparing their GC retention indices, mass spectra with publish data (Adams, 2007) and National Institute of Standards and Technology mass spectra library data, provided by the software of GC–MS system. Essential oil components are reported as a relative percentage of the total oil by peak area.

2.5. Antibacterial activity

2.5.1. Microbial strains

The microorganisms used for antibacterial activity evaluation were obtained from the American type culture collection (ATCC) as well as the culture collection of the Microbiology Laboratory from Technology Pavilion, Maranhão Federal University, Brazil. The Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Micrococcus flavus* (ATCC 25923), *Micrococcus luteus* (ATCC 9341), *Bacillus cereus* (ATCC 11778); Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Enterobacter aerogenes* (ATCC 13048) and *Salmonella typhi* (ATCC 19430) were used in the antimicrobial assays.

Strains were preserved at 4 °C and grew on Luria–Bertani agar 24 h prior to any assay. Mueller–Hinton (MH) was used for the antibiotic susceptibility tests.

2.5.2. Antimicrobial activity assay

The essential oil was tested for the antimicrobial activity by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2009), using 100 μL of suspension of tested microorganisms, containing 2×10^8 CFU/mL for bacteria. Mueller–Hinton agar was sterilized in a flask and cooled to 45–50 °C and was distributed into sterilized Petri dishes with a diameter of 9 cm (15 mL). The paper-filter disks (6 mm in diameter) were individually impregnated with 10 μL of the oil dissolved in 0.5% at concentration (75 $\mu g.mL^{-1}$), and then placed onto the agar plates which had previously been inoculated with the tested microorganisms.

The Petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37 °C, for 24 h. The diameters of the inhibition zones (mm) were measured including the diameter of the disks. All tests were performed in triplicate. Gentamycin (30 μg/disk), for Gram-positive bacteria, and mikacin (30 μg/disk) – for Gram-negative – served as positive controls.

2.5.3. Determination of minimal inhibitory and minimal bactericidal concentrations

The minimal inhibitory concentration (MIC) was determined using the macro dilution broth method as previously described (Clinical and Laboratory Standards Institute — CLSI, 2009). All tests were performed in MHB. The investigated oils were dissolved in 1% of dimethyl sulfoxide (DMSO) and then diluted until the highest concentration.

Serial doubling dilutions of oils were prepared in a 96-well micropipette over the range of (0.001–75.0 $\mu g \cdot mL^{-1}$). Overnight broth cultures of each strain were prepared and the final concentration in each was adjusted to 5×10^5 CFU/mL for bacteria. The bacteria were incubated for 24 h, at 37 and at 30 °C, respectively. The MIC is defined through the lowest concentration of the essential oil until the microorganism does not demonstrate visible growth. Microorganism growth was indicated by turbidity.

2.6. Statistical analysis

In disk sensitivity, zones of inhibition were measured in millimeters with a centimeter ruler. All the experiments were conducted in triplicate and statistical analysis of data was performed by analysis of variance (ANOVA), using SPSS 21 software. All data are presented with mean values ± standard deviation (SD). In order to satisfy ANOVA, assumptions data were transformed, followed by multiple comparisons tests.

3. Results and discussion

3.1. Chemical composition of volatile oil

The essential oil was obtained by hydrodistillation of fresh leaf sample of *R. graveolens* L., and produced a green color with a yield of 1.29% (v/w). The chemical compositions of essential oil were analyzed by GC and GC–MS and the result was presented in Table 1. In total, seven components were identified, representing 100% of the total amount. 2-Undecanone (47.21%), an aliphatic ketone was found as the main component. 2-Nonanone (39.17%) was the second major aliphatic ketone detected in rue oil, followed by octyl acetate (7.31%), 2-decanone (2,.03%), diethyl phthalate (1.73%), 2-dodecanone (1.53%), pentadecanolide acetate (1.02%), and others were found to be the minor components in the essential oil.

The profile obtained in the present study was very similar to the previous results reported by Gina et al. (2008), who identified 83.4% of