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Essential oil composition and antibacterial activity of two Ageratina species collected in Mérida-Venezuela.

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Abstract

Essential oil from leaves of Ageratina jahnii (B.L.Rob.) R. M. King & H. Rob. and Ageratina pichinchensis (Kunth) R. M. King & H. Rob (Asteraceae) collected in Jan 2010 were analyzed by GC/MS. Oils extracted by hydrodistillation yielded 0.50% and 0.43% w/v, respectively. Fifteen and twenty five components were identified by comparison of their mass spectra with the Wiley GC-MS Library data and by their retention indices (RI). The major components identified in A. jahnii were β-myrcene (31.6%), α-pinene (23.1%), limonene (7.8%) and pentacosane (10.2%) while for A. pichinchensis 8,9-epoxythymyl isobutyrate (21.2%), germacrene-D (20.8%), thymyl isobutyrate (15.8%), eupatoriochromene (5.5%) and encecalol (4.9%) were observed as main compounds. This is the first report regarding the essential oil composition and antibacterial activity of the essential oil of A. jahnii.

INTRODUCTION

Ageratina genus belongs to Asteraceae family[1] and its species are distributed mainly in South America. In Venezuela are located in Amazonas, Aragua, Bolívar, Distrito Federal, Monagas, Zulia, Táchira, Mérida and Trujillo[2]. Species of this genus have been used in traditional medicine for the treatment of mycosis, skin infections and wounds, as well as for its analgesic activity[3,4]. Previous investigations have also reported anti-inflammatory[5] antiviral[6] antibacterial[6,7] molluscicidal[8] and larvicidal activities[9]. Essential oil composition of different Ageratina (Syn. Eupatorium) species has also been investigated, from which a variety of monoterpernes and sesquiterpenes have been identified[7]. Present investigation aim to compare the chemical composition and evaluate the antibacterial activity of essential oils of A. jahnii and A. pichinchesis collected from Mérida-Venezuela.

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MATERIALS AND METHODS

Plant material

Ageratina jahnii (B.L.Rob.) R. M. King & H. Rob. was collected from "Tostós" (8º22´27´´N-71º16´00´´W), Mérida state at 2547 m.a.s.l. while Ageratina pichinchensis (Kunth) R. M. King & H. Rob. was collected from "Las Lajas" (8º36´36´´N-71º23´38´´W), Mérida State at 3621 m.a.s.l. Plants were identified by Dr. Pablo Meléndez and voucher specimens (A. jahnii, LT02 and A. pichinchensis, PM 614) were deposited in the Luis Ruiz Terán Herbarium of the Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela.

Isolation of essential oil

Fresh leaves (A. jahnii, 1250 g) and (A. pichinchensis, 1480 g) were cut into small pieces and submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4ºC.

Gas chromatography-Mass spectrometry (GC-MS)

The GC-MS analyses were carried out on a Hewlett Packard GC-MS system, model 5973, fitted with a fused-silica capillary column of 5% phenylmethyl polysiloxane of 30 m x 0.25 mm, film thickness 0.25 µm (HP-5MS, Hewlett Packard, USA). The source and quadrupole temperatures were 230°C and 150°C, respectively. Helium was used as carrier gas, adjusted to a linear velocity of 34 m/s. The ionization energy was 70 ev, and the scan range 40-500 amu at 3.9 scans/s. The injected volume was 1.0 µl of a dilution of oil in n-heptane (2%). A Hewlett-Packard ALS injector was used with a split ratio of 1:100. The identification of the components was based on a Wiley MS data library (6th ed), followed by comparisons of MS data with published literature[10].

Antimicrobial assay

The antimicrobial assay was carried out according to the disc diffusion method described by Rondón et al., 2005[11]. The strains (Staphylococcus aureus (25923), Enterococcus faecalis (19433), Escherichia coli (25992), Pseudomonas aeruginosa (27853) and Klebsiella pneumoniae (25955)) were maintained in agar at room temperature. Every bacterial inoculum (2.5 mL) was incubated in Mueller-Hinton broth at 37ºC for 18 h. The bacterial inoculum was diluted in sterile saline solution (0.85%) to obtain turbidity visually comparable to a McFarland Nº 0.5 standard (106-8 CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a filter paper disc (6 mm in diameter) saturated with 10 µL of essential oil. The plates were left for 30 min at room temperature and then incubated at 37ºC for 24 h. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also

used to check the sensitivity of the tested organisms using: Amikacin® (30 µg), Ampicillin® (10 µg) and Erythromycin® (15 µg), these are reference antibiotics commonly used to treat this kind of bacteria. The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. Dilutions of the oil in dimethylsulfoxide (DMSO) within a concentration range of 10-200 mg/mL were applied. MIC was defined as the lowest concentration that inhibited the visible bacterial growth[12]. The experiments were repeated three times.

RESULTS AND DISCUSSION

Fresh leaves of Ageratina jahnii (AJ) and Ageratina pichinchensis (AP) yielded (6.5 mL, 0.52% w/v, AJ), and (1.9 mL, 0.13 % w/v, AP) of essential oil, showing the presence of 15 and 25 components, respectively. The major components identified in AJ β-myrcene (31.6%), α-pinene (23.1%), limonene (7.8%) and pentacosane (10.2%), while for AP 8,9-epoxithymyl isobutyrate (21.2%), germacrene-D (20.8%), thymyl isobutyrate (15.8%), eupatoriochromene (5.5%) and encecalol (4.9%) were detected in major concentrations. Antibacterial activity of the essential oils was also evaluated against Gram-positive (S. aureus ATCC 25923), E faecalis ATCC 19433 and Gram-negative (E. coli ATCC 25922, P. aeruginosa ATCC 27853 and K. pneumoniae ATCC 25955) bacteria using the disc diffusion agar method showing activity against S. aureus and E. faecalis with MIC values of 49.5 mg/mL for AJ and 104 mg/mL for AP. Now days the study of antibacterial agents has become an important issue, due to the constant development of resistance mechanisms of microorganisms to conventional antimicrobials. Consequently, search for new agents, those of plant origin must be emphasized, thus, the results observed in this investigation might be of interest for the natural products research.

CONCLUSSION

In the present investigation, chemical composition and antibacterial activity of essential oils of fresh leaves of A. jahnii and A. pichinchensis were evaluated. β-myrcene, α-pinene, limonene, germacrene-D, isobutyrate de 8,9-epoxitimilo, isobutyrate de timilo, eupatoriochromene and encecalol were the components observed in major proportions. Furthermore, antibacterial activity was observed in AJ and AP essential oil samples against S. aureus and E. faecalis, those important pathogens responsible for several infections in humans.