CIENCIA 16(1),5 - 9 , 2008
Maracaibo, Venezuela

Chemical composition and antibacterial activity of the essential oil from aerial parts of *Porophyllum ruderale* (Jacq.) Cass. collected in Venezuela

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Recibuido: 22-06-07 Aceptado: 23-10-07

Abstract

Essential oil from the fresh aerial parts of *Porophyllum ruderale* (Jacq.) Cass. (Asteraceae) collected in January 2005 was analyzed by GC/MS. The oil was obtained by hydrodistillation with a 0.18% w/v extraction yield. Twenty three components were identified by comparison of their mass spectra with the mass spectra of a GC-MS Library data and the retention indices calculated for every compound. The main constituents were a mixture of limonene and β-phellandrene (50.3%), sabinene (20.2%), 1-undecene (4.7%), 4-terpineol (3.8%) and α-pinene (2.9 %). Antibacterial activity of the essential oil of this species was evaluated against Gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria using the disc diffusion agar method. The results showed a broad spectrum of activity with minimal inhibitory concentration (MIC) values ranging from 20 to 200 μg/mL.

Key words: Asteraceae; antibacterial activity; essential oil; GC-MS; *Porophyllum ruderale*.

Composición química y actividad antibacteriana del aceite esencial de las partes aéreas de *Porophyllum ruderale* (Jacq.) Cass. colectado en Venezuela

Resumen

El aceite esencial de las partes aéreas de *Porophyllum ruderale* (Jacq.) Cass. (Asteraceae) fue analizado por CG/EM. El aceite extraído por hidrodestilación dio un rendimiento de 0,18% p/v. Veintitrés componentes fueron identificados por comparación de sus espectros de masas con los espectros de masas de la base de datos Wiley GC-MS y los índices de retención calculados para cada compuesto. Los componentes mayoritarios fueron una mezcla de limoneno y β-felandreno (50,3%), sabineno (20,2%), 1-undeceno (4,7%), 4-terpineol (3,8%) y α-pineno (2,9%). La actividad antibacteriana del aceite esencial de esta especie fue evaluada sobre bacterias Gram positivas (*Staphylococcus aureus*, *Enterococcus faecalis*) y bacterias Gram negativas

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(*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), usando el método de difusión en agar con discos. Los resultados mostraron actividad antibacteriana de amplio espectro, con un rango de concentración inhibitoria mínima (CIM) entre 20 μg/mL y 200 μg/mL.

Palabras clave: Asteraceae; aceite esencial; actividad antibacteriana; CG/EM; *Porophyllum ruderale*.

Introduction

Porophyllum genus (Asteraceae) comprises 28 species distributed in the subtropical America. *Porophyllum ruderale* (Jacq.) Cass., is a shrub of 1.50 m of height approximately and it is characterized by its strong fragrance (1). The aerial parts of the plant have been used in folk medicine as diaphoretic, emmenagoge, sedative (2), to treat genital inflammation and to alleviate epilepsy (3). The alcoholic extract from aerial parts of the plant has also shown activity *in vitro* against the promastigote forms of *Leishmania* (4). Investigations have been carried out to assess the resistance role of this species against insect herbivore. α-Terthienyl, a phytotoxic polyacetylenic derivative and a variety of monoterpenes and sesquiterpenes have been reported in *P. gracile* and *P. ruderale* and the hypothesis that these volatiles exert a synergistic effect on the insecticidal properties of α–terthienyl was also proved (5). Studies on the chemical composition of the essential oil of some species of this genus have revealed the presence of thiophene and thymol derivatives (6-8) and dithienylacetylene was also isolated from the aerial parts of *Porohyllum ruderale* (9). The chemical composition of the essential oil of *P. rudearale* growing in Bolivia (10), Brazil (11-12) and Mexico (3) have also been previously reported. In the present study we are reporting the chemical composition of an essential oil sample obtained from aerial parts of *P. ruderale* collected from a xerophytic area of Venezuela and the antibacterial activity against Gram positive and Gram negative bacteria using the disc diffusion agar method.

Materials and Methods

Aerial parts of *Porophyllum ruderale*, were collected at the flowering stage in January 2005 in a xerophyitic zone of Venezuela (San Juan de Lagunillas) at 1099 m above sea level. Voucher specimen (703) has been deposited in the Herbarium MERF of the Faculty of Pharmacy and Biomedical Sciences of University of Los Andes.

Isolation of essential oil

Fresh leaves and inflorescences (1.250 g) were cut into small pieces and subjected to hydrodistillation for 4 h, using a Clevenger-type apparatus. Distilled water (5 lt) was used for the extraction of the essential oil. The yellow oil was dried over anhydrous sodium sulphate and stored at 4°C.

Gas chromatography: GC analyses were performed on a Perkin-Elmer AutoSystem gas chromatograph equipped with flame ionization detectors. Two capillary columns of different polarities were used: a 5% phenylmethyl polysiloxane fused-silica column (HP-5MS, Hewlett Packard, USA) 60 m x 0.25 μm, film thickness 0.25 μm, and a polyethylene glycol fused-silica column (AT-WAX, Alltech Associates Inc., Deerfield, IL) of the same dimensions. The initial oven temperature was 60°C; it was then heated to 260°C at 4°C/min, and the final temperature was maintained for 20 min. The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium at 1.0 mL/min. The sample was injected using a split ratio of 1:100. Retention indices were calculated relative to C_8-C_{24} *n*-alkanes, and compared with values reported in the literature (13-14).

Gas chromatography-mass spectrometry: The GC-MS analyses were carried out on a Hewlett Packard GC-MS system, Model 5973, fitted with a 30 m long, cross-linked 5% phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25°m). The source temperature was 230°C, the quadrupole temperature 150°C, the carrier gas helium, adjusted to a linear velocity of 34 m/s, the ionization energy 70 eV, and the scan range 40-500 amu at 3.9 scans/s. The injected volume was 1.0 μl of a 2% dilution of oil in *n*-heptane. A Hewlett-Packard ALS injector was used with split ratio 1:100. The identification of the oil components was based on a Wiley MS Data Library (6th edn), followed by comparisons of MS data with published literature (13).

Microbiological analysis

Bacterial strains

The microorganisms used were *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25992), *Klebsiella pneumoniae* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

Antimicrobial method

The antimicrobial activity was carried out according to the disc diffusion assay described by Rondón *et al* (15). The strains were maintained in agar at room temperature. Each bacterial inoculum (2.5 mL) was incubated in Mueller-Hinton broth at 37°C for 18 hours. The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to a McFarland Nº 0.5 standard (10^{6-8} CFU/mL). Every inoculum was spread over plates containing Mueller-Hinton agar and a paper filter disc (6 mm) saturated with 10 µL of essential oil. The plates were left for 30 min at room temperature and then incubated at 37°C for 24 h. The inhibitory zone around the disc was measured and expressed in mm. A positive control was also assayed to check the sensitivity of the tested microorganisms using the following antibiotics: Ampicillin-sulbactam® (10 µg/10 µg), Vancomycin® (30 µg), Netilmicin® (30 µg), Aztreonam® (30 µg) and Cefoperazone® (75 µg) (Table 2).

The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the essential oil in dimethyl sulphoxide (DMSO) pipetting 10 µL of each dilution onto a filter paper disc.

Table 1
Composition of the essential oil of aerial parts of *Porophyllum ruderale* (Jacq.) Cass. collected in Venezuela*

Components	% w/v	RI
tricyclene	0.2	926
α-pinene	**2.9**	**932**
sabinene	**20.2**	**967**
β-pinene	0.7	971
myrcene	2.7	982
p-mentha-1-(7),8-diene	2.2	996
α-terpinene	1.1	1009
p-cimene	0.3	1018
limonene + β-phelandrene	**50.3**	**1024**
trans-β-ocimene	1.2	1042
γ-terpinene	1.7	1055
1-undecene	**4.7**	**1092**
linalool	0.3	1102
cis-*p*-2-menten-1-ol	0.3	1124
4-terpineol	**3.8**	**1181**
1-tridecene	0.5	1302
β-cariophyllene	1.5	1424
germacrene-D	0.4	1488

*The composition of the essential oil was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention index (RI).

Table 2
Antibacterial activity of essential oil of aerial parts of *Porophyllum ruderale* (Jacq.) Cass.

Microorganism	Inhibition zone (mm)						MIC (μg/mL)
	Essential Oil	Reference Compounds					
		Amp-S	Va	Net	Azt	Cef	
Staphylococcus aureus ATCC (25923)	19*	35*					20
Enterococcu. faecalis ATCC (29212)	18*		21*				120
Escherichia coli ATCC (25922)	18*			23*			100
Klebsiella pneumoniae ATCC (23357)	12*				31*		130
Pseudomonas aeruginosa ATCC (27853)	9*					28*	200

* Inhibition zone, diameter measured in mm, disc diameter 6 mm, The data shown above is an average of two assays.
MIC: Minimal inhibitory concentration.
Amp-S: ampicillin-sulbactam; Va: vancomycin; Net: netilmicin; Azt: aztreonam; Cef: cefoperazone.

Dilutions of the oil within a concentration range of 10-230 μg/mL were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (16). A negative control was also included in the test using a filter paper disc saturated with DMSO (10 μL) to check possible activity of this solvent against the bacteria assayed. The experiments were repeated at least twice.

Results and Discussion

Essential oil from aerial parts of *Porophyllum ruderale* was analyzed by GC-MS. The average oil yield was 0.18% w/v. Twenty three compounds were identified in the oil, being a mixture of limonene and β-phellandrene (50.3%), sabinene (20.2%), 1-undecene (4.7%), 4-terpineol (3.8%) and xx-pinene (2.9%) the major constituents. A summary of the chemical composition of the essential oil is shown in Table 1.

These results were compared to the composition described in the literature for the essential oil of *P. ruderale* that grows in Ceará (Brazil) (11) where the main components are limonene (74.6%) and (*E*,*E*)-dodecadienal (21.8%); *P. ruderale* that grows in Rio de Janeiro (Brazil) (12) contained *trans*-β-ocimene (53 %), myrcene (16%), limonene (13%), α-pinene (5.4%), 1-undecene (10 %) and 2-β-pinene (2.7%) and the main components reported for *P. ruderale* from Bolivia (10) were sabinene (64.1%) and 4-terpineole (10.3%).

The results of the antibacterial activity showed a broad spectrum of activity of the oil, displaying values of MIC ranging from 20 to 120 μg/mL for the Gram positive (*S. aureus* y *E. faecalis*) and 100 to 200 μg/mL for the Gram negatives (*E. coli*, *K. pneumoniae* and *P. aeruginosa*). The complete results of this experiment are shown in Table 2. This is the first time the antibacterial activity of the essential oil of *P. ruderale* has been reported.

Antimicrobial activity of essential oils is difficult to correlate to a specific compound due to their complexity and variability. It has been mainly explained through C_{10} and C_{15} terpenes with aromatic rings and phenolic

hydroxyl groups able to form hydrogen bonds with active site of target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils (17). However, β-phellandrene and limonene, observed at important concentrations in the essential oil of the species analysed in the present investigation, are well known to possess antibacterial activity. Previous investigations have reported activity of these compounds against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi*, *Salmonella Choleraesuis* and *Bacillus subtilis,* (18, 19, 20). Thus, the antibacterial results observed in this investigation might be related to the presence of these compounds.

Acknowledgment

The authors would like to acknowledge to Alfredo Usubillaga, Faculty of Pharmacy and Biomedical Sciences, University of los Andes for helping in performing the analysis of GC/MS and the Fondo Nacional de Ciencia, Tecnología e Innovación (Fonacit), Ministerio de Ciencia y Tecnología, Caracas, Venezuela, for the partially financial support (F-2000001633) of this investigation.

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