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SHORT COMMUNICATION

Chemical Composition of the essential oil of *Morella parvifolia* (Benth.) Parra-O. from the Venezuelan Andes

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ABSTRACT

Venezuela is a country with high biodiversity. The specie *Morella parvifolia* belongs to the Myricaceae family, which is rich in essential oils. The study of M. pubescens essential oil is the only reported in this genus. Its major component is germacrene - B (~ 32%). The present work reports the chemical composition of the essential oil of *Morella parvifolia* (Benth.) Parra -O. collected in Venezuela. The essential oil (0.3 - 0.5 % yield) of the fresh leaves of *M. parvifolia* from three different plants, from the same location, were obtained by hydrodistillation using a Clevenger type apparatus. The chemical constituents were identified by GC-MS analysis. From twenty three to twenty nine compounds (96.06 – 97.31 % of the samples) were identified. The major constituents found were α - bisabolol (50.56 - 58.9 %) and α - pinene (12.88 -16.79%). Analysis of antimicrobial activity on Gram (+) and Gram (-) strains was performed but no antibacterial activity was observed.

Keywords: α-bisabolol; Essential oil; Morella parvifolia; Myricaceae

INTRODUCTION

Venezuela is among the ten biodiversity-rich countries in the world (UNEP-WCMC, 2010). Therefore, a huge part of this biodiversity still needs to be studied. In the tropical Andes, the paramo is located, usually on the line just above where the cloud forest ends and below the snow line. The paramo is the most biodiverse high mountain ecosystem in the world. The herbaceous paramo formed a heterogeneous array of plant communities, which changes according to local topographic and environmental variations (Jorgensen, 1994; Luteyn, 1999). However, the vegetation has a clear dominance of shrubs and grasses. *Morella parvifolia* (Benth.) Parra-O. belongs to Myricaceae (Hokche, 2008) family. It is a shrub of almost 4m high with leaves and fragrant fruits that grows wild in the Venezuelan Paramo (Parra, 2002). It is commonly known as encinillo.

The essential oil of leaves and fruits of the specie *M. pubescens* of Colombia has been studied. Fifty-five constituents have been identified and characterized mainly as sesquiterpene hydrocarbons. Germacrene B was the major constituent of the oil also with considerable amounts of selina -3,7-(11)-diene and δ-cadinene (Sandoval, 2010). According to the authors' knowledge the essential oil from the leaves of *Morella parvifolia* has not been subjected to previous studies. This paper aims to identify the chemical components of essential oil of Venezuela *Morella parvifolia* and determine its antibacterial activity against Gram (+) and Gram (-) bacteria.

MATERIALS AND METHODS

Plant material

Aerial parts of *Morella parvifolia* were collected at Las Piñuelas, Gavidia, Municipio Rangel, Mérida State, Venezuela. A voucher Nº FMBS052 was deposited at Facultad de Farmacia y Bioanálisis Herbarium, Universidad de Los Andes, Mérida, Venezuela (MERF Herbarium).

Isolation of the essential oil

Fresh leaves (1000 g) were cut into small pieces and subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil (0.3 - 0.5 % yield) was dried over anhydrous sodium sulphate and stored at 4 ºC (Rojas et al., 1999).

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Gas chromatography (GC)

GC analyses were performed using a Perkin-Elmer AutoSystem gas chromatograph equipped with a flame ionization detector and data handling system. A 5% phenylmethyl polysiloxane fused-silica column (AT-5, Alltech Associates Inc., Deerfield, IL), 60 m x 0.25 mm, film thickness 0.25 µm, was used. The initial oven temperature was 60°C; it was then heated to 260°C at 4°C/min, and the final temperature 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 (1µL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. Retention indices were calculated relative to C8-C24 *n*-alkanes, and compared with values reported in the literature (Adams, 2007; Davies, 1990).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were carried out on a Model 5973 Hewlett-Packard GC-MS system fitted with a HP-5MS fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm, Hewlett-Packard). The oven temperature program was the same as that used for the HP-5 column for GC analysis; the transfer line temperature was programmed from 150ºC to 280ºC; source temperature, 230ºC; quadrupole temperature, 150ºC; carrier gas, helium, adjusted to a linear velocity of 34 cm/s; ionization energy, 70 eV; scan range, 40:500 amu; 3.9 scans/s. The sample was diluted with diethyl ether (20µL in 1 mL) and 1µL was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their GC retention indices, by comparison of their MS spectra with those of standard compounds available in the laboratory, and by a library search (Nist, 05) (Adams, 2007; Davies, 1990).

Bacterial strains and antibacterial assays

The inhibitory activity of essential oil of *M. parvifolia* was tested against Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 23357, and *Pseudomonas aeruginosa* ATCC 27853) strains provided by the Department of Microbiology and Parasitology, Faculty of Pharmacy and Bioanalysis, University of Los Andes). The antibacterial activity was performed according to the agar diffusion method with disks (Velasco et al, 2007). Ciprofloxacin was used as positive control. Assays were performed in duplicate.

RESULTS AND DISCUSSION

Hydrodestilation of *M. parvifolia* leaves produced slightly yellow oil which yielded from 0.3 - 0.5 %. The analysis of the essential oils obtained by GC-MS enabled the identification of the compounds, which are shown in Table 1 and represented 97.31, 96.37 and 96.06% of the samples A, B and C respectively (Fig. 1). No significant differences were found in the composition of the oil from the three samples. More than 65 % of the samples consist of two compounds: α-bisabolol and α-pinene. In this work, no antibacterial activity was observed against the tested bacteria. However, bisabolol as the major component of this oil has been tested for anti-inflammatory (Rocha et al, 1011), and antibacterial activity against some specific strains. Also it is known for its deodorizing effect (Forrer et al, 2013). a-Pinene has been found in several essential oils with antimicrobial activity (Rivas, 2012; Hernandez, 2013). Our results lead us to suppose that the a-pinene has an antibacterial synergistic effect for compounds with antibacterial activity. a-Pinene in combination with bisabolol seems not to have this effect or the enantiomeric non active form of this compound is in the oil. Further

Table 1: Chemical composition of the essential oil of *Morella parvifolia* samples A, B and C (*)

Compound	LRI	Peak area percent		
		A	B	C
3-hexenol	849	0.29	0.12	-
α-thujene	931	0.23	0.32	0.28
α-pinene	937	12.88	16.48	16.79
benzaldehyde	941	-	0.15	-
camphene	952	0.25	0.40	0.41
β-pinene	978	0.70	1.00	0.96
α-terpinene	1013	-	0.25	0.16
p-cymene	1015	3.18	3.91	4.98
limonene	1025	2.16	3.11	3.03
1,8-Cineol	1033	-	0.15	0.16
γ-terpinene	1061	3.58	5.14	4.11
α-terpinolene	1090	0.37	0.6	0.47
linalool	1100	2.62	2.68	2.76
nonanal	1104	-	0.16	0.17
borneol	1159	-	0.24	0.25
terpineol-4	1155	0.34	0.42	0.37
α-terpineol	1178	0.64	0.76	0.72
fenchyl acetate	1205	-	0.23	0.28
bornyl acetate	1293	0.38	0.42	0.49
eugenol	1331	0.95	-	0.83
E- caryophyllene	1434	2.15	2.25	1.55
α-bergamotene	1434	0.60	0.56	0.50
ar-curcumene	1484	-	0.13	0.14
E-β-farnesene	1486	0.68	0.58	0.51
α-zingiberene	1496	-	0.15	-
β-bisabolene	1510	0.54	0.45	-
(*Z*)-α-bisabolene	1542	2.22	1.78	1.56
caryophyllene oxide	1578	0.63	0.49	0.65
β-eudesmol	1641	0.33	-	-
bisabolol oxide	1654	2.67	2.22	3.37
α-bisabolol	1689	58.92	51.22	50.56
Total		97.31	96.37	96.06

(*) The chemical composition was determined by comparison of mass spectra of each compound database Wiley GC/MS library and Kovats index (LRI). Kovats index were determined on HP-5 capillary column

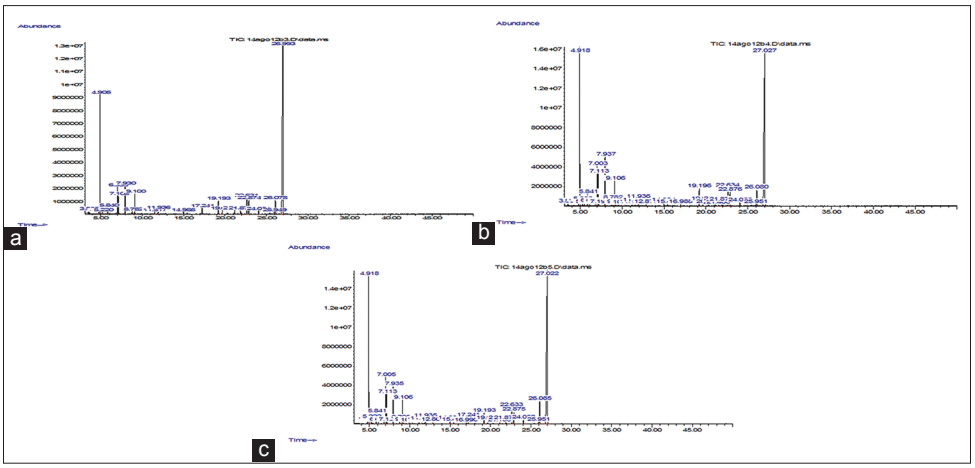

Fig 1. Chromatograms of the essential oil of *Morella parvifolia*, a) sample A, b) sample B, c) sample C

investigations are necessary to determine the antibacterial activity of these compounds, either pure or combined.

CONCLUSION

According to the results observed in this study, the essential oil of *Morella parvifolia* from the Venezuelan Andes (Mérida State) has a chemical composition with high content in monoterpenes and sesquiterpenes. The major compounds found in the oil were: α-bisabolol and α-pinene. No microbiological activity against selected Gram (+) and Gram (-) strains was determined. This study contributes to the knowledge of chemical components and biological activity of the essential oil of this specie.

Authors contribution

M.F.D. wrote the project, obtained the essential oil and chemical analysis, H.V. performed antimicrobial activity, C.J. and S.B. did the taxonomic identification and collect the specie, R.L.B. Chemical analysis of the essential oil using gas chromatography and mass spectrometry. All authors wrote, read and approved the final manuscript.

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