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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *MYRCIANTHES MYRCINOIDES* (KUNTH) GRIFO (MYRTACEAE) COLLECTED IN THE VENEZUELAN ANDES

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Abstract

The essential oil of the aerial parts from *Myrcianthes myrsinoides* (Kunth) Grifo (Myrtaceae) was analyzed by gas chromatography and gas chromatogrphy-mass spectroscopy (GC/MS). Altogether 28 compounds representing 90.2% of the total of the essential oil were identified. The major constituentes were *p*-Terpinen-4-ol (32.22%), o-cymene (8.15%), spathulenol (7.59%) and caryophyllene oxide (7.14%). It was tested against *Gram* positive and Gram negative bacteria at different concentrations and presented an important effect against *B. cereus* (MIC 100-200 µg/mL; MBC 200 µg/mL); *B. subtilis* and *S. epidermidis* (MIC 200-400 µg/mL; MBC 800 µg/mL). This is the first report on the antibacterial activity of the essential oil of *M. myrsinoides* (Kunth) Grifo.

Keywords: Antibacterial activity; Essential Oil; *Myrcianthes myrcinoides*

Introduction

Many infectious diseases remain a public health problem around the world due at resistance to antibiotics, especially diseases such as pneumonia, diarrhoeal disease, AIDS, hospital-acquired infections, leishmaniasis, malaria, and common worms. Some of the recommendations of the World Health Organization include increasing research for new drugs and vaccines, increasing availability of essential drugs, and making effective medicines accessible to the poor as a solution to a this problem (1). So far, many researches have been developed in the area of natural products with the aim of finding new therapeutic alternatives for controlling infectious diseases and as an alternative therapies against conventionally resistant infections. Some of these studies have focused on plants that have been used since ancient times by the population. Many species of diverse botanic family exhibit important antimicrobial activity against a wide spectrum of pathogenic microorganisms due at the presence of essential oil in this species (2). Some of these species belong to the Myrtaceae family such as *Eucalyptus* (3), *Eugenia* (4), *Myrcia (5) Pimenta (6)* and *Psidium* (7) genus among other, which have been studied for their antimicrobial proprieties. *Myrcianthes* O. Berg genus includes 35 species (8) although DNA-based systematic study has allied it to *Eugenia* L genus (9). *Myrcianthes* ranging from Mexico and Caribbean to northern Argentina, Uruguay and north central Chile, although most are Andean (10). Some *Myrcianthes* species has showed antimicrobial activity *in vitro*, such as *Myrcianthes cysplatensis* (Cambess) O. Berg (11), *M. hallii* (O. Berg) Mc. Vaugh (12), and *M. pseudo-mato* (Legr.) Mc. Vaugh (13). Scientific research has demonstrated that other species exhibit various biological activities as *Myrcianthes pungens* (O. Berg) D. Legrand that exhibited anticholinesterase activity (14); antioxidant activity was observed in *M. leucoxyla* (15), and *M. pungens* (16) species, while that cytotoxic activity was exhibited in *Myrcianthes* sp. from Monte Verde in Costa Rica (17). *Myrcianthes myrsinoides* (Kunth) Grifo (Syn *Eugenia triquetra* O. Berg) grows in the Andes from Bolivia, Colombia, Ecuador, and Venezuela (18). A review of scientific literature showed only the repot of the larvicidal activity of the essential oil of this species as well as the chemical composition of the essential oil (19). So far, this has been the only report found in the scientific literature on this specie.

Materials and Methods

Plant Material:

Leaves of *Myrcianthes myrsinoides* (Kunth) Grifo, were collected in Sector Los Frailes in Mérida State, Venezuela at 2173 in February 2014, and identified by Ing. Juan Carmona Arzola. Voucher specimens (MER013) was deposited in the Dr. Luis E Ruiz Terán Herbarium, Faculty of Pharmacy and Biomedical Science, University of Los Andes.

Essential oil isolation:

600 g of botanical material was subjected to hydrodistillation for 4 h using a Clevenger- type apparatus. The obtained oil was dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C in the dark until analyzed and tested. The yield (0.5 %) was calculated based on the dry weight of the plant material.

Gas Chromatography:

Gas chromatography analyses were performed on a Perkin Elmer Auto System gas chromatograph equipped with flame ionization detector. A 5 % phenylmethylpolysiloxane fused-silica capillary column (AT-5, Alltech Associates Inc., Deerfield, IL), 60 m x 0.25 mm, film thickness 0.25 µm, was used for the GC analysis. The initial oven temperature was 60°C; this was then raised 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 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 (20).

GC/MS Analysis:

GC/MS analyses carried out on a Hewlett Packard GC-MS system, model 5973, fitted with a 30 m long, cross-linked 5 % phenylmethylsiloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 µm). The source temperature was 230°C, the quadrupole temperature 150 °C, and the carrier gas helium, 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 scan/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 a split ratio of 1:100. The identification of the oil components was based on the Wiley MS Data Library (6^{th} Ed), followed by comparison of MS data with published literature (21).

Antibacterial Activity

Antibacterial activity was determined against *Gram* positive (*Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* CECT232, *Bacillus subtilis* CECT39, *Bacillus cereus* CECT496, *Enterococcus faecalis* CECT735) and *Gram* negative (*Escherichia coli* CECT99, *Pseudomonas aeruginosa* AK958, *Klebsiella pneumoniae* ATCC23357, *Proteus mirabilis* CECT170,) bacterial.
The bacteria cultures were developed in nutrient broth (NB) or brain-heart infusion broth (for *E. faecalis*). All media were purchased Oxoid. The minimal inhibitory concentration (MIC) and minimal bactericidal (MBC) were determined for each sample in triplicate, using the broth microdilution method (22). All samples were dissolved in DMSO, several wells were also filled with the same proportions of DMSO as controls and never exceeded 1% (v/v). The starting microorganism concentration was approximately 1-5 x 10^5 CFU/mL, growth was monitored by measuring the optical density increasing at 550 nm (OD550) using a microplate reader (Multiskan Plus II). The MIC was defined as the lowest concentration of the essential oil where growth inhibition was observed after 24 h of incubation in a rotatory shaker at 37 °C. All well with no visible growth were sub-cultured by transferring 100 µL to nutrient and brain-heart infusion agar plates. After overnight incubation, colony counts were performed and the MBC was defined as the lowest concentration of the essential oil that produced ≥ 99.9 % killing of the initial inoculum.

Results

Chemical composition of the essential oil

The essential oil was analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectroscopy (GC-MS). All compounds (28, representing 94.39% of the total oil) were characterized by comparison of each MS with the Wiley GC/MS library data and also from its retention index (RI). *p*-Terpinen-4-ol (32.22%), o-cymene (8.15%), spathulenol (7.59%) and caryophyllene oxide (7.14%) were the major compounds observed in the essential oil. A list of the identified components along with their percentage of the total oil is given in Table 1.

The monoterpenes oxigenated fraction was the most abundant (40.84%) follow by sesquiterpenes oxigentaed (32.30%) in the essential oil of *M. myrsinoides*. This result are according with previous research on the chemical composition of the essential oil obtained from genus *Myrcianthes* species growing in others South American´s countries such as *M. cisplatensis* (Camb). Berg. from Brasil (23) and Uruguay (24), *M. pseudo-mato* (Legr.) McVaugh from Argentina (13) and Bolivia, (25) *M. leucoxyla* (Ortega) McVaugh from Colombia (15) and *Myrcianthes* sp nov. "Black Fruit" from Costa Rica (17). All these species showed a high proportion of monoterpenes in their chemical composition (56.4 – 81.3 %) and 1,8-cineole was the majority compound in all the cases (15.17-55.70%). Surprised that 1,8-cineole in our sample was present in a very low concentration (0.92%). For other hands, while that *p*-Terpinen-4-ol was the major compounds in our sample (32.22%), this compound isn´t present in other species or is in low proportion. However, α-terpineol, α-pinene and β-pinene were presents in the essential oil genus *Myrciantes* species as majority compounds too (17, 24, 25, 26). This might be due that the chemical composition of the essential oils dependent of physical factors like temperature, atmospheric pressure, increasing precipitation, altitude geographic variation, environmental and agronomic conditions lead to differentiation among and within species (27).

Antibacterial Activity

The antibacterial assay (MIC and MBC) of the essential oil of *M. myrsinoides* is given in the Table 2. The best results were observed against *B. cereus* (MIC 100-200 µg/mL; MBC 200 µg/mL); *B. subtilis* and *S. epidermidis* (MIC 200-400 µg/mL; MBC 800 µg/mL). Some authors think that for MIC values less than 100 µg/mL, the antimicrobial activity can be considered good; from 100 to 500 µg/mL, weak; and over 1000 µg/mL, inactive (28). While that the antimicrobial activity of natural products with MIC values below 100 µg/mL can be considered promising (29). According with this criterion, the antibacterial proprieties of the essential oil of *M. myrsinoides* can be considered weak against *S. epidermidis*, *B. subtilis* and *B. cereus* and inactive against *S. aureus*, *E faecalis* and *Gram* negative bacterial used in this work.
A similar results was observed in the essential oil of *M. pseudo-mato* from Argentina which showed a high degree of inhibition against *B. cereus* (MIC 230 µg/mL) (13). On the other hands, *p*-Terpinen-4-ol has a role as substance that kills or slows the growth of bacteria. Additionally, is the most active ingredient to Tea Tree Oil followed of 1,8-cineole and α-terpineol, which are widely used as antibacterial agent in some skin-wash formulas and has been evaluated against *S. aureus*, *E. coli* and *P. aeruginosa* (30,31). For other hand, although that there is research showing that α-terpineol may induce morphostructural alterations in *E. coli* which suggests that the α-terpineol has excellent antibacterial activity,

this not was observed in this work probably due to low concentration of this compounds in the essential oil (32). For our knowledge, this is the first report on antibacterial activity of the essential oil of *Myrcianthes myrsinoides* (Kunth) Grifo. Because the essential oil is obtained with excellent yield, the authors suggest wide the study of antimicrobial activity of the essential oil to other pathogens with the aim of knowing the spectrum of action of this essential oil.

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Table 1: Chemical composition of *Myrcianthes myrsinoides* (Kunth) Grifo essential oil

N°	Compuesto[a]	TR	% área	IK[b]	IK[c]
1	α-pinene	4.97	1.01	933	932
2	Sabinene	5.83	0.42	966	969
3	β-pinene	5.92	0.74	969	974
4	*o*-cymene	7.09	8.15	1006	1022
5	limonene	7.20	3.76	1009	1024
6	1,8-cineole	7.28	0.92	1012	1026
7	linalool	9.19	1.88	1090	1095
8	*cis-p*-Menth-2-en-1-ol	9.87	0.68	1116	1118
9	*trans-p*-Menth-2-en-1-ol	10.43	0.64	1135	1136
10	*p*-Terpinen-4-ol	11.67	32.22	1176	1174
11	*p*-Cymen-8-ol-	11.87	0.43	1182	1179
12	α-terpineol	12.05	4.11	1188	1186
13	Trans-piperitol	12.58	0.39	1203	1207
14	α-cubebene	17.12	0.72	1350	1345
15	α-copaene	17.96	0.78	1375	1374
16	(E)- caryophyllene	19.32	2.07	1415	1417
17	α-humulene	20.35	1.72	1451	1452
18	Trans-calamenene	22.44	1.45	1522	1521
19	(E)-nerolidol	23.60	0.37	1558	1561
20	spathulenol	24.04	7.59	1572	1577
21	Caryophyllene oxide	24.20	7.14	1576	1582
22	Isoaromadendrene epoxide	24.60	3.81	1588	1579
23	1,5,5,8-tetramethyl-12-oxabiciclo[9.1.0]dodeca-3,7-diene	24.94	2.95	1598	1607
24	β-oplopenone	25.03	3.92	1601	1606
25	1,10-di-epi-Cubenol	25.46	0.98	1613	1618
26	cubenol	25.85	1.79	1626	1642
27	t-muurolol	26.19	2.52	1641	1640
28	Amorpha-4,9-dien-2-ol	27.06	1.23	1676	1700
	Total identified (%)		**94.39**		
	Monoterpenes		**14.08**		
	Oxigenated Monoterpenes		**40.84**		
	Sesquiterpenes		**6.74**		
	Oxigenated Sesquiterpenes		**32.30**		
	Phenylpropanoids		**0.43**		

[a] Compounds listed in sequence of elution from a DB-5 MS column; TR: Time retention; [b] IK calculated; [c] IK from literature (20.21)

Table 2: Antibacterial activity of *Myrcianthes myrsinoides* (Kunth) Grifo essential oil.

Microorganism	Essential oil (µg/mL)	
	MIC	MBC
***Gram* positive bacterial**		
***S. aureus* CECT232**	800-1600	3200
***S. epidermidis* CECT232**	200-400	800
***B. subtilis* CECT39**	200-400	800
***B. cereus* CECT496**	100-200	200
***E. faecalis* CECT735**	400-800	1600
***Gram* negative bacterial**		
***E. coli* CECT99**	1600-3200	3200
***P. aeruginosa* AK958**	3200-6400	6400
***K. pneumoniae* ATCC23357**	1600-3200	3200
***P. mirabilis* CECT170**	1600-3200	3200

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration