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Aromatic Plants from Yungas. Part IV: Composition and Antimicrobial Activity of *Myrcianthes pseudo-mato* Essential Oil

M.S. Demo[1], M.M. Oliva[1], M.P. Zunino[2], M.L. Lopez[2] and J.A. Zygadlo[2]

[1]Universidad Nacional de Río Cuarto, Departamento de Microbiologia e Inmunologia, Argentina; [2]Catedra de Química Orgánica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, IMBIV-CONICET, Córdoba, Argentina

Abstract

The essential oil of *Myrcianthes pseudo-mato* (Legr.) Mc. Vaugh, from the Yungas area (Argentina), has been studied for the first time. The essential oil obtained was analyzed by GC/MS and assayed for anti-microbial activity. Height different genera of bacteria and one yeast species were used in this study as test organisms. The oil showed a high degree of inhibition against *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus*. Twenty components were characterized representing 97.5% of the total components detected with 1,8-cineole (32.5%) and β-caryophyllene (18.9%) as major constituents.

Keywords: *Myrcianthes pseudo-mato*, antimicrobial activity, essential oil composition, traditional medicine.

Introduction

The Yunga area is enriched with plants and many are used in herbal medicines. The rural population of Yungas heavily depends on traditional herbal drugs, because no adequate medical facilities are available.

Many species of the genus *Myrcianthes* are popularly used, in Argentina, for the treatment of several infectious diseases (Kott et al., 1999; Penna et al., 2001). Thus, as part of our investigations on local medicinal-aromatic plants, we now report the antimicrobial activity of essential oil of *Myrcianthes pseudo-mato* (Legr.) Mc Vaugh (Myrtaceae).

Materials and methods

Plant materials

The leaves of *Myrcianthes pseudo-mato* (Legr.) Mc. Vaugh (Myrtaceae) were collected from Oran, Salta province of Argentina, in October 2000. Voucher specimens are kept in the Herbarium of the Museo Botanico de la Facultad de Ciencias Exactas Fisicas y Naturales of the Universidad Nacional of Cordoba (CORD 852).

Procurement of the essential oil

Dried leaves of *Myrcianthes pseudo-mato* were hydrodistilled in Clevenger-like apparatus to yield 0.3% of the oil. The oil obtained was dried over anhydrous sodium sulphate and stored in a refrigerator until analysis.

GC analyses

Analyses were accomplished using a Shimadzu GC-R1A (FID) gas-chromatograph, fitted with a 30 m × 0.25 mm (0.25 µm film thickness) fused silica capillary column coated with a DB-5 (J&W) and then Supelcowax-10 (Supelco Co.). The GC operating conditions were the following: oven temperature programmed from 40 to 230 °C at 2 °C/min, injector and detector temperatures 240 °C, the carrier gas was nitrogen with a constant flow of 0.9 ml/min. Identification of the components was performed by comparison of their retention times with those of pure authentic samples. GC/MS analyses were performed with a Perkin Elmer Q-700

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Address correspondence to: M.P. Zunino, Catedra de Química Orgánica, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, IMBIV-CONICET, Vélez Sarsfield 1600, 5000 Córdoba, Argentina.

equipped with a SE-30 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm film). Analytical conditions: the oven temperature varied from 40 °C to 230 °C at 2 °C/min, the carrier gas was helium at a constant flow of 0.9 ml/min, the source was 70 eV. The oil components were identified by two computer library MS searches using retention indices as a pre-selection routine, and visual inspection of the mass spectra from literature for confirmation.

Testing for antimicrobial activity

Antimicrobial activity was assayed against nine microorganisms. *Micrococcus luteus* (ATCC 9341), *Bacillus cereus, Staphylococcus aureus* (ATCC) 25512*, Staphylococcus epidermidis*, *Escherichia coli, Proteus mirabilis, Enterococcus faecalis* (ATCC) 29212*, Klebsiella spp* and *Candida albicans*. All the strains tested were maintained at 4 °C in Triptein-Soy Agar and were subcultured every month. The fungus was stored at the same temperature as bacteria in Sabourand Agar and subcultured every month. The paper disc diffusion method was used for testing antimicrobial activity. It was performed using an 18 h culture, growth at 37 °C and adjusted to approximately 10^6 cfu/ml; 200 µl of the inoculum were spread over plates containing Mueller-Hinton Agar and a paper filter disc, 6 mm, impregnated with 10 µl of the essential oil was placed on the surface of the media. The plates were left 30 min at room temperature to allow the diffusion of the oil, then they were incubated at 37 °C during 24 h. After this time the inhibition zone around the disc was measured with a calliper.

The minimum inhibitory concentration (MIC) was probed only with microorganisms that shown inhibitory zones larger than 9 mm. It was determined by the dilution of essential oil in dimethyl sulfoxide comprising 10 µl of each dilution on a filter paper disc. Dilutions of the emulsions of essential oils were made over the concentration range of 10 to 45,000 µg/ml. MIC was defined as the lowest concentration that inhibited visible growth (DeFeo et al., 1998).

Table 1. Essential oil composition of *Myrcianthes pseudo-mato*.

Compounds	Retention Index		%	Methods of identification
	DB-5	Supelcowax-10		
α-pinene	939	1021	6.5	ms, co, st
sabinene	976	1132	6.6	ms, co, st
β-pinene	980	1118	0.5	ms, co, st
limonene	1031	1190	0.3	ms, co, st
1,8-cineole	**1033**	**1213**	**32.5**	**ms, co, st**
terpineol-4	1177	1589	0.5	ms, co, st
α-terpineol	1189	1714	2.7	ms, co, st
β-caryophyllene	**1418**	**1631**	**18.9**	**ms, co, st**
γ-elemene	1433		0.7	ms
aromadendrene	1439	1599	5.4	ms
α-humulene	1454	1653	2.1	ms
aromadendrene (allo)	1463	1643	1.3	ms
γ-muurolene	1471	1676	0.5	ms
δ-cadinene	1524	1744	1.4	ms
nerolidol (trans)	1564	2040	3.5	ms
spathulenol	1576	2110	3.3	ms, co, st
caryophyllene oxide	1581	2032	0.6	ms
cadinol tau	1640	2155	3.4	ms
muurolol tau	1641	2171	4.5	ms
β-eudesmol	1649	2283	2.3	ms
M^+220 $C_{15}H_{24}O$	2111		0.5	ms
M^+220 $C_{15}H_{24}O$	2118		0.6	ms
M^+220 $C_{15}H_{24}O$	2168		0.2	ms
M^+220 $C_{15}H_{24}O$	2204		0.3	ms
M^+220 $C_{15}H_{24}O$	2281		0.5	ms
M^+220 $C_{15}H_{24}O$	2298		0.4	ms

In elution order from a DB-5 column, co = peak identifications are based on standard comparison with relative retention time. ms = peak identifications are based on MS comparison with file spectra. st = peak identifications are based on MS comparison with a pure standard.

Table 2. Antimicrobial activity of the essential oils of *Myrcianthes pseudo-mato* (IZ: inhibition zone diameter measured in mm, disc diameter: 6 mm). na: not active, nt: not tested.

	Essential oil		1,8-cineole		β-caryophyllene		Positive control	
Microorganism	IZ	MIC µg/ml	IZ	MIC µg/ml	IZ	MIC µg/ml	Gentamicin 10 µg/ml	Amphotericin B 20 µg/ml
S. aureus	12	230	na		9.5	910	15	
S. epidermidis	na		na		na		30	
B. cereus	18	230	11	930	8		25	
M. luteus	17.5	110	na		na		18	
E. faecalis	na		na		na		nt	
E. coli	9		na		na		18	
Klebsiella spp.	na		na		na		22.5	
P. mirabilis	na		na		7		23	
C. albicans	na		nt		na			20

Two controls were also included in the test. The first one was a filter paper disc treated with 10 µl of dimethyl sulfoxide. The second was a control involving the antibiotic gentamicin (10 µg) for bacteria and anphotericin B (2×10^2 µg/ml) for *C. albicans.*

Antifungal experiments were made in the same way using Malt-Extract Broth for the culture and Sabouraud Agar for the plates.

The same methodology was used to study the pure components of the essential oils which were present in the composition with a percentage larger than 15%.

Results and discussion

The components of the oil are reported in Table 1. The oil was particularly rich in 1,8-cineole (32.5%) and β-caryophyllene (18.9%). Table 2 shows the antibacterial activities of the oils. The essential oil of *M. pseudo-mato* manifested the highest activity against *B. cereus* and *M. luteus*. The antimicrobial activity of the 1,8-cineole and β-caryophyllene were lower than the oil, it can be ascribed to the presence of some minor components of which previous studies have shown an strong antimicrobial activity, such terpineol-4 or α-terpineol (Lis-Balchin et al., 1998; Zygadlo & Juliani, 2000).

In Yungas area *M. pseudo-mato*, also called "alpamato," "güili," "güili blanco" and "laurel," is a generalist medicinal plant used for diverse diseases such as cough and stomachache. In accordance with published data on the biological activity of the main components of the essential oil of leaves of *M. pseudo-mato*, the following statements on the above mentioned applications of leaves can be given (Setzer et al., 1999). The high content of β-caryophyllene seems to explain the folk medicinal use of this essential oil as an anti-inflammatory drug (Martin et al., 1993) with gastric cytoprotective effect (Tambe et al., 1996) and anaesthetic activity (Ghelardini et al., 2001). Moreover, the analgesic and anti-inflammatory effects of 1,8-cineole have been reported (Santos & Rao, 2000; Aydin et al., 1999).

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