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Aromatic plants from Yungas. Part III. Composition and antimicrobial activity of *Myrrhinium atropurpureum* Schott var. *octandrum* Bentham essential oil

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ABSTRACT: The essential oil of *Myrrhinium atropurpureum* Schott var. *octandrum* Bentham was analysed by means of GC and GC–MS. From 24 identified compounds, representing 95.6% of the oils, α-pinene was the major component. The oil showed antimicrobial activity against Gram-positive bacteria. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: *Myrrhinium atropurpureum* var. *octandrum*; Myrtaceae; α-pinene; essential oil composition; antimicrobial activity

Introduction

The search for biologically active compounds from natural sources has the purpose of looking for new drugs useful to the pharmaceutical industry.[1,2] Recently, the antimicrobial activity of different species belonging to the family Myrtaceae have been studied, and a high inhibitory effect against bacteria and fungi has been found.[3–7] Thus, in our continuing research on the essential oils from Argentine plants and their antimicrobial activity, we have investigated the essential oil of *Myrrhinium atropurpureum* Schott var. *octandrum* Bentham obtained from the phytogeographic area of Yungas.[8] However, the essential oil of *M. atropurpureum* has been studied from a sample of southern Brazil.[9]

Materials and Methods

Plant Material

Myrrhinium atropurpureum Schott var. *octandrum* Bentham (Myrtaceae). Plants were collected in El Fuerte, Department of Santa Barbara, Jujuy Province, in Argentina. The collection was made in December 2000. A specimen is kept in the Herbarium of the Facultad de Ciencias Agrarias of Jujuy, Universidad Nacional of Jujuy (Rotman 1359 JUA).

Preparation of Essential Oil

Dried leaves of *M. atropurpureum* var. *octandrum* were hydrodistilled for 2 h in a Clevenger apparatus, yielding 0.1% v/w of the oil. It was dried over anhydrous sodium sulphate and stored in a refrigerator until its analysis.

Gas Chromatography Analyses

Analyses were performed on a Shimadzu GC-R1A (FID) gas chromatograph, fitted with a fused silica capillary column coated with a phase 5% phenyl 95% dimethylpolysiloxane (non-polar DB-5) (30 m × 0.25 mm i.d., film thickness 0.25 μm) and then a polar Supelcowax 10 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm), phase polyethyleneglycol, was used. The GC operating conditions were as follows: oven temperature programmed from 40 °C to 230 °C at 2 °C/min; injector and detector temperatures, 240 °C; carrier gas, nitrogen at a constant flow of 0.9 ml/min. The constituents of the

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essential oils were identified on the basis of their GC retention indices (RI) with reference to a homologous series of *n*-alkanes (C_{12}–C_{25}), by comparison of their retention times with those of pure authentic samples from Sigma, Fluka and Palma companies, peak enrichment on co-injection with authentic standards were performed wherever possible, by GC–MS library search (Wiley and NIST) and using visual inspection of the mass spectra from the literature, for confirmation. GC–MS analyses were performed with a Perkin–Elmer Q-700 equipped with an apolar SE-30 capillary column, phase 100% dimethylpolysiloxane (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The analytical conditions were: oven temperature from 40 °C to 230 °C at 2 °C/min; carrier gas, helium at a constant flow of 0.9 ml/min; ion source, maintained at 70 eV.

Testing for antimicrobial activity

Antimicrobial activity was assayed against 10 microorganisms, four Gram-positive, five Gram-negative and a yeast: *Micrococcus luteus* (ATCC 9341); *Bacillus cereus* (food-borne pathogen); *Staphylococcus aureus* (ATCC 25512); *Staphylococcus epidermidis* (clinical sample); *Escherichia coli* (water sample); *Proteus mirabilis* (clinical sample); *Enterococcus faecalis* (ATCC 29212); *Pseudomonas aeruginosa* (water sample); *Klebsiella* sp. (clinical sample); and *Candida albicans* (clinical sample). Strains obtained from water, food and clinical samples were typified in the Microbiology and Immunology Department Laboratory of the National University of Rio Cuarto (Argentina). 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 the 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, grown at 37 °C and adjusted to approximately 10^6 cfu/ml. 200 μl each inoculum was spread over plates containing Mueller–Hinton agar and a paper filter disc (6 mm) impregnated with 10 μl the essential oil was placed on the surface of the media. The plates were left for 30 min at room temperature to allow the diffusion of the oil, and then they were incubated at 37 °C for 24 h. After this time, the inhibition zone around the disc was measured usily a vernier caliper.

The minimum inhibitory concentration (MIC) was determined only with microorganisms that showed inhibitory zones > 10 mm, following as the criterion that an inhibitory area of essential oil of 10 mm is approximately 50% of the inhibitory area of gentamycin (10 μg/ml). MIC was determined by the dilution of essential oil in dimethyl sulphoxide, pipetting 10 μl each dilution onto a filter paper disc. Dilutions of the emulsions of essential oils were made over the concentration range 10–45 000 μg/ml. MIC was defined as the lowest concentration that inhibited visible growth.[10]

A negative control was also included in the test, in which a filter paper disc loaded with 10 μl dimethyl sulphoxide (DMSO), used as an oil diluent, was added to the plate to check a possible antimicrobial activity of DMSO against these microorganisms. The positive control was the antibiotic gentamycin (10 μg/ml) for bacteria and amphotericine B (2×10^2 μg/ml) for *C. albicans*, in order to check the sensitivity of the test organism.

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 main components of the essential oil.

Results and Discussion

Twenty-four compounds were identified by GC and GC–MS, representing about 95.6% of the oil, and main component was α-pinene (75.8%) (Table 1). However, the main components of essential oil of *M. atropurpureum* from southern Brazil were limonene (35.0%), 1,8-cineole (23.4%) and α-pinene (12.2%).[9]

M. atropurpureum var. *octandrum* essential oil was active against the Gram-positive bacteria. The MIC values were 490 μg/ml for *S. aureus* and *M. luteus* and 250 μg/ml for *B. cereus*.

The essential oil did not present activity against Gram-negative bacteria and *C. albicans* (Table 2); in the case of Gram-negative bacteria, this could be due to their outer membrane.[11,12]

α-Pinene showed better activity than the essential oil against *B. cereus* and the yeast *C. albicans*; this could be interpreted in terms of variations in the rate of monoterpene penetration through cell wall and cell membrane structures, because the ability of monoterpenes to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at minimum inhibitory levels.[13,14] Previous studies have showed that *B. cereus* and *C. albicans* are inhibited by α-pinene.[15,16] However, α-pinene was not capable of strongly inhibiting *S. aureus*, *M. luteus*, *E. coli* and *Klebsiella* sp.

The use of *M. atropurpureum* var. *octandrum* in folk medicine for the treatment of several illnesses, such as intestinal disorders, headache and infections, could be explained by the relaxant effect,[17] analgesic properties[18] and antimicrobial activity[3] of α-pinene, the main component of the essential oil.

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Table 1. Essential oil composition of *Myrrhinium atropurpureum* var. *octandrum*

Compounds	Retention Index		(%)	Methods of identification
	DB-5	Supelcowax-10		
α-Pinene	939	1028	75.8	MS, CO
β-Pinene	980	1108	2.4	MS, CO
α-Phellandrene	1005		1.8	MS, CO
p-Cymene	1025	1268	0.8	MS
1,8-Cineole	1033	1213	1.4	MS, CO
Limonene	1031	1190	2.1	MS, CO
γ-Terpinene	1045	1255	0.5	MS
Terpinolene	1082	1281	0.5	MS
Borneol	1152		0.3	MS
α-Terpineol	1189	1714	1.4	MS, CO
β-Caryophyllene	1419	1579	2.0	MS, CO
β-Gurjunene	1432		0.6	MS
Aromadendrene	1439		1.1	MS
α-Humulene	1454	1653	0.2	MS
allo-Aromadendrene	1463	1643	0.2	MS
1*s*-*cis*-Calamenene	1521	1826	0.5	MS
β-Calacorene	1550		0.3	MS
δ-Cadinene	1524	1744	0.3	MS
trans-Nerolidol	1564	2040	0.2	MS
Spathulenol	1576	2110	0.6	MS
Caryophyllene oxide	1581	2032	0.1	MS
τ-Cadinol	1640	2155	0.9	MS
τ-Muurolol	1641	2171	0.7	MS
β-Eudesmol	1649	2283	0.9	MS
Monoterpene hydrocarbons			83.9	
Oxygenated monoterpenes			3.1	
Sesquiterpene hydrocarbons			5.2	
Oxygenated sesquiterpenes			3.4	
Total			95.6	

Components are listed in order of elution on DB-5 column. MS, compounds that have been identified by mass spectra. CO, compounds that have also been identified by standards.

Table 2. Antimicrobial activity of essential oils of *Myrrhinium atropurpureum* var. *octandrum* (inhibition zone diameter measured in mm, disc diameter 6 mm)

Microorganism	Inhibition zone				MIC of essential oil (μg/ml)
	Essential oil	α-Pinene	Positive control Gentamicin (10 μg/ml)	Amphotericin B (20 μg/ml)	
S. aureus	14	8	20.5	NT	490
S. epidermidis	NA	NA	26	NT	NT
B. cereus	11	17	26	NT	250
M. luteus	12.5	6	20.5	NT	490
E. fecalis	NA	NA	18.5	NT	NT
E. coli	NA	8	18	NT	NT
Klebsiella sp.	NA	7	19	NT	NT
P. mirabilis	NA	NA	20	NT	NT
P. aeruginosa	NA	NA	20	NT	NT
C. albicans	NA	11	NT	20	NT

NA, not active; NT, not tested.

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