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Phytoconstituents and antimicrobial activity of *Melaleuca leucadendron* leaf essential oil from Venezuela

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Abstract

The essential oil composition of Venezuelan *Melaleuca leucadendron* leaves was determined. The oil was extracted by hydrodistillation and analyzed by HRGC and GC-MS. Among the 44 constituents identified, 1,8-cineole (38.4%), nerolidol (28.7%), alloaromadendrene (14.4%) and a-terpineol (12.6%) were the most abundant. The 1,8-cineole content was an indication that this essential oil belongs to the chemotype I. The essential oil was active against *Bacillus cereus* and *Staphylococcus aureus*, but was inactive against *Escherichia coli* and *Pseudomonas aeruginosa*. It also showed toxicity in the brine shrimp (*Artemia salina*) lethality test (LC50 (24 h) = 22. 25 µg/ml).

Key words: Antibacterial activity; essential oil; GC-MS: HRGC; *Melaleuca leucadendron*.

Fitoconstituyentes y actividad antimicrobiana del aceite esencial de la hoja de *Melaleuca leucadendron* de Venezuela

Resumen

La composición del aceite esencial de las hojas de *Melaleuca leucadendron* venezolano fue determinada. El aceite esencial fue extraído por hidrodestilación y analizado por una combinación de HRGC y GC-MS. Entre los 44 constituyentes identificados los más abundantes fueron 1,8-cineol (38,4%), nerolidol (28,7%), alloaromadendreno (14,4%) y α-terpineol (12,6%). El contenido de 1.8 cineol fue una indicación de que este aceite esencial pertenece al quimiotipo I. El aceite esencial fue activo contra *Bacillus cereus* y *Staphylococcus aureus*, pero no fue activo contra *Escherichia coli* y *Pseudomonas aeruginosa*. También mostró toxicidad contra *Artemia salina* (LC50 (24 h) = 22,25 µg/mL).

Palabras clave: Aceite esencial; actividad antibacteriana: GC-MS: HRGC; *Melaleuca leucadendron*.

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Introduction

Melaleuca leucadendron L. is a perennial tree appreciated for its cork production. This species is endemic of Australia, New Guinea and Indonesia, but is well adapted to some Venezuelan regions such as Táchira State. Like most plants belonging to the Myrtaceae, a fragrant essential oil can be obtained by hydrodistillation of the leaves. This essential oil is a mixture of volatile terpenoid compounds and has been used in soaps, perfumes, and skin-care products to treat infections, because of its antimicrobial properties (1-3).

Essential oils from *Melaleuca* species have been reported as a natural source of terpinen-4-ol, a compound with a high biological activity (3-5). In fact, according to Australian specifications, a terpinen-4-ol content greater than 30% and a 1,8 cineole content of less tan 15% is the standard of quality (4-6).

Investigations on the essential oils of *M. leucadendron*, *M. quinquenervia* and *M. alternifolia*, revealed the existence of several chemotypes, even within the same species, with differences in the type and amount of constituents (7-10): chemotype I, rich in 1,8 cineole (40-50%) (9,11,12), chemotype II, rich in nerolidol (40-80%) (13), chemotype III, rich in methyl-isoeugenol (>88%) (7), chemotype IV, rich in methyl-eugenol (>99%) (7), chemotype V, rich in viridiflorol (48%) (12), chemotype VI, rich in linalool (14). These seem to be associated with the biosynthetic pathways of monoterpenoids (5, 7, 15). Genetic inheritance and plant age together with environmental factors are the first and second reasons, respectively, influencing compositional differences in essential oils of similar plant species (16). For instance, the habitat modified essential oils content of *Melaleuca halmaturorum* and *Melaleuca tropical quinquenervia* in Madagascar (8). Therefore, it is considered that tropical ecological conditions of Venezuelan trees might affect physico-chemical properties, biological activities and essential oil compositions of leaves.

The objective of this work was to identify the constituents of Venezuelan *M. leucadendron* leaf essential oil by HRGC and GC-MS and to determine its physical-chemical properties and biological activity.

Materials and Methods

Plant material

Leaves of *M. leucadendron* were collected from adult trees in a commercial orchard at Rubio, Táchira State (Venezuela). The plant was identified in the MER-Herbarium from Universidad de Los Andes, Mérida (Venezuela) by comparison with the Revista Mantisa 1,105 from Australia.

Essential oil extraction

The essential oil of fresh leaves (20 Kg) was obtained by hydrodistillation in a Clavenger-type apparatus for 4 hours.

Physical-chemical properties

Density, refraction index and specific rotation of the oil were determined at 22°C.

HRGC analyses

HRGC analyses were performed with a Perkin Elmer Gas Chromatograph Autosystem Model, equipped with a FID detector and a OV-17 capillary column (50 m x 0.32 mm i.d.). The analytical conditions used were: injector and detector temperature 220°C, oven temperature programmed isothermically at 60°C for 3 min, followed by a rate of 4°C/min from 60°C to 220°C for 4 min. The nitrogen carrier gas flow rate was 25 cm/s. The injection volume was 1 µL of essential oil. The identity of each compound was determined by co-chromatography employing known standards. In general, standards of essential oil components were obtained from Sigma Chemical Co. (USA) and Aldrich Co. (USA), with a purity greater than 95%. The results were averaged from three runs.

GC-MS analyses

For confirmation, mass spectra were performed with a Perkin Elmer Gas Chromatograph equipped with a Perkin Elmer 910 Q mass selective detector operating at 70 eV. A 5% phenylmethylsilicone capillary column (30 m x 0,25 mm i.d.) and helium as carrier gas were used. Analytical conditions were identical with those of the HRGC analyses. The compounds were identified by comparison with the NIST/NBS library spectra and with published data (17, 18).

Microbiological assays

The antibacterial properties of the *Melaleuca leucadendron* essential oil was tested against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. All cultures were provided by Centro de Referencia Bacteriológica del Hospital Universitario de Maracaibo, del Estado Zulia, Venezuela. Stock cultures were maintained on trypticase soy agar slants and transferred to trypticase soy broth before its use as inoculum. Incubation was at 35 to 37°C for 24 h. The assay of the antibacterial activity was made employing the paper-disc agar diffusion method (2) using sterile filter paper disks (Whatman N° 2, 4 mm in diameter) saturated with essential oil. The impregnated disks (~ 3,3 mg) (14) were irradiated with high intensity UV light during different time lapses (0, 2, 4, 6 and 7 h) and placed on the surface of inoculated medium (Muller-Hilton - Meck, Germany) with a culture previously standardized of test bacteria (10^6/ml final concentration). After 24 h of incubation at 37°C, the inhibitory effect of *M. leucadendron* leaves oil was determined measuring the clear zone around the disks with a Vernier. This assay was made in duplicate.

The toxic properties of the oil to brine shrimp (*Artemia salina*) was determined. The test was carried out with eggs of *A. salina* acquired in a pet shop. Upon being placed in plates with sea water, the eggs hatch within 48 h at 25°C to provide large number of larvae (nauplii) for experimental use. The essential oil was tested at concentrations of 0.01, 0.1, 1, 10, 100 and 1000 ppm in vials containing 6 ml of brine shrimp each, three replicates were made. Survivors were counted after 24 h at 25°C (19, 20). The LC50 value was estimated using a single program on a personal computer with 95% confidence intervals for statistically significant comparisons of potencies.

Results and Discussion

The leaves of *Melaleuca leucadendron* yielded a mean of 1% of essential oil with an specific gravity of 0.88. The refraction index of the oil was 1.46 and the specific rotation, 2.47°.

The GC analysis revealed a total of 44 constituents: 29 mono and sesquiterpene hydrocarbons and 15 oxygenated compounds could be identified in the essential oil. All compounds, listed in Table 1, have been confirmed by GC-MS analysis of the whole oil. As can be seen, monoterpene hydrocarbons are found in amounts of 1,7%. α-pinene (0.9%) and β-pinene (0.3%) are the most abundant constituents of this monoterpene fraction. The most prominent component of the oil is 1,8 cineole with a percentage of 38.4%. Therefore, this essential oil belongs to the chemotype I. Due to the 1,8-cineole content, the Venezuelan essential oil is similar to Vietnamese essential oil (9) but differs from the other chemotypes which have high concentrations of methyl eugenol and E-methylisoeugenol as principal components (7). The oils with high cineole content are not recommended for medicinal use because this compound irritates mucous membranes and skin (5). Among the other oxygenated compounds there is a high proportion of nerolidol (28.7%) and α-terpineol (12.6%). Terpinen-4-ol was not found. According to Australian specifications this essential oil has intermediate quality characters since does not contain terpinen-4-ol,

Table 1
Phytoconstituents of *M. leucadendron* leaf essential oil from Venezuela

Compound	% w/w	Cited
Monoterpenes:		
α-pinene	0.9	3, 5, 8
Camphene	traces	3, 8
β-pinene	0.3	3, 5, 8
Myrcene	0.1	3, 5, 8
3-carene	traces	8
γ-terpinene	0.2	3, 5, 8
Terpinolene	0.1	3, 5, 8
3,7-dimethyl-1,3,7-octatriene[1]	0.1	
4-carene	traces	
Ocymene	traces	
Sub-total:	1.7	
Oxygenated:		
1,8 cineole	38.4	3,5,8
Benzaldehyde	0.2	8
Methyl-benzoate	0.1	8
Dehydrocarveol	0.1	
5-caranol[1]	traces	
γ-terpineol[1]	0.4	
α-terpineol	12.6	3, 5, 8
Cinnamic acid	traces	
4-hydroxi-2-methyl-acetophenone[1]	traces	
Isoeugenol	traces	
Nerolidol	28.7	8
Ledol	traces	8
Oxygenated:		
α-bisabolol[1]	traces	
Patulenol[1]	traces	
Sub-total:	80.5	
Sesquiterpenes:		
Germacrene[1]	traces	8
α-copaene[1]	traces	3, 8
β-maaliene[1]	0.1	
β-caryophyllene	0.8	3, 5, 8
Iso-caryophyllene[1]	traces	
γ-gurjunene[1]	traces	
α-caryophyllene	traces	
Eremophyllene[1]	0.3	
γ-selene[1]	traces	
γ-muurolene[1]	traces	3
β-elemene[1]	0.1	3
Sativene[1]	0.1	
Ledene[1]	0.7	
α-farnesene	traces	
γ-cadinene[1]	0.1	3
Cadinene[1]	0.2	8
α-cubebene[1]	traces	3, 8
Aromadendrene[1]	traces	3, 8
Alloaromadendrene	14.4	3, 5, 8
Sub-total:	16.8	
Total:	99.0	

Table 2
Antibacterial activity of *M. leucadendron* leaf essential oil from Venezuela

Organism \ Irradiation time	Inhibition zone (mm)				
	0h	2h	4h	6h	7h
Bacillus cereus	0	3	4	4	4
Staphylococcus aureus	0	4	7	7	7
Escherichia coli	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0

but possesses a high amount of 1,8 cineole (4,5). Sesquiterpene compounds were found in amounts of 16.8%. Allaaromadendrene was the most prominent component with a percentage of 14.4%, followed by β-caryophyllene (0.8%) and ledene (0.7%).

The results of antibacterial activity assays are shown in Table 2. The non-irradiated essential oil did not exhibit antibacterial activity against any of the strains. However, with *B. cereus* and *S. aureus* this activity increased when the essential oil was irradiated or photo-stimulated with UV (10), while it was inactive against *E. coli* and *P. aeruginosa*. The *M. leucadendron* essential oil was more effective in reducing Gram-positive bacteria than Gram-negative bacteria. These results are similar to Dabbah et al. (21).

A brine shrimp (*Artemia salina*) lethality bioassay on the essential oil showed that it was very toxic to the brine shrimp with a LC50 of 22.25 µg/ml. Some authors proved that compounds such as 1,8 cineole and α-terpineol, isolated from *M. cayuputi* essential oil, showed antibacterial activity (22). The presence of these compounds at high levels suggests their influence on the inhibitory and toxic properties of the essential oil.

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