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Volatile Constituents of the Essential Oil from Dried Leaves of *Calycolpus moritzianus* (O. Berg) Burret obtained by using two methods

Constituyentes volátiles del aceite esencial de las hojas secas de Calycolpus moritzianus (O. Berg) Burret obtenido mediante el uso de dos métodos

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ABSTRACT

The Myrtaceae family has species that produce essential oils of great interest, such as Melaleuca alternifolia and Eucaliptus globulus. We have researched some species growing in the East Region of Colombia. The essential oils of leaves of Calycolpus moritzianus (O. Berg) Burret from five localities of Norte de Santander (Chinácota, Ocaña, Pamplonita, Salazar and Toledo) in Colombia were obtained by two extraction methods: Microwave assisted-Hydrodistillation (MWHD) and Steam Distillation (SD). The chemical constituents were identified by HRGC-FID and HRGC-MS. The main constituents were 1,8-Cineole (20.2-42%), Limonene (21.2-40%), α-Pinene (3.2-8%), α-Terpineol (2.1-6.3%), Guaiol (1.7-14%) and β-Caryophyllene (1.7-3.0%).

Keywords: *Calycolpus moritzianus, Myrtaceae, essential oil, 1,8-cineole, limonene, α-pinene.*

RESUMEN

La familia Myrtaceae agrupa varias especies vegetales que son productoras de aceites esenciales de reconocida importancia a nivel mundial, tales como, Melaleuca alternifolia y Eucaliptus globulus. En nuestro grupo, se han investigado algunas mirtáceas de ocurrencia natural en la región oriental de Colombia. Los aceites esenciales de las hojas de Calycolpus moritzianus (O. Berg) Burret, recolectadas en cinco municipios (Chinácota, Ocaña, Pamplonita, Salazar y Toledo) del departamento de Norte de Santander, en Colombia, se obtuvieron mediante dos métodos: Hidrodestilación asistida por radiación de microondas y Destilación por arrastre con vapor de agua. Los componentes químicos de los

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aceites se identificaron por Cromatografía de Gases de Alta Resolución. Los compuestos mayoritarios fueron: 1,8-Cineol o Eucaliptol (20.2-42%), Limoneno (21-40%), α-Pineno (3.2-8%), α-Terpineol (2.1-6.3%), Guaiol (1.7-14%) y β-Cariofileno (1.7-3.0%).

Palabras clave: *Moritzianus Calycolpus, Myrtaceae, aceite esencial, 1,8-cineol, limoneno, α-pineno.*

INTRODUCTION

The Myrtaceae family consists of some 75 genera and nearly 3000 species of mainly tropical evergreen trees and shrubs. The main areas of distribution are the American and Asian tropics and Australia (Weiss, 1997).
Calycolpus O. Berg is a genus of ca. 15 species ranging from Central America to Brazil with the greatest diversity in northeastern South America in the Guayana Highlands. It closely resembles *Psidium* L. and is distinguished from it by a combination of floral, inflorescence and seed coat characters. *C. moritzianus* (O.Berg) Burret, syn. *Psidium caudatum* McVaugh, syn. and *Psidiopsis moritziana* (O. Berg), commonly known in the Colombia as "arrayán" or “cínaro”, is a tree ca. 15 m high, whose leaves are green and elliptic (Landrum, 2008).

In a previous work, the chemical composition of the essential oil of *Psidium caudatum* McVaugh grown in Pamplona (Colombia) was investigated and thirty-two compounds were identified by HRGC-MS. The main constituents were terpinen-4-ol (47.72%), γ-terpinene (11.58%), α-terpinene (6.70%), limonene (5.20%), α-pinene (4.49%), 1,8-cineole (eucalyptol) (3.95%), α-terpineol (3.05%), β-terpinene (2.89%), β-pinene (2.66%), p-cymene (2.22%) and α-terpinolene (2.22%) (Yáñez *et al*., 2002), whereas the same plant collected in other region of Colombia (Cesar), showed as main components, eucalyptol (20.4%), *trans*-β-caryophyllene(8.3%), γ-eudesmol (7.6%), α–terpineol (6.1%), guaiol+viridiflorol (5.2%) and α-pinene (3.1%) (Castañeda *et al*., 2007). In a recent study on the essential oil of this plant collected in Mérida (Venezuela), thirty components were identified, of which the seven major ones were β-caryophyllene (21.9%), α-pinene (10.9%), viridiflorol (9.7%), α-copaene (6.3%), β-selinene (6.1%), α-selinene (5.3%) and γ-eudesmol (5.1%). The oil was found to have antibacterial activity against *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212 with MIC (Minimal Inhibitory Concentration) values of 60 μg/mL and 180 μg/mL, respectively (Díaz *et al*., 2008). Similarly, the essential oil of *C. moritzianus* collected in Colombia, was effective against *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Proteus mirabilis* ATCC 4307 with MIC values of 39 μg/mL, 106 μg/mL, 836.4 μg/mL and 892.2 μg/mL, respectively. The analysis of antibacterial activity coefficient of the main components showed that the α-Copaene was the most influential in that activity (Mojica *et al.*¸ 2011). On the other hand, the oil of *C. moritzianus* collected in regions of Salazar and Pamplonita (Colombia) showed antifungal properties against *Candida crusei* with MIC values of 500 μg/mL and the oils of plants from regions of Salazar and Pamplonita (Colombia) were the most cytotoxic on HeLa cells at concentrations of 37.8 and 40.6 μg/mL, respectively (Yáñez *et al.*¸ 2009). The effect of the treatment of the leaf about the variability of essential oil content of the leaves of the *C. moritzianus* from five areas from North of Santander (Colombia), obtained by using Microwave-Assisted Extraction was investigated and the major yield obtained was about 1%, for the essential oil from dried and split leaves collected of the Pamplonita region (Yáñez *et al.*¸ 2011). Besides, another previous work showed the composition of the oil obtained by using Simultaneous Distillation-Extraction (SDE) and statistical analysis was performed (Vanegas *et al.*¸ 2011). This paper presents additional analysis of the oil composition of *C. moritzianus* from differentes populations from the northwest region of Colombia. In addition, the oil compositions of dried "cínaro" leaves are compared by using two methods of extraction.

MATERIALS AND METHODS

Plant material

The leaves of *Calycolpus moritzianus* (O. Berg) Burret, syn. *Psidium caudatum* McVaugh were collected during raining periods from April to Juny and September to November around of five localities in Norte de Santander State (Chinácota, Ocaña, Pamplonita, Salazar and Toledo) ranging in elevation from 1000 to 2600 m. Voucher specimens have been deposited in the Herbarium Catatumbo-Sarare of the University of Pamplona (Pamplona, Colombia).

Isolation of the oils

Dried and split leaves as samples were used. The leaves were air-dried at room temperature in the shade by 15 days. The oils were obtained by using two methods, Microwave assisted-Hydrodistillation (MWHD) and Steam Distillation (SD).

Microwave assisted - Hydrodistillation (MWHD)

400g of leaves and 2000 mL of distilled water were placed in a 4000 mL round-bottom flask and connected to a Clevenger-type apparatus placed in a modified microwave oven (Samsung). The oven was operated for 30 min (Stashenko *et al*., 2004).

Steam Distillation (SD)

400g of leaves and 2000 mL of distilled water were placed in a 4000mL round-bottom flask and subjected to steam distillation for 2 h after boiling. The oils obtained by MWHD and SD methods were dried over anhydrous sodium sulphate and stored in a refrigerator until analysis by HRGC.

Gas Chromatography (GC-FID)

An HP 6890A Series II with flame ionization detector and with a fused silica HP-5 capillary column (60 m x 0.25 mm x 0.25 µm film thickness) was used. The oven temperature was maintained at 50ºC for 4 min after injection, then programmed at 2ºC/min to 90°C, then held for 3 min, 90-220°C at 4°C/min. Air and hydrogen flow rates were 300 and 30 mL/min. Nitrogen was used as a make-up gas at a flow rate of 30 mL/min. The carrier flow rate (He) was1 mL/min. Injector and detector temperatures were kept at 250ºC. 1.0µL of the solution of essential oil in dichloromethane was injected with split ratio of 10:1. The identity of the components was assigned by comparison of their retention indices, relative to C_8-C_{32} n-alkanes (Adams, 2005).

Gas Chromatography-mass spectrometry (GC/MS)

An HP 6890 Series II coupled to a HP5973N mass detector with the column HP-5MS (30 m x 0.25 mm x 0.25 µm film thickness) was used. A similar temperature program as in the GC-FID technique was used. Mass spectra were recorded in the electron impact mode at 70 eV.

Qualitative and quantitative analysis of volatile compounds

Constituentes were identified by comparison of their mass spectra with those in NIST-2005 data base, and confirmed in some compounds by their relative retention indices with authentic standards. Mass spectra from the literature were compared (Adams, 2005). Quantitative determinations were carried out in GC-FID by the internal standard method (0.5 μL de n-tetradecano) and in terms of relative (percent) areas for HD and MWHD.

RESULTS AND DISCUSSION

A total of 28 compounds at concentrations above 0.1% were identified in the essential oil of *C. moritzianus* in all extracts. The results for each population according to MWHD and SD procedures are described (Table 1).

The major compounds were 1,8-cineol (42.0%), limonene (25.0%) and α-Terpineol (5.0%) by using MWHD method, whereas 1,8-cineol (38.0%), limonene (30.0%) and β-caryophyllene (5.7%) were the main compounds by using SD method in the oils from plants of the Chinácota region.

Table 1

Constituents of the essential oil of *C. moritzianus* from five populations of Norte de Santander (Colombia)

Compound	*RI* (1)	CHINÁCOTA		OCAÑA		PAMPLONITA		SALAZAR		TOLEDO	
		MWHD% (2)	*SD%* (2)	*MWHD%*	*SD%*	*MWHD%*	*SD%*	*MWHD%*	*SD%*	*MWHD%*	*SD%*
α-pinene[3]	935	3.2	4.3	7.2	8.1	3.2	8.0	4.0	6.8	7.1	7.0
camphene	950	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
β-mircene[3]	992	1.0	1.0	1.0	1.2	1.0	1.2	0.9	1.3	1.1	1.2
α-phellandrene[3]	1005	0.2	0.2	0.1	0.3	0.2	0.3	0.1	0.3	0.1	0.1
α-terpinene	1020	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
limonene[3]	1032	25.0	30.0	26.1	27.0	39.5	33.0	21.4	40.0	28.2	24.0
1,8-cineole[3]	1035	42.0	38.0	32.1	30.0	41.2	32.0	31.0	20.2	40.4	38.0
γ-terpinene	1062	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.2	0.3	0.4
α-terpinolene	1090	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
linalool	1100	1.1	1.1	2.3	1.6	1.1	0.7	0.6	0.6	2.7	2.0
borneol	1172	0.3	0.1	0.2	0.2	0.3	0.2	0.2	0.5	0.3	0.3
terpinen-4-ol	1182	0.7	0.5	0.4	0.4	0.7	0.5	0.4	0.5	0.8	0.8
α-terpineol	1195	5.0	3.1	4.2	4.0	6.3	4.2	4.7	2.1	4.0	3.6
α-copaene	1380	0.8	1.3	1.0	0.5	1.0	1.1	1.2	2.0	0.8	1.2
β-caryophyllene	1432	3.7	5.7	3.3	6.3	3.8	5.0	4.6	9.0	2.0	4.0
α-humulene[3]	1466	0.3	0.1	0.5	0.3	0.4	0.2	0.5	0.2	0.2	0.7
aromadendrene	1474	0.1	0.5	0.5	0.7	0.2	0.5	0.5	1.0	0.2	0.5
β-selinene	1501	0.3	0.4	0.3	1.0	0.3	0.5	0.7	1.5	0.5	0.9
α-selinene	1508	0.7	0.8	0.2	1.0	1.0	1.0	0.8	2.0	0.2	1.4
γ-cadinene	1515	0.2	0.2	0.2	0.6	0.3	0.8	0.2	0.8	0.3	0.6
(Z)-nerolidol	1530	0.3	0.4	0.8	0.4	0.3	0.4	0.4	0.5	0.2	0.3
caryophyllene	1580	2.0	2.3	2.8	2.0	1.7	3.0	2.2	0.8	2.0	3.0
viridiflorol	1602	0.2	0.1	0.4	0.3	0.2	0.2	0.4	0.5	0.2	0.4
guaiol	1613	4.7	2.2	2.0	1.7	2.7	2.6	14.0	4.2	3.5	3.5
cubenol	1620	0.2	0.2	0.2	0.3	0.2	0.2	0.9	0.3	0.6	0.4
eudesmol	1626	0.6	0.1	0.3	0.2	0.6	0.1	0.5	0.2	0.5	0.3
γ-eudesmol	1642	1.0	2.0	1.6	2.1	1.1	0.4	2.1	1.3	0.7	0.5
selin-11-en-4-ol	1663	0.7	0.2	0.5	1.1	0.3	0.5	1.2	0.6	0.3	0.2

(1) Retention Index relative to C_8-C_{32} on HP-5 column.
(2) Relative percentage from three extractions by each method.
(3) Standard samples were used.

The major compounds were 1,8-cineol (30.0-32.1%), limonene (26.1-27.0%) and α-pinene (7.2-8.1%) by using MWHD and SD procedures in plants of the Ocaña region.

The major compounds were 1,8-cineol (41.2%), limonene (39.5%) and α-Terpineol (6.3%) by MWHD method, whereas limonene (33.0%), 1,8-cineol (32.0%), and α-pinene (8.0%) were the main compounds by SD method in the oils of plants of the Pamplonita region.

The main compounds were 1,8-cineol (31.0%), Limonene (21.4%) and guaiol (14.0%), by MWHD method, whereas limonene (40.0%), 1,8-cineol (20.2%), and β-caryophyllene (9.0%) were the main compounds by SD method in the oils of dried leaves of the Salazar region.
The oils of leaves collected in TOLEDO region showed high content of 1,8-cineol (38.0-40.4%), limonene (24.0-28.2%) and α-pinene (7.0-7.1%) by the two methods.

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