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Uruguayan essential oils. Composition of leaf oil of *Myrcianthes cisplatensis* (Camb.) Berg. ('Guayabo colorado') (Myrtaceae)

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ABSTRACT: *Myrcianthes cisplatensis* leaf oil, obtained by steam distillation, was analysed by GC–FID and GC–MS. Twenty-six components were identified in the oil (90% of the total composition); the enantiomeric distribution of α-pinene, β-pinene, limonene, linalool, terpinen-4-ol and α-terpineol was studied by multidimensional HRGC–HRGC. The major component was 1,8-cineole (54%). The enantiomeric purity for the (+) enantiomers for the monoterpenes studied was 96% for α-pinene, 49% for β-pinene, 100% for limonene, 94% for linalool, 50% for terpinen-4-ol and 64% for α-terpineol. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: *Myrcianthes cisplatensis*; 'Guayabo colorado'; Myrtaceae; essential oil composition; GC–FID; GC–MS; 1,8-cineole; α-pinene; limonene; terpinen-4-ol; chiral analysis

Introduction

Myrcianthes cisplatensis (Camb.) Berg. ('Guayabo colorado'), initially classified as *Eugenia cisplatensis* (Camb.)[1] is a tree of dense foliage and pleasant aspect for its stems with bark of light brown colour. It grows near the rivers in almost all the fluvial forests with preference for the external parts of them, and is widespread in Uruguay, Argentina and South of Brazil.[1] The quality of its wood has made the occurrence of this beautiful tree diminish. The composition of the leaf oil of *M. cisplatensis* is reported in this paper.

A literature survey on *M. cisplatensis* yielded two publications on the chemical composition of leaf oils, both for Argentina,[2,3] and one related to its antioxidative activity.[4]

The aim of this work is to analyse the oil from *M. cisplatensis* from Uruguay in order to compare it with those reported in the literature and to characterize the oil according to the enantiomeric distribution of some monoterpene components.

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Experimental

Plant Material and Extraction

The fresh leaves were collected in the South of Uruguay around a low hills area ('cerros pelados'), Canelones province, in September 1998. Voucher specimens of the plant were identified and deposited at the Herbarium of the Facultad de Agronomía in Montevideo (MVFA 7246). The leaves were dried by exposure to the air and then extracted by steam distillation for 2 h in a modified Clevenger apparatus.[5] The *M. cisplatensis* leaf oil was analysed by GC–FID and by GC–MS.

GC Analysis

The composition of the oil was carried out by GC on a Shimadzu 14 B gas chromatograph equipped with a FID and a Shimadzu data processor software EZ-Chrom, using two capillary columns. The first was a SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m $\times$ 0.32 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.40–0.45 μm phase thickness); column temperature, 60 °C for 8 min, rising to 180 °C at 3 °C/min, 180–250 °C at 20 °C/min, then 250 °C for 10 min. Injector temperature, 250 °C; detector temperature, 280 °C; injection mode, split; split ratio, 1 : 30; volume injected, 0.2 μl of the oil. Carrier gas was

hydrogen, 55 kPa. The second was a Carbowax 20M (Ohio Valley, USA) bonded fused-silica capillary column (25 m × 0.32 mm i.d.), coated with polyethylene glycol (0.25 μm phase thickness); column temperature, 40 °C for 8 min, rising to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min. Injector temperature, 250 °C; detector temperature, 250 °C; injection mode, split; split ratio, 1 : 30; volume injected, 0.2 μl of the oil. Carrier gas was hydrogen, 30 kPa.

GC–MS Analysis

GC–MS analysis was conducted using a Shimadzu QP 5050 apparatus equipped with reference libraries[6,7] using two capillary columns. The first was a SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m × 0.25 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness); column temperature, 60 °C for 8 min, rising to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min. Injector temperature, 250 °C; injection mode, split; split ratio, 1 : 40; volume injected, 0.2 μl of the oil. Helium was used as the carrier gas, using 122.2 kPa (51.6 cm/s); interface temperature, 250 °C; acquisition mass range, *m*/*z* 40–400. The second was a BP 20 (SGE, Australia) bonded fused-silica capillary column (25 m × 0.25 mm i.d.), coated with polyethylene glycol (0.25 μm phase thickness); column temperature, 40 °C for 8 min, rising to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min. Injector temperature, 250 °C; injection mode, split; split ratio, 1 : 40; volume injected, 0.2 μl of the oil. Carrier gas was He, 92.6 kPa (55.9 cm/s); interface temperature, 250 °C; acquisition mass range, *m*/*z* 40–400.

Identification and quantification

The components of the essential oil were identified by comparison of their linear retention indices (LRIs) on the two columns, determined in relation to a homologous series of *n*-alkanes, with those from pure standards or reported in literature. Comparison of fragmentation patterns in the mass spectra with those stored on databases,[6,7] was also performed. The quantification of the components was performed on the basis of their GC peak areas, without corrections for factor of response.

Chiral Analysis

Enantiomeric ratios of α-pinene, β-pinene, limonene, linalool, terpinen-4-ol and α-terpineol were obtained by multidimensional GC, using a development model[8] set up with two GC ovens. The first oven was equipped with a column coated with SE-52, and the second with a chiral column coated with a derivatized β-cyclodextrin, a hot interface, a rotary switching valve and a system to maintain a constant flow during the transfer. With this system, a heart-cut of the relevant fractions can be made and these fractions transferred from the non-chiral to the chiral column in the following experimental conditions: precolumn, SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (30 m × 0.32 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.40–0.45 μm phase thickness); column temperature, 45 °C for 6 min, rising to 280 °C at 2 °C/min, 280 °C for 15 min; analytical column, fused-silica capillary column (25 m × 0.25 mm i.d., 0.25 μm phase thickness), coated with 2,3-di-*O*-ethyl 6-*O*-*t*-butyldimethylsilil-β-cyclodextrin in PS 086 (13% phenylmethyl-polysiloxane) (Mega, Legnano, Italy); injection temperature, 250 °C; column temperature, 50 °C for 6 min, rising to 90 °C at 2 °C/min, 90 °C for 20 min, 90–180 °C at 2 °C/min, 180 °C for 10 min, interface temperature, 200 °C; detector FID, 280 °C (for both chromatographs). Volume injected, 1 μl of an oil dilution 1 : 10 in *n*-hexane; injection mode, split; split ratio, 1 : 15. Carrier gas was helium at 90 kPa (precolumn), 110 kPa (analytical column).

Results and Discussion

M. cisplatensis oil is yellow and has a pleasant persistent spicy odour, which initially hides the aromatic note of 1,8-cineole and α-terpineol. Table 1 gives the relative percentages of single components and classes of compounds of *M. cisplatensis* oil. Table 1 shows the presence of 26 identified components, which represent about 90% of the total oil.

The oil contains a high amount (65.3%) of oxygenated compounds; monoterpene hydrocarbons represent 22.0%, while sesquitepene hydrocarbons represent only 2.6%. 1,8-cineole (53.8%) is the main component, followed by α-pinene (16.6%). In addition to 1,8-cineole, the monoterpene oxygenated fraction is made up especially of alcohols; α-terpineol (4.2%), terpinen-4-ol (0.6%) are the main monoterpene alcohols.

Oils of *M. cisplatensis* occurring in Argentina are cited in the literature.[2,3] In comparison to the results previously reported from Argentina, and with regard to the main components cited, there were considerable differences qualitatively and especially quantitatively. The main differences in composition are related to the α-pinene (4.3% Argentina and 16.6% Uruguay), limonene (22.1%; 4.1%), 1,8-cineole (40.7%; 53.8%) and linalool (4.8%; 0.2%) contents; other variations were reported depending on the origin of the oil. The results indicated a high biodiversity of the native populations of *M. cisplatensis*.

Table 1. Percentage composition of the essential oil of *Myrcianthes cisplatensis* (Camb.) Berg. and linear retention index (LRI) of its components

Compound*		(%)**	R	S	LRI*** SE-52	LRI*** CW 20M
1	α-Thujene	0.3			923	
2	α-Pinene [1R-(+), IS-(−)]	16.6	96.3	3.7	930	1006
3	β-Pinene [1R-(+), IS-(−)]	0.3	49.3	50.7	971	1079
4	Myrcene	0.1			1000	
5	δ-3-Carene	0.3			1007	
6	Limonene [4R-(+), 4S-(−)]	4.1	100.0		1020	
7	1,8-Cineole	53.8			1029	1186
8	γ-Terpinene	0.4			1054	1218
9	*trans*-Linalool oxide	0.1			1084	
10	Linalool [3R-(−), 3S-(+)]	0.2	6.1	93.9	1100	1529
11	*trans*-Pinocarveol	0.4			1132	1600
12	Pinocarvone	0.1			1155	1500
13	4-Terpineol [4R-(−), 4S-(+)]	0.6	50.1	49.9	1171	1569
14	α-Terpineol [8R-(+), 8S-(−)]	4.2	64.0	36.0	1186	1664
15	(*E*)-Caryophyllene	0.5			1414	1557
16	Aromadendrene	0.8			1434	
17	α-Humulene	0.2			1448	
18	*allo*-Aromadendrene	0.5			1456	
19	β-Selinene	0.4			1481	
20	α-Selinene	0.3			1490	
21	Spathulenol	1.0			1572	2074
22	Caryophyllene oxide	1.1			1576	
23	Thujopsan-2-α-ol	2.0			1579	
24	Globulol	0.8			1586	
25	1-*epi*-Cubenol	0.5			1630	
26	β-Eudesmol	0.5			1649	2064
Identified components		89.9				
Grouped components						
Monoterpene hydrocarbons		22.0				
Oxygen-containing monoterpenes		59.4				
Sesquiterpene hydrocarbons		2.6				
Oxygen-containing sesquiterpenes		5.9				

*The components are reported according to their elution order on SE-52.

**Relative proportions of the essential oil constituents were expressed as percentages obtained by peak-area normalization, all relative-response factors being taken as one. Percentages were obtained on SE-52 except for those of limonene and 1,8-cineole, which were obtained on Carbowax 20M.

***Peak identifications are based on comparison of their linear retention indices (LRIs) on the two columns with those from pure standards or reported in literature, and MS comparison with file spectra.

The combination of chemical analysis of an oil and chiral analysis of selected optically active components has been reported as a powerful tool and a versatile indicator of its characterization and origin.[9,10] By using this approach, and in order to characterize the oil of *M. cisplatensis* growing wild in Uruguay, we also report here the enantiomeric distribution of three monoterpene hydrocarbons (α-pinene, β-pinene and limonene) and three monoterpene alcohols (linalool, terpinen-4-ol and α-terpineol). Table 1 reports the enantiomeric ratios of the components analysed.

The good indicator compounds that emerge from these data are limonene and linalool, which could be used to assess the origin of *M. cisplatensis* oil obtained by similar extraction and processing methods.[11]

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