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Composition and Stereoanalysis of *Cymbopogon winterianus* Jowitt Oil from Southern Brazil

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ABSTRACT: The hydrodistilled essential oil from aerial parts of *Cymbopogon winterianus* Jowitt, cultivated in Southern Brazil, was analysed by GC–MS. Thirty-one components, representing 96% of the oil, were characterized. Enantiomeric ratios of limonene, linalool, citronellal and β-citronellol were obtained by multidimensional gas chromatography, using a developmental model set up with two GC ovens. The enantiomeric distributions are discussed as indicators of origin authenticity and quality of this oil. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: *Cymbopogon winterianus* Jowitt; citronella; volatile fraction composition; enantioselective gas chromatography

Introduction

Citronella oils are produced from two distinct types of citronella grass: *Cymbopogon nardus* L. Rendle, known as *lenabatu* or Ceylon citronella, and *C. winterianus* Jowitt, known as *maha pengiri* or Java-type citronella. The Ceylon type is the original cultivated variety and the Java type seems to have arisen as a distinct form of the Ceylon type.

Until the early part of this century the Ceylon type citronella was the most widely produced oil. Gradually the Java type has come to dominate the market, due to its higher yield of oil. It is grown commercially on a worldwide scale in places like Haiti, Central America, the South Pacific and tropical Africa. More recently other countries, such as Brazil, have contributed to the citronella oil production.[1]

Java citronella oil is used directly in perfumery, but is also one of the most important sources of natural citronellal, this component being present at higher concentration than in Ceylon oil.[2,3]

Before the advent of chemical sprays, Ceylon citronella was used in combination with Virginian red cedarwood oil in commercial insect repellents. Probably due to its association with insect repellency, citronella is usually overlooked as an aromatherapy oil ingredient.

A comprehensive survey on the composition of citronella oils has been published,[4] showing considerable differences in the components of the Java oils in relation to genetic, climatic and geographical origins, as well as on the stage of development of the plants used for the oil distillation.

Given the wide use of citronella oils and their frequent use as a basis of blends with other oils, as well as their variability, chemical evaluation of their monoterpenoid constituents is of fundamental interest. Furthermore, the enantiomeric distribution of the different monoterpenic components has been reported as criteria for genuinity in citronella oils.[5–8]

As part of our research on Brazilian essential oils, we report here the results relative to the composition and enantiomeric distribution of four monoterpenes (limonene, linalool, citronella and β-citronellol) of *Cymbopogon winterianus* Jowitt cultivated in the South of Brazil.

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Experimental

Materials

The aerial parts of Java citronella (*C. winterianus* Jowitt) were harvested from a small-scale experimental area cultivated at the Prefeitura de Santo Antonio da Patrulha in Rio Grande do Sul State (Brazil), in April 1997. The essential oil was obtained from the aerial parts after 2 h of hydrodistillation in the pilot plant of the Instituto de Biotecnologia, Universidade de Caxias do Sul. The average yield was 0.74 (w/w).

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 × 0.32 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.40–0.45 μm phase thickness); column temperature, 60°C (8 min) to 180°C at 3°C/min, 180–250°C at 20°C/min, 250°C (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 (8 min) to 180°C at 3°C/min, 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 were conducted using a Shimadzu QP 5050 equipped with Adams library,[9] 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 (8 min) to 180°C at 3°C/min, to 230°C at 20°C/min. Injector temperature 250°C; injection mode, split; split ratio 1.40; volume injected, 0.2μl of the oil. Helium was used as a carrier, using 122.2 kPa (51.6 cm/s); interface temperature 250°C; acquisition mass range 40–400; solvent cut, 2 min.

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 (8 min) to 180°C at 3°C/min, 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 40–400; solvent cut, 2 min.

Individual components were identified by comparison of both mass spectrum and their GC retention data with those of authentic compounds previously analysed and stored in the data system. Other identifications were made by comparison of mass spectra with those in the data system libraries and cited in the literature.[9,10] The linear retention index (LRI) of individual components were measured on both columns (SE 52 and Carbowax 20M).

Chiral Analysis

Enantiomeric ratios of limonene, linalool, citronellal and β-citronellol were obtained by multidimensional gas chromatography, using a model under development[11] set up with two GC ovens, the first equipped with a column coated with SE-52 and the second with a chiral column coated with a derivatized β-cyclodextrin, and 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 could be made and these fractions transferred from the non-chiral column to the chiral one 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 (6 min) to 280°C at 2°C/min; 280°C (15 min). Two different analytical columns were used, the separation of limonene and linalool enantiomers was achieved using a 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*-butyldimethylsilyl β-cyclodextrin in PS 086 (13% phenylmethyl-polysiloxane) (Mega, Legnano, Italy) while citronellal and β-citronellol enantiomers were separated with a fused-silica capillary column (25 m × 0.25 mm i.d., 0.25 μm phase thickness), coated 2,3-di-*O*-acetyl 6-*O*-*t*-butyldimethylsilyl β-cyclodextrin in OV 1701 (14% cyanopropylphenyl-methylpolysiloxane) (Mega, Legnano, italy); injection temperature, 250°C; column temperature, 45°C (6 min) to 90°C at 2°C/min, 90°C (20 min); 90°C to 180°C at 2°C/min, 180°C (10 min); interface temperature, 200°C; detector FID, 280°C (for both chromatographs). Volume injected, 1 μl of an oil dilution 1 : 10; injection mode, split; split ratio 1 : 15. Carrier gas was Ar, 90 kPa (precolumn), 110 kPa (analytical column). Evaluation of the elution order of the enantiomers was achieved using monoterpene mixtures with known enantiomeric ratio.

Results and Discussion

Thirty-one components could be identified in the oil. The basic composition of the oil of *C. winterianus* cultivated in Southern Brazil was similar to that reported for other Brazilian regions.[12] Differences were observed due to higher values of citronellyl acetate and geraniol and the absence of elemicin and α-cadinol. The volatile constituents identified in the oils are listed in Table 1. Figure 1 shows the total ion chromatogram of the oil. Compositional changes could be explained by extrinsic conditions associated to the climate and geographical origin of the cultivation areas. The enantiomeric ratio of four components (limonene, linalool, citronellal and β-citronellol) was established by subsequent transfer during different analysis using two chiral stationary phases. Under the experimental conditions, limonene and linalool were best resolved on diethyl *tert*-butylsilyl-β-cyclodextrin (2,3-DiEtTBS-β-CDX), while citronellal and β-citronellol were resolved on diacetyl *tert*-butylsilyl-β-cyclodextrin (2,3-DiAcTBS-β-CD). The heart cutting and cut-times for the essential oil are listed in Table 3. Figures 2 and 3 show the chromatograms obtained with the chiral analysis.

Enantiomeric ratios of the components analysed (Table 2) are similar to those reported in the literature.[6] The most marked difference was observed for linalool, which showed a (+)/(−) enantiomeric ratio of 61.3 : 38.7. This value was higher than that reported in the literature.[6]

Summarizing, the chiral essential compounds studied exhibit an enantiomeric composition which is in practice in correspondence with reported results for this species. The enantiomeric distribution for limonene in the oil of *C. winterianus* cultivated in Brazil is first reported in this work.

Furthermore, the results indicate the utility of the combination of chemical analysis of an oil and chiral

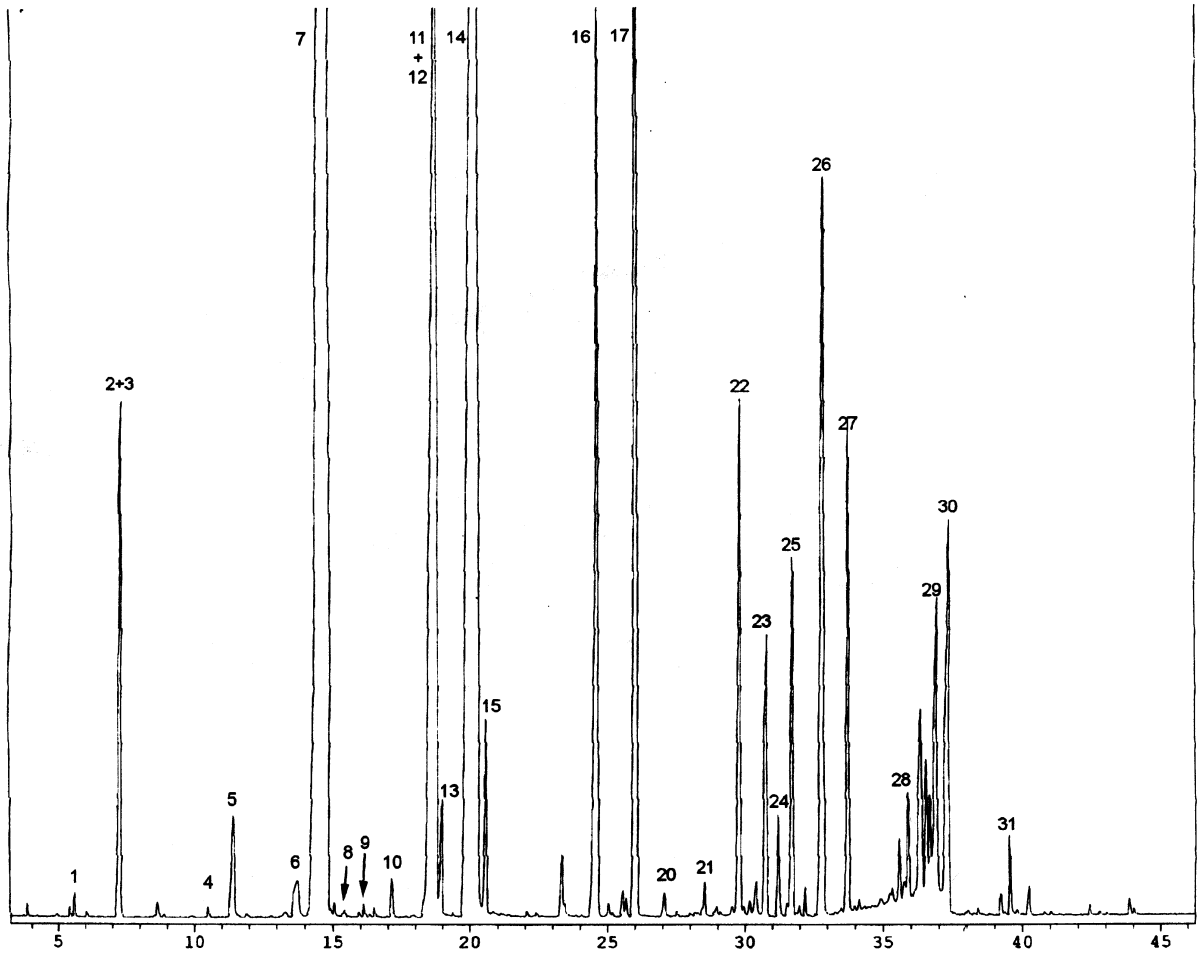

Figure 1. Chromatogram of *Cymbopogon winterianus* oil in SE-52

Table 1. Percentage composition of the essential oil of *Cymbopogon winterianus* Jowitt

Compound	(%)*	L.R.I.	
		SE-52**	CW
1 Myrcene	0.1	987	1120
2 Limonene	3.0	1026	1153
3 β-Phellandrene	0.1		1155
4 Terpinolene	0.1		1233
5 Linalool	0.6	1100	1513
6 Isopulegol	0.1	1142	1535
7 Citronellal	36.1	1158	1432
8 4-Terpineol	<0.1	1177	
9 α-Terpineol	<0.1	1189	
10 Decanal	0.1	1204	
11 β-Citronellol	9.9	1231	1740
12 Nerol	0.3	1231	1767
13 Neral	0.4	1239	1659
14 Geraniol	19.9	1259	1823
15 Geranial	0.6	1270	1684
16 Citronellyl acetate	3.5	1354	1610
17 Geranyl acetate	3.8	1388	1700
18 β-Bourbonene	0.2	1385	
19 β-Elemene	1.6		1525
20 (*E*)-Caryophyllene	0.1	1414	
21 α-Humulene	0.1	1446	1600
22 Germacrene D	2.6	1477	1642
23 α-Murolene	0.4	1500	
24 γ-Cadinene	0.4	1510	
25 δ-Cadinene	1.9	1521	1685
26 Elemol	5.8	1549	2052
27 Germacrene D-4-ol	1.7	1573	2008
28 10-*epi*-γ-Eudesmol	0.2	1617	
29 *epi*-α-Muurolol	1.0	1640	2200
30 α-Eudesmol	1.6	1653	
31 Farnesol	0.2	1721	1944
Identified components	96.3		
Grouped components			
Monoterpene hydrocarbons	3.2		
Oxygen-containing monoterpenes	75.1		
Sesquiterpene hydrocarbons	7.4		
Oxygen-containing sesquiterpenes	10.6		
Others	0.1		

* These percentages were obtained on SE-52 except for those of β-phellandrene, terpinolene, nerol and β-elemene which were obtained on Carbowax 20M.
** The components are reported according to their elution order on SE-52.

Table 2. Enantiomeric ratios for limonene, linalool, citronellal and β-citronellol in *Cymbopogon winterianus* Jowitt

Limonene		Linalool		Citronellal		β-citronellol	
4R-(+)	4S-(−)	3S-(+)	3R-(−)	3R-(+)	3S-(−)	3R-(+)	3S-(−)
13.0	87.0	61.3	38.7	91.2	8.8	84.7	15.3

Table 3. Heart-cutting times on the essential oil

	Cut-times (min)	
	2,3-DiEtTBS-β-CD	2,3-DiAcTBS-β-CD
Limonene	13.80–14.05	
Linalool	18.40–18.70	
Citronellal		18.40–18.70
β-citronellol		22.10–22.35
		27.40–27.65

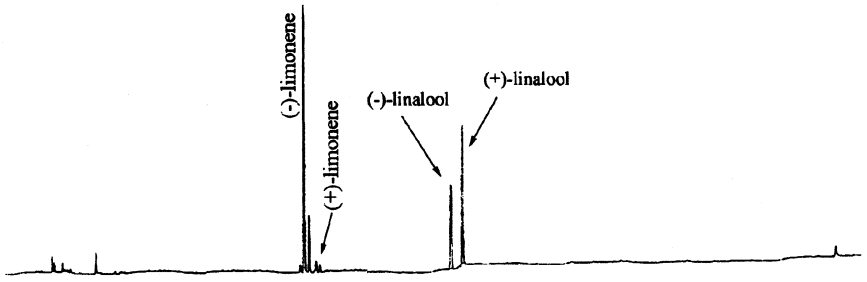

Figure 2. Chiral gas chromatogram of limonene and linalool of *Cymbopogon winterianus* oil on DiEtBuSilβ CDX (PS 086) column

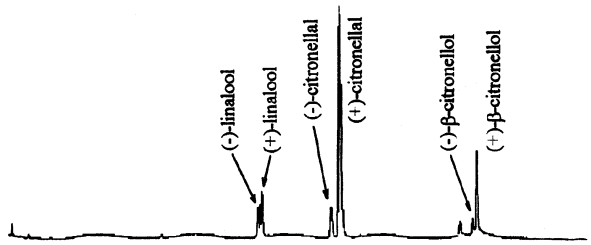

Figure 3. Chiral gas chromatogram of linalool, citronellal and β-citronellol of *Cymbopogon winterianus* oil on DiAcTBuSilβ CDX (OV 1701) column

analysis of selected optically active ingredients as a powerful tool to describe an essential oil.

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