The chemical composition and antimicrobial activity of the leaf oil of *Cupressus lusitanica* from Monteverde, Costa Rica

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ABSTRACT

The essential oils from the leaves of three different individuals of *Cupressus lusitanica* were obtained by hydrodistillation and analyzed by gas chromatography - mass spectrometry. A total of 49 compounds were identified in the leaf oils. The major components of *C. lusitanica* leaf oil were α-pinene (40%-82%), limonene (4%-18%), isobornyl acetate (up to 10%) and *cis*-muurola-4(14),5-diene (up to 7%). The essential oil was screened for antimicrobial activity, and it showed antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger*.

Key words: *cis*-muurola-4(14), 5-diene, α-pinene, antimicrobial, composition, *Cupressus lusitanica*, essential oil, isobornyl acetate, limonene

INTRODUCTION

There are 13 species of *Cupressus* (Cupressaceae) distributed throughout North America.[1] *Cupressus lusitanica* Mill, known in Costa Rica as *ciprés*, normally ranges from central Mexico to Honduras but has been cultivated in other parts of the world. In Monteverde, it has been planted as a windbreak to protect dairy cows from harsh winds.[2] The leaves of this plant are used to cure some skin diseases caused by dermatophytes and have also been used to ward off insects from stored grain.[3] In Costa Rica, a drink made by steeping a branch in alcohol is taken to alleviate coughs and cold symptoms.[4] Essential oil compositions of *C. lusitanica* from Portugal[5,6] and from Cameroon[3] have been reported, but these show wide variation. In this report, we present the leaf essential oil composition and antimicrobial activity of *C. lusitanica* from Monteverde, Costa Rica.

MATERIALS AND METHODS

Plant material

Leaves of *C. lusitanica* were collected from three different mature trees growing in Monteverde, Costa Rica (10.3059 N, 84.8144W, 1380 m above sea level), on May 9, 2009. The plant was identified by William Setzer.[2] The fresh leaves were chopped and hydrodistilled for 4 hours using a Likens-Nickerson hydrodistillation apparatus[7] with continuous extraction with $CHCl_3$ (50 mL). The chloroform extract was then evaporated to yield yellow essential oils [Table 1].

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Gas chromatographic-mass spectral analysis

A gas chromatographic-mass spectral analysis was performed on the essential oils of *C. lusitanica* using an Agilent 6890 GC with Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/s), and a fused silica capillary column (HP 5 ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The carrier gas was helium with a flow rate of 1 mL/min, and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 minutes at 40°C, then ramp to 200°C at 3°C/min and finally to 220°C at 2°C/min. The interface temperature was 280°C. A 1% w/v solution of each sample in $CHCl_3$ was prepared, and 1 μL was injected using a splitless injection technique. Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature,[8] and stored on the MS library [NIST database (G1036A revision D.01.00)/ChemStation data system

Table 1: Yields and chemical compositions of *Cupressus lusitanica* leaf oils

Collection		Tree A	Tree B	Tree C
Mass of fresh leaves (g)		121.0	144.7	132.0
Yield of leaf oil (mg)		379.7	859.6	368.2
Chemical composition RI	**Compound**		**% Composition**	
860	(3*Z*)-Hexenol	2.1	1.0	1.1
940	α-Pinene	82.3	39.9	60.0
953	Camphene	1.3	2.3	0.2
974	Sabinene	0.5	0.5	1.1
976	β-Pinene	0.4	1.5	1.6
992	Myrcene	1.6	2.9	2.7
1001	α-Phellandrene	-	tr	0.1
1013	δ-3-Carene	-	-	tr
1015	α-Terpinene	tr	0.2	1.3
1030	Limonene	4.2	17.6	8.4
1036	1,8-Cineole	tr	tr	tr
1043	2-Heptyl acetate	-	0.3	tr
1047	(*E*)-β-Ocimene	-	tr	tr
1058	γ-Terpinene	0.1	0.4	1.1
1088	Terpinolene	0.4	1.1	2.2
1094	2-Nonanone	-	0.6	tr
1105	Unidentified ($C_{10}H_{16}$)	-	1.8	0.1
1132	Unidentified	-	0.8	0.7
1146	Camphor	-	1.0	-
1149	Isopulegol	-	0.1	-
1162	Isoborneol	tr	0.1	-
1167	Borneol	tr	0.3	-
1173	Umbellulone	0.4	0.7	0.7
1177	Terpinen-4-ol	0.2	0.5	tr
1188	α-Terpineol	tr	0.1	0.2
1286	Isobornyl acetate	4.6	9.6	0.2
1351	α-Terpinyl acetate	0.4	0.8	0.7
1390	β-Elemene	-	tr	tr
1419	(*E*)-Caryophyllene	0.3	1.0	0.3
1449	*cis*-Muurola-3,5-diene	0.1	2.6	2.6
1453	α-Humulene	-	tr	tr
1468	*cis*-Muurola-4(14),5-diene	0.3	6.4	6.7
1483	Germacrene D		0.1	tr
1503	Epizonarene	tr	1.8	1.5
1513	α-Alaskene	tr	-	-
1516	β-Curcumene	0.3	0.3	0.2
1523	*trans*-Calamenene	-	tr	tr
1526	δ-Cadinene	-	0.4	0.6
1531	Zonarene	-	tr	tr
1549	*cis*-Muurol-5-en-4β-ol	-	0.1	0.2
1559	*cis*-Muurol-5-en-4α-ol	-	0.1	0.2
1583	Caryophyllene oxide	tr	tr	-
1618	1,10-di-*epi*-Cubenol	-	tr	0.2
1632	α-Acorenol	0.3	tr	tr
1635	β-Acorenol	tr	tr	tr
1642	τ-Cadinol	-	tr	0.2
1654	α-Cadinol	-	0.3	0.8
1987	Manool oxide	-	tr	-
2125	Nezukol	-	2.6	4.0
2278	*cis*-Totarol	-	-	tr
2303	*trans*-Totarol	-	tr	0.1
	Total Identified	99.8	97.2	99.0
	Monoterpene hydrocarbons	90.7	68.1	78.9
	Oxygenated monoterpenoids	5.6	13.3	1.8
	Sesquiterpene hydrocarbons	1.0	12.5	11.9
	Oxygenated sesquiterpenoids	0.3	0.6	1.5
	Diterpenoids	0.2	2.8	4.1
	Others	2.1	2.7	1.8

(G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical compositions of the *C. lusitanica* leaf oils are summarized in Table 1.

Antimicrobial screening

The essential oil was screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique.[9] Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 μL of 1% w/w solutions of crude extracts in DMSO plus 50 μL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony-forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hours; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Geneticin was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* (ATCC No. 90028) in yeast-mold (YM) broth with concentration of approximately 7.5×10^7 CFU/mL. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

RESULTS AND DISCUSSION

The hydrodistillation of the fresh leaves of *C. lusitanica* produced pale yellow essential oils in yields ranging from 0.28% to 0.58%. The main components in all three essential oils were monoterpene hydrocarbons, which included α-pinene (82.3%, 39.9% and 60.0%) and limonene (4.2%, 17.6% and 8.4%) as the major components. This is in contrast to essential oil compositions growing in Portugal (dominated by the diterpene abietadiene, 11%-24%)[6] or Cameroon (composed principally of umbellulone, 17%-18%).[3] It is however qualitatively similar to the oil reported by Carmo and Frazão[5] (18.0%, α-pinene; 13.2%, β-pinene + sabinene), but these workers had only identified 79% of the composition. *C. lusitanica* leaf oil (combined samples) was screened for antimicrobial activity against *Bacillus cereus* (MIC = 78 μg/mL), *Staphylococcus aureus* (MIC = 625 μg/mL), *Escherichia coli* (MIC = 1250 μg/mL), *Pseudomonas aeruginosa* (MIC = 1250 μg/mL), *Candida albicans* (MIC = 625 μg/mL) and *Aspergillus niger* (MIC = 78 μg/mL). Thus, *C. lusitanica* leaf oil showed appreciable activity against the Gram-positive bacterium *B. cereus* and the mold *A. niger* only.

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