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Essential Oil of *Cymbopogon winterianus* Jowitt from Tanzania: Composition and Antimicrobial Activity

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Abstract: The hydro-distilled essential oil (1.6%) of fresh aerial parts of wild *Cymbopogon winterianus* Jowitt was analyzed by GC-MS. Fifty compounds representing 96.5% of the oil were identified. The main components of the oil were linalool (27.4%), citronellol (10.9%), geraniol (8.5%), α-calacorene, *cis*-calamenene (4.3%), β-elemene (3.9%) and longifolene (3.5%). The oil exhibited low antimicrobial activity.

Key Words: *Cymbopogon winterianus*, essential oil composition, linalool, citronellol, geraniol, antimicrobial activity.

Introduction: *Cymbopogon winterianus* Jowitt (*Andropogon nardus java* de Jong) is a grass (Gramínea family) that is cultivated because it yields citronella oil, whose main constituents are citronellal and geraniol. It is referred to as 'mahapengiri' and it is closely related to *Cymbopogon nardus* Rendle (*Andropogon nardus ceylon* de Jong) which is called 'lenabatu'. The two species can be distinguished morphologically because *C. winterianus* usually has shorter and broader leaves than *C. nardus*. The former gives a

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higher yield of oil and the oil is of better quality than that of *C. nardus*, since it has a higher geraniol and citronellal content. However, *C. winterianus* requires more fertile soil, more rainfall and care then the hardier *C. nardus*. The former grows mainly in the islands of Java and Formosa, Guatemala, Honduras and Haiti, and hence the name Java oil. The latter, called Ceylon oil, is producced almost exclusively on the Island of Ceylon (Sri Lanka). Java oil is used as a starting material for extraction of important isolates like geraniol and citronellal, which can be converted to useful aromatics like citronellol, hydroxycitronellal, synthetic menthol and esters of geraniol and citronellol[1,2]. Various studies have shown that citronella oil has antifungal[3,4] and mosquito repellant[5] activity.

In this study, we report, for the first time the composition of the citronella oil from Tanzania and its antimicrobial activity.

Experimental

Plant material and oil isolation: Leaves of wild *Cymbopogon winterianus* Jowitt were collected in 1998 from Mombo, at the slopes of Usambara mountains in Tanzania. Voucher specimens were deposited in the Herbarium of Institute of Traditional Medicine, Muhimbili University College of Health Sciences, University of Dar es Salaam. Fresh leaves were hydro-distilled to yield 1.6 % oil which was dried over anhydrous sodium sulphate and stored at 4°C until analysis.

Gas Chromatography: The essential oils were analyzed with a Shimadzu GC-R1A gas chromatograph equipped with a fused silica column (30 m x 0.25 mm) coated with DB-5. The temperature of the column was programmed from 60°C to 240°C at 4°C/min. The injector and detector temperatures were at 250°C. The carrier gas was helium, at a flow rate of 1 ml/min. Peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas obtained, without FID response factor correction. Programmed temperature retention indices of the compounds were determined relative to n-alkanes[6].

Gas Chromatography-Mass Spectrometry: GC-MS analyses were performed on a Perkin Elmer Q-910 using a 30 m x 0.25 mm capillary column coated with DB-5. The temperature of the column and the injector were the same as those from GC. The carrier gas was helium, at a flow rate of 1 ml/min. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices, mass spectra with those of authentic samples, by peak enhancement, with published data[7], mass spectra library of National Institute of Standards and Technology (NIST 3.0) and the mass spectra library which contains references mass spectra and retention indices of volatile compounds.

Antimicrobial assay: A collection of 8 bacteria were used, including gram-positive bacteria *Bacillus cereus* (from rice), *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25212), and *Staphylococcus epidermidis* (from cow milk), Gram-negative strains *Escherichia coli* (from water) *Klebsiella* spp. (from bird food) and *Proteus mirabilis* (from human urine). All the samples of microorganisms were characterized at the Department of Microbiology, National University

of Rio Cuarto, Argentina and voucher specimens were preserved. All the strains tested were maintained at 4°C in Tryptone Soy Agar and were subcultured every month. The fungus (*Candida albicans*) was stored at the same temperature as bacteria in Sabouraud Agar and subcultured every month. The paper disc diffusion method was used to test antibacterial activity. It was performed using an 18 h culture, growth at 37°C and adjusted to approximately 10^6 cfu/ml. The inoculum (200 l) was spread over plates containing Mueller-Hinton Agar and a paper filter disc (4 mm) impregnated with 10 µl of the essential oil was placed on the surface of the media. A gentamycin disc (Brittania Co.) containing 10 µg was used as a positive control. The plates were left 30 min at room temperature to allow the diffusion of the oil, then they were incubated at 37°C for 24 h. After this time the inhibition zone around the disc was measured with a calliper. Antifungal experiments were made in the same way using Extracto de Malta Broth for the culture and Sabouraud Agar for the plates, and using amphotericin as the positive control. The zones of inhibition were measured in mm, with the disc diameter of 4 mm being included.

Results and discussion: Fifty constituents, representing 96.5% of *C. winterianus* leaf oil (yield 1.6%) were identified by GC and GC-MS. As can be seen from the results in table 1, the oil is rich in linalool (27.4%), citronellol (10.9%), geraniol (8.5%), α-calacorene (6.0%), *cis*-calamenene (4.3%), β-elemene (3.9%) and longifolene (3.5%). Cultivated citronella oil normally contains citronellal and geraniol as the main constituents[1,2]. The quality of the oil may vary depending on the soil. Manure or compost increases both herbage and oil yield[8], while presence of cadmium in the soil reduces both the herbage and oil quality[9]. Disease may also significantly affect herbage, yield and oil composition. In one study, plants with yellowing and crinkling disease had geraniol, elemol, citronellal and citronellol as the major constituents[9]. Plants suffering from iron chlorosis had lower herbage and yields. The plant under study was obtained from the wild and the many variables may contribute to the high linalool content. The oil zones of inhibition against *Bacillus cereus, Micrococcus luteus* and *Staphylococococcus aureus* were 7.5, 7.3 and 6.0 mm, compared to the gentamicin zones of inhibition of 25, 20 and 15, respectively. The oil had no activity against the bacteria *Escherichia coli, Enterococcus faecalis, Klebsiella spp*., *Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus epidermidis* as well as the fungus *Candida albicans*. The variation in the oil constituents may be responsible for the low antimicrobial activity.

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

Compounds	%	DB-5 RI[a]	Supelcowax-10 RI[b]	Methods of identification
Triclyclene	0.1	926	-	MS
α-Thujene	0.1	931	-	MS
α-Fenchene	0.3	953	-	MS
Sabinene	0.3	976	-	MS
Myrcene	0.4	991	-	MS
O-Cymene	0.1	1022	-	MS
Limonene	2.3	1031	1193	MS-CO
β-Phellandrene	1.1	1031	1203	MS-CO
1,8-Cineole	1.3	1033	-	MS-CO
(Z)-β-Ocimene	0.1	1040	-	MS
cis-Linalool oxide (furanoid)	0.1	1074	-	MS
Terpinolene	0.1	1088	-	MS
Linalool	27.4	1098	-	MS
α-Thujone	tr	1102	-	MS
β-Thujone	tr	1114	-	MS
cis-Limonene oxide	tr	1134	-	MS
Camphor	1.3	1143	-	MS-CO
Borneol	2.6	1165	-	MS-CO
α-Terpineol	1.3	1189	-	MS-CO
Citronellol	10.9	1229	-	MS-CO

table 1. (continued)

Compounds	%	DB-5 RI[a]	Supelcowax-10 RI[b]	Methods of identification
Geraniol	8.5	1256	-	MS-CO
Geranial	0.9	1270	-	MS-CO
Bornyl acetate	0.8	1285	-	MS
δ-Elemene	0.1	1339	-	MS
α-Cubebene	tr	1351	-	MS
Citronellyl acetate	0.6	1354	-	MS
α-Ylangene	0.4	1373	-	MS
Geranyl acetate	tr	1383	-	MS
β-Bourbonene	0.4	1385	-	MS
β-Cubebene	0.3	1390	-	MS
β-Elemene	3.9	1394	-	MS
α-Gurjunene	1.2	1403	-	MS
Longifolene	3.5	1404	-	MS
β-Caryophyllene	tr	1418	-	MS-CO
α-Humulene	0.3	1454	-	MS
β-Chamigrene	1.2	1482	-	MS
γ-Cadinene	0.3	1513	-	MS
cis-Calamenene	4.3	1521	-	MS
δ-Cadinene	3.4	1524	-	MS
α-Selinene	0.5	1531	-	MS
α-Calacorene	6.0	1542	-	MS
β-Calacorene	1.5	1556	-	MS
Elemol	2.6	1549	-	MS
Spathulenol	2.1	1576	-	MS-CO
Caryophylene oxide	1.5	1581	-	MS
γ-Eudesmol	1.4	1625	-	MS
Cadinol tau	1.6	1640	2155	-
α-Muurolol (torreyol)	1.8	1645	2171	-
β-Eudesmol	1.2	1649	-	MS
α-Eudesmol	2.3	1652	-	MS
α-Cadinol	1.4	1653	2212	MS
Total	96.5			

a: Compounds are listed in order of their elution from a DB-5 column.
b: Further confirmation for some compounds by their elution from a Supelcowax 10 column.
CO: peak identifications based on coinjection.
MS: peak identifications based on MS comparison with file spectra.