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Constituents of the Essential Oil of *Cymbopogon afronardus* Stapf.

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The pleasantly smelling light yellow essential oil isolated by hydrodistillation from the leaves of *Cymbopogon afronardus* Stapf. (Graminea) (yield 0.4 %) was analysed by GC and GC-MS. Forty five compounds constituting about 95.9 % of the oil were identified. The major constituents were 5-epiparadisol and intermedeol (40.9 % together), 6S:7R bisabolone (39.5 %) and 6R:7R-bisabolone (4.4 %). The essential oil of *C. afronardus* was significantly different from that of *C. nardus* (L.) Rendle. The antimicrobial activity of the oil is also reported.

Key Words: *Cymbopogon afronardus* Stapf., Graminea, essential oil, GC-MS, 5-epiparadisol, intermedeol, bisabolone, antimicrobial activity.

INTRODUCTION

Cymbopogon afronardus Stapf. (Graminea) [Syn. *C. nardus* (L.) Rendle, *C. confertiflorus* (Steud) Stapf., *C. validus* (Stapf.) Burtt-Davy, *C. validus* (Stapf.) Burtt-Davy var *lysocladus*, *C. claessensii* Robyns, *C. prolixus* (Stapf) Phillips, *C. nardus* var *confertiflorus* (Steud) Bor, *Andropogon nardus, A. confertiflorus* Steud, *A. nardus* L. var *prolixus* Stapf., *A. nardus* L. var *validus* Stapf.] is a tufted perennial 75-300 cm high with persistent basal sheaths. Leaf-blades mostly flat, 20-80 cm long, 3-20 mm wide; ligule 3-9 mm long. The false panicle is 15-60 cm in length; racemes 10-20 mm long with the lowest internode and not swollen. Lower glume of sessile spikelet slightly convex to shallowly concave between sharp keels; the pedicelled spikelet lanceolate 3.5-7 mm long with 3-7 intercarinal nerves[1,2]. The tough and almost unpalatable grass in pasture areas is widely spread in deciduous bushland and upland grassland in Kenya, Uganda and Tanzania, and from Sudan to South Africa. It has an agreeable aroma on crushing.

There is scanty information on essential oils from wild Kenyan Graminea family. However, oil from *C. afronardus* has been mentioned in old literature as containing geraniol as the major constituent[3,4,5]. The odour of the oil was also said to resemble that of palmarosa.

In our continuing research of Kenyan flora containing essential oils, we report on the analysis of *C. afronardus* oil using gas chromatography and gas chromatography-mass spectrometry. Antibacterial activity of the oil was also investigated.

EXPERIMENTAL

Plant Material

The leaves of Cymbopogon *afronardus* were collected at Kianjege West, Kirinyaga District, Kenya in July 1997. The identity of the plant was established at the Botany Department, Egerton University. Voucher specimens were deposited at the same and at Department of Pharmacology and

Pharmacognosy, University of Nairobi. The plant was also introduced into cultivation in the botanical garden at the Faculty of Pharmacy, University of Nairobi.

Essential oil isolation

The semi-dried leaves were subjected to hydrodistillation for 3 hours in a Clevenger-like apparatus to give the essential oil (0.4 %). The oil was dried over anhydrous sodium sulphate and stored at 4 °C.

Gas chromatography

Gas chromatography was performed on the following:

i) DEISI 121 chromatograph equipped with a CPWAX 52 CB capillary column (25 m x 0.3 mm; df 0.5 micrometre) and a flame ionisation detector. Carrier gas nitrogen , column output flow rate: 1 ml/min, split ratio: 1/60, pressure: 0.8 bar, flammable gas: hydrogen, flow rate 30 ml/min under 1 bar pressure, oxidizing gas: compressed air, flow 3000 ml/min under 1 bar pressure, temperatures: injector 240 °C, detector: 250 °C, oven temperature programming: isothermal step at 50 °C for 5 min, then gradient of 2 °C/min for 85 min upto final temperature 220 °C, injected volume: 0.05microlitre of oil.

ii) A Shimadzu GC-R1A (FID) gas chromatograph fitted with a 30 m x 0.25 mm (0.25 µm film thickness) fused silica capillary column coated with a DB-5 (J&W). The GC operating conditions were as follows: oven temperature programmed from 40 ^{0}C to 230 ^{0}C at 2 ^{0}C/min, injector and detector temperatures 240 ^{0}C, carrier gas was nitrogen at a constant flow at 0.9 ml/min. Identification of the components was performed by comparison of their retention times with those of pure authentic samples.

Gas chromatography-Mass Spectrometry

GC-MS was done using:

i) GC-MS was done using a Hewlett Packard 5970 quadrupole mass selective detector with electron impact ionization (70 eV), coupled to a sigma 300 chromatograph equipped with a CPWAX 51 CB column (50 m x 0.3 mm; df 0.15 µm). The operating conditions were as above; the final temperature was 250 °C for 5 min.

ii) Analysis was also performed with a Perkin Elmer Q-700 equipped with a SE-30 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm film). Analytical conditions; oven temperature from 40 ^{0}C to 230 ^{0}C at 2 ^{0}C/min, carrier gas helium at a constant flow at 0.9 ml-min, source 70 eV. The oil components were identified by two computer library MS searches using retention indices as a preselection routine, and visual inspection of the mass spectra from literature for confirmation [6,7].

Antimicrobial activity

A collection of 8 microorganisms were used: *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Klebsiella spp, Micrococcus luteus (ATCC 9341)* and *Enterococcus faecalis (ATCC 29212)*. All the sample of microorganisms non ATCC, were characterized at the Department of Microbiology, National University of Rio Cuarto, Argentina and specimens were preserved. All the strains tested were maintained at 4°C in Tryptone-Soya Agar and were subcultured every month. Two different techniques were used to test antibacterial activity: the paper disc diffusion method was used for preliminary screening and the well diffusion one for quantitative determination. Both were performed using an 18 hours culture, grown at 37 °C and adjusted to

approximately 10^6 cfu/ml. 200µl of the inoculum were spread over plates containing Mueller-Hinton Agar and a paper filter disc (6 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 (the minimum inhibitory concentration used for microorganisms sampled from the hospital) was used as the reference. The plates were left for 30 minutes at room temperature to allow the diffusion of the oil, then they were incubated at 37°C during 24 hours. After this time the inhibition zone around the disc was measured with a clipper. The well diffusion method was carried out with 4 mm diameter wells in the agar and filling with 50 µl of the essential oil. The inhibition zone diameter included the well diameter. A solution of gentamycin (10 µg /50 µl) was used as a reference.

RESULTS AND DISCUSSION

The chemical composition of the oil is shown in table 1. Both GC-MS methods gave the same profile. A total of 45 compounds accounting for 95.9 % of the oil were identified. The most abundant compounds were the structurally related sesquiterpene alcohols (5-epiparadisol and intermedeol-40.9 % together) and sesquiterpene ketones (6S:7R bisabolone, 39.5 % and 6R:7R-bisabolone, 4.4 %).

The plant under study was identified as *C. nardus* at both National Herbarium of Kenya and Botanical Gardens at Kew, but as *C. afronardus* at Botany Department of Egerton University, Kenya. Its chemical composition was clearly different from that of previous work on *C. nardus*, which contains mainly geraniol, citronellol, citronellal, cis/trans –rose oxides, linalol and terpinen-4-ol [5,8,9].

Table 1. Chemical constituents of the essential oil from the leaves of *Cymbopogon afronardus*

Component	%	Component	%
myrcene	0.3	geraniol	0.3
limonene	t	muurol-4-ene-α-ol	0.2
decanal	t	muurol-4-ene-β-ol	0.3
β-bourbolene	0.1	α-caryophyllene oxide	0.2
heptylpropylcetone	t	germacrene-D-4-ol	0.7
linalol	1.3	1,10-diepicubenol	0.2
octanol	t	1-epicubenol	0.2
trans-α -bergamotene, β-elemene	0.7	sesquiphellandrene hydrate	1.3
		10-epieudesmol	0.2
β-caryophyllene	T	neointermedeol	t
(Z)-β-farnesene	0.1	γ-eudesmol	0.1
α-humelene	t	δ-cadinol	1.0
β-chamigrene	0.8	sesquiphellandrene hydrate	0.2
germacrene-D	0.2	α-epi-T-muurolol	2.7
β-selinene	t	α-muurolol	0.5
α-muurolene, bicyclogermacrene	0.3	α-bisabolol, epi-α-bisabolol	0.2
δ-cadinene	1.7	5-epiparadisol, intermedeol	40.9
γ_2-cadinene	t		
7-epi-α-selinene	0.2	6R 7R bisabolone	4.4
β-sesquiphellandrene	t	6S 7R bisabolone	39.5
α-cadinene	t	hydroxycadalene	t
calamene	0.1		

t = trace (<0.05 %)

A recent literature search indicates that previous work on *C. nardus* oil constitution is different from the one obtained in this study.

The essential oil content of *C. afronardus* has been reported to range from trace to 1% and

Table 2. Antibacterial activity of the essential oil of *Cymbopogon nardus* leaves

Organism	Zones of inhibition (mm)	
	Essential oil	Gentamycin (10μg)
Bacillus cereus	9.5	25
Staphylococcus aureus(ATCC 25212)	7.0	15
Staphylococcus epidermidis	7.0	30
Proteus mirabilis	8.0	23
Escherichia coli	NI	18
Klebsiella spp	NI	22
Micrococcus luteus (ATCC 9341)	11.0	20
Enterococcus faecalis (ATCC 29212)	7.0	13

NI: No inhibition

contained alcohol (calculated as geraniol-84.2% by bisulfite method), esters (calculated as geranyl acetate-8.2%) and aldehydes and ketones (3%) [4,5]. The leaf oil of '*C. validus (C. afronardus*)' from Zimbabwe has also been found to contain myrcene (15-20%), ocimene (9-14%), α-pinene(4-6%), camphene(4-6%), linalool (3-4%), gemacrenes (10%), cadinenes (25%) and murolenes (8%)[6]. These findings also different in composition from the present work.

In describing '*C. nardus* (Syn. *C. afronardus* Stapf)' Clayton and Renvoize [1] state that a 'tendency to fewer nerves in the glume of the pedicelled spikelet among the East African specimens has formed the basis of a segregate species (*C. afronardus*), but the variable occurrence of shorter intermediate nerves robs the character of all precision'. It seems they were dealing with two species with very minor differences and therefore concluded that the species was *C. nardus*. The results of the present study indicate that *C. nardus* and *C. afronardus* are two different species and that this can form a basis for chemotaxonomy. The present work also indicates that there is need to re-evaluate the taxonomy of these two species in particular and the whole *Cymbopogon* genus in general.

The neat oil was assessed for antimicrobial activity against 8 bacteria organisms. Gentamycin was used as a reference compound. The results are shown in table 2. Comparative antimicrobial activity against 10 μg gentamycin ranged from 0 % for *E. coli* and *Klebsiella* to 55% for *M. luteus*.

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