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Composition and Antimicrobial Activity of *Cymbopogon giganteus* (Hochst.) Chiov. Essential Flower, Leaf and Stem Oils from Cameroon†

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Abstract

The essential oils of fresh flowers (2 samples), leaves and stems of *Cymbopogon giganteus* (Hochst.) Chiovenda from Cameroon were investigated by GC and GC/MS. More than 55 components have been identified in the samples 1 (flowers 1), 2 (leaves), 3 (stems) and 4 (flowers 2) with main compounds possessing the p-menthadiene skeleton as follows: *cis-p*-mentha-1(7),8-dien-2-ol (1: 22.8%, 2: 27.7%, 3: 29.1%, 4: 20.5%), *trans*-p-mentha-1(7),8-dien-2-ol (1: 24.9%, 2: 21.6%, 3: 28.1%, 4: 26.5%), *trans*-p-mentha-2,8-dien-1-ol (1: 17.3%, 2: 22.1%, 3: 21.4%, 4: 16.3%) and *cis*-p-mentha-2,8-dien-1-ol (1: 8.3%, 2: 5.4%, 3: 4.6%, 4: 9.7%). Additional components in higher concentrations, responsible for the characteristic aroma impressions of these samples are especially limonene, *trans*-verbenol and carvone as well as some other mono- and sesquiterpenes. Antimicrobial activities of the four oils were found against Gram-(+)- and Gram-(-)-bacteria as well as the yeast *Candida albicans*, and these results were discussed with the compositions of each sample.

Key Word Index

Cymbopogon giganteus, Poaceae, essential oil compositions, *cis*-p-mentha-1(7),8-dien-2-ol, *trans*-p-mentha-1(7),8-dien-2-ol, *trans*-p-mentha-2,8-dien-1-ol, antimicrobial activity.

Plant Name

Cymbopogon giganteus (Hochst.) Chiovenda syn. *Andropogon giganteus* Hochst., Poaceae.

Source

Fresh plants of *C. giganteus* were collected at the Waza-Park near Mora-City, North-Province, Cameroon, during February 2005 (dry season, morning-time). The plants were identified by botanists of the University of Yaounde (Cameroon) and a voucher specimen (no. 13971 SFRCAM) has been deposited in the specially maintained Herbarium of Yaounde.

Plant Part

Fresh flowers, leaves and stems were hydrodistilled for 4 h using a Clevenger-type apparatus. The flower sample was separated into two parts. The oils of leaves, stems and one part of the flowers were obtained by decantation of water and dried by filtration over anhydrous sodium sulfate. Oil yields were 0.4% for leaves (sample 2), 0.01% for stems (sample 3) and 1.4% for flowers (sample 1). The second part of the collected flower distillate was extracted with diethyl ether (2 x 100 mL). The ether extract was dried over anhydrous sodium sulphate. After evaporation of the ether the yield of the flower oil (sample 4) was 1.1%.

Previous Work

Cymbopogon giganteus (Hochst.) Chiov. syn. *Andropogon giganteus* Hochst. (common names: Tsauri grass; Cameroon: wadjalo; French: beignefata; German: Großes Lemongrass (1), Poaceae) is growing in the Asian and African tropical Savannahs (2). *Cymbopogon giganteus* is a loosely tufted perennial herb, the basal sheaths soon falling away; culms are robust and 1–3m high, are erect, sometime supported by stilt roots at the base;

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leaf blades are linear to narrowly lanceolate, 15–60cm long and 8–30cm wide, smooth, mostly dark green, firm. False panicles are linear 20–70cm and racemes are 10–15mm long; the lower most inter node and pedicel are connate (3). *Cymbopogon giganteus* plant parts are used in local African medicine for various treatments, such as against rheumatism, fever, cough, skin disorders (decoctions of leaves and flowers) and arterial hypertension (2,4). Some antimalarial activities have also been studied in animal experiments (5). The essential oils of this as well as of other *Cymbopogon* species (e.g. the more common *C. citratus*) are important aromatic substances, used for food flavoring (1) and perfumery/cosmetic products (6), and also show antioxidative and antiradical (6) as well as anti-inflammatory and analgesic properties (7). Analyses of the oil composition of *C. giganteus* samples from Africa, such as Burkina Faso, Ivory Coast and Mali, showed p-menthadienols as main constituents (2,4,6–8). Only few papers discuss the composition of oils of *C. giganteus* from Cameroon (9). To the best of our knowledge, no information is available on the oil composition of different plant parts of *C. giganteus* from Cameroon and their antimicrobial activities against various microorganisms.

Present Work

The essential oils of *C. giganteus* from Cameroon were described as being intense fresh, green-herbal, spicy and weak floral (leaves); fresh-green, floral and weak herbal (flowers); fresh-spicy, weak floral-fruity and herbal-musty in the background (stems) as well as fresh-floral, green-herbal and weak spicy (flowers, ether-sample). The oil composition was analyzed by a combination of GC and GC/MS. GC analysis of the oil was carried out on a Shimadzu GC-14A (FID) and a Varian GC-3700 (FID) gas chromatograph fitted with a 30 m x 0.32 mm (film thickness: 0.25 µm) chemically bonded apolar FSOT-RSL-200 (Biorad) and with a 30 m x 0.32 mm (film thickness: 0.50 µm) Stabilwax (Restek) fused silica column, respectively. The sample was injected by splitter using H as carrier gas. The column temperature was programmed from 40°C (5 min) to 280°C (20 min) at 6°C/min. The compound identification was partly possible by coinjection of pure compounds and correlation with published retention-time data (10–13). GC/MS analysis was carried out on a Shimadzu GC-17A/QP5000, on a HP-5890GC/HP-5970MSD and on a Finnigan MAT GCQ (carrier gas: He, EI mode, 70 eV, scan-range: 40–450 amu and ion-source temperature 200°C each, columns see GC-part) equipped with Wiley/NBS- and NIST libraries. For additional mass spectral correlations, published data (10,12–14) were used.

By means of these combinations more than 55 constituents of the oils of the flowers (sample 1 and 4), leaves (sample 2) and stems (sample 3) from *C. giganteus* from Cameroon could be identified. The significant main compounds (concentration calculated as relative %-peak area of GC-FID analysis, apolar column) were *cis*-p-mentha-1(7),8-dien-2-ol (1: 22.8%, 2: 27.7%, 3: 29.1%, 4: 20.5%), *trans*-p-mentha-1(7),8-dien-2-ol (1: 24.9%, 2: 21.6%, 3: 28.1%, 4: 26.5%), *trans*-p-mentha-2,8-dien-1-ol (1: 17.3%, 2: 22.1%, 3: 21.4%, 4: 16.3%), *cis*-p-mentha-2,8-dien-1-ol (1: 8.3%, 2: 5.4%, 3: 4.6%, 4: 9.7%), limonene (1: 5.2%, 2: 4.9%, 3: 1.3%, 4: 7.4%) and *trans*-carveol (1: 4.8%, 2: 2.9%, 3: 2.6%, 4: 1.9%). Further identified constituents of *C. giganteus* were mainly mono- and sesquiterpenes.

The compounds identified in the oils of *C. giganteus* are listed in order of elution (retention indices = RI, calculated using a mixture of a homologous alkane series from n-hexane up to n-hexadecane) from an apolar FSOT-RSL (DB-5 like) column and the percentage calculated by relative %-peak-area calculations of GC analysis (see Table I.).

Comparing the compositions of the different oils of this *Cymbopogon* species from Cameroon with sensory data published elsewhere (15–19), it is to assume that the fresh-green-herbal aroma can be correlated to the p-menthadienols, p-menthenols, verbenols, camphor and some further mono- and sesquiterpenes. Floral-fruity odor-notes are known from limonene, terpinen-4-ol, perillaldehyde and perillyl alcohol. Herbal-spicy odor attributes can be given to carveols, carvone, thymol, β-caryophyllene and germacrene D.

For antimicrobial testings, the oils of *C. giganteus* and the reference compounds, eugenol (Sigma-Aldrich, Vienna, Austria; W24,670-0), Lidaprim^{R}-infusion-bottle (Nycomed, Vienna, Austria, 250mg containing 0.8g sulfametrol and 0.16g trimethoprim) and tetracycline hydrochloride (achromycine hydrochloride . 25g, T3383-25G) were prepared as 20% solutions of ethanol and dissolved in a 0.9% NaCl solution (ratio of 1:10). As test microorganisms (colony-forming-units=cfu/cm^3), Gram-(+)-bacteria *Staphylococcus aureus* ATCC 6538P ($1x10^{13}$) and *Enterococcus faecalis* (clinically isolated, $1x10^{13}$); Gram-(-)-bacteria *Escherichia coli* ATCC 8739 ($2x10^{12}$), *Pseudomonas aeruginosa* G 28 ($1.2x10^{9}$), *Klebsiella pneumoniae* (clinically isolated, $1x10^{13}$), *Proteus vulgaris* (clinical isolated, $3x10^{13}$) and *Salmonella* sp. (clinically isolated, $3x10^{12}$) as well as the yeast *Candida albicans* ATCC 10231 ($1x10^{11}$) – all products from the National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria – were used.

The antimicrobial activity was studied by two methods in accordance to previous studies (20–22): Agar diffusion disc method using 6 mm paper discs and quantities of 6 µL of the sample. After cultivation of the bacteria and the yeast at 37°C for 24 h, the diameter of the inhibition zone (IZ) was measured. Agar serial tube dilution method with results as minimum inhibitory concentration (MIC) as follows: The pure oils and reference compounds were separately added to brine, containing 1.0% (v/v) Tween 80 at the appropriate volumes to produce final concentrations of the samples in the range of 100–1.000 ppm; the Petri dishes were inoculated by pipetting 0.1cm^3 of the desired culture and 0.6 µL of the samples as well as the reference compounds (the tablettes of the additional tested synthetic antibioticum Ciproxin^{R} (Bayer Co., Vienna, Austria, 500mg-tabletes with 1 tablete = 582mg ciprofloxacine hydrochloride / water) were added as solution in brine at a quantity of 300 µg) on paper discs (6 mm) and then incubated at 37°C for 24 h.

The four oils of *C. giganteus* from Cameroon showed medium to high antimicrobial activities against all strains of microorganisms using both agar methods (see Table II). The p-menthadienols, as main compounds in all of the oils with a total amount of 73.3% (sample 1), 76.8% (2), 83.2% (3) and 73.0% (4) as well as the monoterpene hydrocarbon limonene may be responsible for these significantly high antimicrobial

Table I. Percentage composition of the essential oils of different plant parts of *Cymbopogon giganteus* from Cameroon

Compound	RI	flower oil (1)	leaf oil	stem oil	flower oil (2)
(*E*)-3-hexenol	847	0.1	0.1	0.1	0.1
(*Z*)-3-hexenol	855	0.1	0.5	0.1	0.1
(*E*)-2-hexenal	857	nd	0.2	0.1	nd
(*Z*)-2-hexenol	860	nd	0.1	0.1	nd
(*E*)-2-hexenol	862	nd	0.2	nd	nd
hexanol	865	0.2	0.1	nd	0.1
α-thujene	933	0.2	0.1	0.1	0.1
heptanol	967	nd	nd	0.2	nd
sabinene	974	0.2	0.1	0.1	0.3
1-octen-3-ol	978	nd	0.2	0.2	nd
p-cymene	1024	0.2	0.1	0.1	0.3
limonene	1028	5.2	4.9	1.3	7.4
benzyl alcohol	1031	0.1	nd	0.1	0.1
(*E*)-β-ocimene	1048	nd	0.1	0.1	nd
p-cymenene	1077	0.2	0.1	nd	0.1
cis-p-menth-2-en-1-ol	1086	0.3	0.1	nd	0.2
linalool	1096	0.2	0.1	nd	0.1
trans-p-mentha-2,8-dien-1-ol	1109	17.3	22.1	21.4	16.3
trans-p-menth-2-en-1-ol	1111	0.4	0.1	nd	0.2
cis-p-mentha-2,8-dien-1-ol	1122	8.3	5.4	4.6	9.7
trans-limonene oxide	1129	0.2	0.1	nd	0.5
camphor	1135	0.6	0.5	0.1	0.5
trans-verbenol	1141	1.4	1.7	1.4	1.8
4-isopropenylcyclohex-2-enone*	1143	0.1	nd	nd	0.1
p-methylacetophenone	1151	0.3	0.9	0.6	0.4
cis-verbenol	1164	0.1	0.2	0.3	0.1
nonanol	1167	0.1	0.1	1.6	0.1
3,9-epoxy-mentha-1,8(9)-diene	1173	0.7	0.3	0.1	0.9
trans-p-mentha-1(7),8-dien-2-ol	1176	24.9	21.6	28.1	26.5
terpinen-4-ol	1180	0.4	0.1	0.3	0.6
α-terpineol	1183	0.2	nd	nd	0.4
isopulegone*	1185	1.3	0.1	0.3	1.1
isopiperitenol I	1188	1.8	2.2	1.2	1.9
p-menthen-9-al*	1193	0.4	0.2	0.1	0.3
isopiperitenol II	1202	0.9	0.7	0.3	1.0
verbenone	1206	0.2	0.1	0.2	0.3
trans-carveol	1209	4.8	2.9	2.6	1.9
cis-p-mentha-1(7),8-dien-2-ol	1213	22.8	27.7	29.1	20.5
cis-carveol	1221	0.2	0.1	0.2	0.5
carvone	1230	1.8	0.9	0.4	1.4
piperitone	1243	0.1	0.2	nd	0.2
isoamyl hexanoate	1258	0.5	0.8	0.3	0.3
carvone oxide	1262	0.2	0.1	nd	0.3
thymol	1269	0.3	0.1	0.7	0.8
isopiperitenone	1275	0.3	0.2	0.1	0.3
perillaldehyde	1279	0.7	0.2	0.1	0.6
2-undecanone	1294	nd	0.1	0.9	0.1
perillyl alcohol	1297	0.3	0.2	0.1	0.2
eugenol	1358	nd	0.1	0.4	0.1
isopulegone oxide*	1366	nd	0.1	0.1	nd
β-caryophyllene	1435	0.2	0.8	0.1	0.1
α-humulene	1454	0.1	0.5	nd	nd
isoamyl octanoate	1458	nd	0.2	0.5	nd
germacrene D	1483	0.2	0.1	nd	0.2
2-tridecanone	1495	nd	0.1	0.5	nd
spathulenol	1575	nd	0.1	nd	nd
caryophyllene oxide	1582	0.1	0.3	nd	0.1
(*E*)-nerolidol	1661	0.1	0.1	nd	0.1
(*E*,*E*)-farnesol	1724	0.1	0.2	nd	0.1

nd not detected;*correct isomer not identified

Table II. Antimicrobial activities of essential oils of *C. giganteus* as well as reference compounds (- no inhibition observed)

Inhibition Zones (IZ) in mm and Minimum Inhibitory Concentrations (MIC) in ppm of test-microorganisms										
Compounds	***Staphylococcus aureus/ Enterococcus faecalis***		***Escherichia coli/ Proteus vulgaris***		***Pseudomonas aeruginosa/ Salmonella sp.***		***Klebsiella pneumoniae***		***Candida albicans***	
	IZ	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**
Leaf oil (sample 2)	12 / 12	60 / 60	15 / 12	60 / 60	9 / 14	60 / 60	11	60	15	60
Stem oil (sample 3)	12 / 8	60 / 600	12 / 12	60 / 60	8 / 12	60 / 60	10	60	15	60
Flower oil 1 (sample 1)	15 / 20	60 / 60	20 / 15	60 / 60	8 / 20	600 / 60	15	60	20	6
Flower oil 2 (sample 4)	10 / 8	60 / 600	10 / 10	60 / 60	9 / 12	60 / 60	10	60	20	60
Eugenol	30 / 30	600 / 600	28 / 28	600 / 600	25 / 25	600 / 600	28	600	32	600
Ciproxin^R	35 / 33	600 / 600	22 / 25	600 / 600	32 / 10	600 / 600	25	600	-	-
Lidaprim^R	27 / 27	600 / 600	11 / 23	60 / 600	- / 8	- / 60	-	-	-	-
Tetracycline hydrochloride	15 / 22	600 / 600	11 / 13	600 / 600	15 / 10	600 / 600	20	600	-	-

effects (see also the correlation with natural and synthetic reference compounds). While antimicrobial testings with limonene as single compound showed similar activities in a previous study (22), pure p-menthadienols must be tested by the use of the same methods and strains to prove the above presented results.

In summary, we can report that the oils of the flowers, leaves and stems of *C. giganteus* from Cameroon were found to be rich in p-menthadienols, limonene and *trans*-carveol. These compounds as well as further mono-and sesquiterpenes were also of high importance for the characteristic aroma of these oils. Limonene and the identified p-menthadienols may also be responsible for the high antimicrobial activity of the oils against Gram-(+)- and Gram-(-)-bacteria as well as the yeast *Candida albicans*, which has been investigated for the first time.

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