

Aromatic Plants from Cataractes Plateau (Congo Basin): Chemical Composition of Inflorescence Essential Oils from *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson Var. *Albescens* Acclimatized in Congo-Brazzaville

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Abstract

Cymbopogon flexuosus (Nees ex Steud.) W. Wats, a lemongrass native to eastern India introduced to Congo-Brazzaville, has been acclimatized for more than 10 years on the Cataract Plateau (Congo Basin). A lemongrass chemotype (*Cymbopogon flexuosus* var. *albescens*) was unexpectedly identified among the lemongrasses in the field, grown in a local experimental lemongrass. The essential oils of the inflorescences of the new chemotype had not been studied until now. The essential oils from the leaves and inflorescences, extracted by steam distillation and analysed by chromatography (GC/FID and GC/MS) for characterisation, mainly contained geraniol (33.0% (inflorescences)/29.1% (leaves)), citral (22.1/15.2%), citronellal (8.3/25.6%), geranyl acetate (7.5/2.7%), citronellol (6.7/9.7%), limonene (2.0/3.5%), citronellyl acetate (1.5/2.3%) and 6-methyl-heptan-5-en-2-one (1.4/0.0%). Compared to that of *C. nardus* L. Rendle, this composition corresponds to a citronella chemotype.

Keywords

Cymbopogon, Inflorescences, Geraniol, Citronellal, Citronellol

1. Introduction

Previous work on *Cymbopogon nardus* (citronella) and *C. citratus* (lemongrass) highlighted the difficulty met in developing *C. citratus* crops at a large scale because of its agronomic requirements and its very low biomass production [1].

C. flexuosus (Nees ex Steud.) Wats, known for its very high biomass production and as an important source of citral (>80%) as *C. citratus*, is a giant poaceae species, with red stems (*flexuosus* variety) or white stems (*albescens* variety). Its leaves are 50 - 120 cm long and 0.25 - 2.00 cm wide. It grows in clumps to a height of 2 m. Its finely branched inflorescences are abundant; it reproduces by both seeds and offshoots [2]-[4]. It was brought to Plateau des Cataractes (Congo-Brazzaville) from the Democratic Republic of Congo (DRC). Ten years later, the occurrence of a citronella chemotype of *C. flexuosus* was noted in experimental fields.

Cymbopogon nardus is a perennial herb that grows in clumps to a height of 1.5 m with violet shoot stems; panicle inflorescences appear one year after planting [5]. An experimental field, associating *C. nardus* (Java type: 0.25 ha) and *C. flexuosus* (citronella chemotype: 0.25 ha), from offshoots, was set up at the Campus Rural of Loukoko (latitude: 4°26'E, longitude: 14°44', elevation: 10 m). For the two species, offshoots 7 cm long were planted and spaced 1 m × 1 m under a palm grove (1 ha) aged about 10 years. Analysis of essential oils extracted from leaves of *C. nardus* by hydrodistillation confirmed the citronella chemotype, and showed a highly stable chemical composition in the experimental field oils [5], recalling previous observations reported by [6], in Bangladesh. The inflorescences of this chemotype had never been studied, unlike those of the citral chemotype [7]. Here, we report analysis of the inflorescence essential oils of *C. flexuosus* var. *albescens*, and compare the results with those obtained: 1) on leaves from the same chemotype, 2) with the citral chemotype of *C. flexuosus*, and 3) on the inflorescence and leaves of *C. nardus* (representative of the citronella chemotype), acclimatized in the same conditions at Plateau des Cataractes.

2. Material and Methods

2.1. Plant Material

For this study, the leaves and inflorescences from *C. flexuosus* var. *albescens* and *C. nardus* were harvested at 12 months in an experimental field. Leaves and inflorescences from *C. flexuosus* var. *flexuosus* were harvested at 18 months [3].

2.2. Isolation of Essential Oils

A pilot extraction was carried out in a 250 L distiller. The plant material and distillation water, placed in the same reactor (0.5 m × 0.5 m × 1 m), were separated by a removable perforated steel partition and brought to a boil for 3 hours. The condenser was a pipe with an internal diameter of 2 cm, equipped with an expansion chamber, passing through a tank (0.5 m × 0.5 m × 1 m) in which the water flowed in the opposite direction to the distillate. The plant material was dried for

7 days and the following proportions were used: 15 kg of leaves per 100 L of distillation water and 10 kg of inflorescences per 90 L of distillation water. If m_1 is the mass of the dried plant material and m_2 is the mass of oil recovered, then the essential oil content is equal to $(m_2/m_1) \times 100$.

2.3. GC and GC/MS Analyses

GC analysis was performed on an Agilent GC 6890 instrument equipped with a split injector (280°C), a flame ionization detector (FID) and a DB-5 column (20 m \times 0.18 mm \times 0.18 μ m). The temperature was ramped from 50°C (3.2 min) to 330°C at 10°C/min. Dihydrogen was used as carrier gas at a flow rate of 1 ml/min. The relative concentrations of the compounds were calculated thanks chemstation, an Agilent Instrument software, from the peak area obtained by gas chromatography without using correction factors.

GC/SM analysis was performed on an Agilent GC 7890/Agilent MS 5975 set-up operating in EI mode (70 eV), equipped with a DB-5 column (20 m \times 0.18 mm \times 0.18 μ m). The injector temperature was 280°C, and the carrier gas was helium at 0.9 mL/min. The temperature was ramped from 50°C (3.2 min) to 330°C at 8°C/min and mass scan mode, m/z : 33 - 450. A mixture of n-alkanes (C8 - C30) was analyzed in the same conditions to calculate retention indices using the equation of [8]. Identification was carried out by comparing retention indices and mass spectra of studied samples with those in data banks [9]-[12] and our own (LEXVA <https://www.lexva-analytique.com>).

2.4. Statistical Treatment

Means, standard deviations and the usual graphs were obtained with Excel 8.0 software.

3. Results and Discussion

3.1. Essential Oils of Leaves and Inflorescences of *C. flexuosus* (Nees ex Steud.) Wats

Table 1 gives the essential oil content and chemical composition of the studied oils which consist of oxygenated monoterpenes (81% - 88%), monoterpene hydrocarbons (2% - 7%), oxygenated sesquiterpenes (0.5% - 1%) and other compounds (1%).

Leaves gave, with a yield of $0.7\% \pm 0.1\%$, an essential oil with 30 identified constituents (content $> 0.1\%$). The first 10 major constituents of the citronella chemotype studied were: geraniol ($29.0\% \pm 3.1\%$), citronellal ($25.6\% \pm 1.4\%$), citronellol ($9.7\% \pm 0.9\%$), geranial ($8.8\% \pm 0.4\%$), neral ($7.1\% \pm 0.3\%$), geranyl acetate ($2.7\% \pm 0.3\%$), citronellyl acetate (2.3 ± 1.0), limonene ($3.5\% \pm 0.6\%$), z - β -ocimene ($2.2\% \pm 1.6\%$) β -caryophyllene ($2.0\% \pm 0.2\%$), and made up 80% of the total oil. This list of major constituents is similar to that drawn up by [2], after compiling data from the literature for the main representative of the citronella chemotype (from *C. nardus*).

Table 1. Composition of essential oils extracted from leaves and inflorescences of *Cymbopogon flexuosus* var. *albescens*.

			Leaves			Inflorescences	
Sample			1	2	3	Mean (SD)	4
Essential oil content (%)			0.7	0.7	0.8	0.7 (0.1)	0.4
RLc	RLit	Constituents (%)					
838	831	4-hydroxy-4methyl-pentan-2-one	0.9	0.0	0.0		0.1
984	981	6-methyl-heptan-5-en-2-one	0.0	0.0	0.0		1.4
991	988	Myrcene	0.1	0.8	0.9		-
1031	1024	limonene	4.1	3.2	3.1	3.5 (0.6)	2.0
1038	1032	z- β -ocimene	0.3	3.2	3.1	2.2 (1.6)	0.3
1049	1044	E- β -ocimene	0.1	0.0	0.0		0.3
1056	1051	bergamal	0.4	0.3	0.3		0.1
1101	1095	linalol	0.9	0.9	1.0		1.0
1151	1144-45	isopulegol	0.7	0.5	0.6		0.3
1156	1148	citronellal	27.3	24.7	24.9	25.6 (1.4)	8.3
1171	1173	rosefurane oxide	0.2	0.0	0.0		0.2
1182	1177	isogeranial	0.5	0.5	0.5		0.3
1208	1201	4E-decenal	0.2	0.0	0.0		-
1230	1223	citronellol	10	8.7	10.5	9.7 (0.9)	6.7
1241	1235	neral	7.1	7.3	6.8	7.1 (0.3)	8.7
1255	1249	geraniol	25.5	31.5	30	29.0 (3.1)	33.0
1271	1264	geranial	8.8	9.2	8.5	8.8 (0.4)	13.4
1350	1350	citronellyl acetate	3.4	1.5	1.9	2.3 (1.0)	1.5
1354	1356	eugénol	0.0	0.0	0.0		0.5
1378	1379	geranyl acetate	3.0	2.4	2.8	2.7 (0.3)	7.5
1390	1389	β -elemene	0.1	0.2	0.2		4.5
1422	1417	β -caryophyllene	1.8	2.1	2.1	2.0 (0.2)	0.3
1458	1452	α -humulene	0.2	0.2	0.2		0.8
1483	1484	germacrene-D	0.3	0.3	0.4		0.4
1515	1513	γ -cadinene	0.6	0.9	1.0		1.1
1519	1529	γ -bisabolene E	0.2	0.2	0.3		1.0
1521	1522	δ -cadinene	0.0	0.0	0.0		0.4
1550	1548	elemol	0.1	0.1	0.2		0.3
1579	1574	germacrene-D-4-ol	0.1	0.2	0.0		0.5
1585	1582	oxyde de caryophyllene	0.2	0.2	0.0		0.3

Continued

Total	97.1	99.1	99.3	95.1
Monoterpene hydrocarbons	4.6	7.2	7.1	2.6
Oxygenated monoterpenes	88	87.5	87.8	81.5
Sesquiterpene hydrocarbons	3.2	3.9	4.2	8.5
Oxygenated sesquiterpenes	0.4	0.5	0.2	1.1
Others	0.9	0	0	1.5

SD: standard deviation; RIC: retention index calculated; RILit: retention index literature.

The major constituent profiles of the two parts of *C. flexuosus* var. *albascens* were very similar: like leaf essential oil, the inflorescence essential oil contained 6-methyl-heptan-5-en-2-one (1.4%), limonene (2.0%), linalool (1.0%), citronellal (8.3%), citronellol (6.7%), citral (neral: 8.7%, geranial: 13.4%), geraniol (33.0%), geranyl acetate (7.5%), and β -elemene (4.5%), but the individual levels of these constituents varied between leaves and inflorescences: (i) geraniol (29.0% leaves, 33.0% inflorescences); (ii) citronellal (25.6% leaves, 8.3% inflorescences); (iii) citral (15.9% leaves, 22.1% inflorescences and (iv) citronellol (9.7% leaves, 6.7% inflorescences). These results can be compared with those in **Table 2**, which were obtained with the citral chemotype of *Cymbopogon flexuosus* acclimatized in Congo and were consistent with our previous results [3].

The essential oils of the inflorescences and leaves of *C. flexuosus* var. *flexuosus* were very similar: same qualitative profile, with fewer components for inflorescences, and a narrow range of variation in the individual contents of these constituents (**Table 2**). This major constituent profile is also consistent with those of *C. flexuosus* (citral chemotype), growing in India [7], with: citral (geranial; 40.0% - 50.0%, neral; 30.0% - 35.0%), borneol (0.0% - 2.0%), citronellal (0.37% - 8.04%), citronellol (0.4% - 4.6%), citronellyl acetate (1.2% - 3.6%), geraniol (1.7% - 40.0%), geranyl acetate (2.0% - 5.1%), limonene (2.4% - 3.7%), methyl eugenol (20.0%), and myrcene (0.1% - 14.2%); however, the essential oil contents of these inflorescences were slightly lower.

This major Constituents profile is also consistent with those of *C. flexuosus* (citral chemotype) growing in India [7] with: citral (geranial; 40.0% - 50.0%, neral; 30.0% - 35.0%), borneol (0.0% - 2.0%), citronellal (0.37% - 8.04%), citronellol (0.4% - 4.6%), citronellyl acetate (1.2% - 3.6%), geraniol (1.7% - 40.0%) geranyl acetate (2.0% - 5.1%), limonene (2.4% - 3.7%) and methyl eugenol (20.0%) myrcene (0.1% - 14.2%).

Studied oils were characterized by a very high level of oxygenated monoterpenes (87% - 95%) and very low level of other constituents: monoterpene hydrocarbons (1% - 2%), oxygenated sesquiterpenes (0.2% - 1.1%) and other compounds (0.5% - 1.0%).

3.2. Essential Oils of Leaves and Inflorescences of *Cymbopogon nardus* L. Rendle

The leaf and inflorescence essential oils of *Cymbopogon nardus* L. Rendle

Table 2. Composition of essential oils extracted from the leaves and inflorescences of *Cymbopogon flexuosus var. flexuosus* [3].

	Leaves	Inflorescences
Essential oil content (%)	0.3	0.2
RI Constituents (%)		
839 4-hydroxy-4methyl-pentan-2-one	0.1	0.0
986 6-méthyl-heptan-5-en-2-one	1.1	0.5
1097 linalool	0.3	0.3
1142 <i>trans</i> -sabinol	0.5	0.0
1150 isopulegol	0.3	0.0
1153 citronellal	0.3	0.2
1156 pinene oxide <beta-	2.1	0.0
1177 Rosefuran epoxide	1.2	0.3
1181 ethyl-3(2-furyl)-propanoate	2.8	0.0
1238 neral	36.2	34.4
1267 geranial	48.9	50.6
1359 damascone<(Z)-alpha	0.4	0.0
1371 NI	0.2	0.0
1381 geranyl acetate	0.7	1.1
1491 β -elemene	0.9	0.0
1418 β -caryophyllene	0.6	1.4
1514 γ -cadinene	0.5	0.2
1529 <i>trans</i> -calamene	0.2	0.0
1550 elemol	0.0	0.2
1581 caryophyllene oxide	0.6	0.0
1836 NI	0.5	0.0
Total (%)	98.4	89.2
Citral (%)	85.1	85.0
Oxygenated monoterpenes	95,1	87,4
Sesquiterpene hydrocarbons	1,3	1,6
Oxygenated sesquiterpenes	1.1	0.2
Others	1,2	0,5

RI: retention index; NI: non-identified.

(reference of citronella chemotype) were examined in the same conditions as *C. flexuosus* (Nees ex Steud.) Wats, to assess the consistency of results with those of this reference (**Table 3**). Studied oils contained oxygenated monoterpenes (65.5%

Table 3. Compositions of essential oils extracted from the leaves and inflorescences of *Cymbopogon nardus* L. Rendle var. *nardus* acclimatized in Congo-Brazzaville.

RIc	RILit	Constituents	Leaves	Inflorescences
838	831	4-hydroxy-4methyl-pentan-2-one	0.1	0.4
984	981	6-methyl-heptan-5-en-2-one	0.0	0.1
1026	1024	limonene	1.7	1.0
1052	1051	bergamal	0.1	0.1
1097	1096	linalol	0.5	0.5
1149	1145	isopulegol	1.1	0.4
1156	1148	citronellol + iso-isopulégol*	37.2	18.7
1160	-	neoisopulegol	0.5	0.1
1181	1174	terpinene-4-ol	0.1	0.1
1227	1227	nerol	0.2	0.2
1231	1223	citronellol	11.1	11.7
1242	1235	neral	0.7	2.0
1256	1249	geraniol	21.0	23.5
1271	1264	geranial	0.8	3.0
1310	1312	citronellic acid	0.2	0.0
1345	1328	8-hydroxyneomenthol	0.2	0.0
1351	1350	citronellyl acetate	0.4	2.2
1355	1356	eugenol	0.8	0.9
1379	1379	geranyl acetate	0.4	2.1
1387	1387	β -bourbonene	0.0	0.2
1392	1389	β -elemene	1.0	2.3
1424	1417	β -caryophyllene	0.1	0.3
1460	1452	α -humulene	0.0	0.1
1478	1478	γ -muurolene	0.0	0.2
1485	1484	germacrene-D	0.8	2.3
1501	1500	α -muurolene	0.2	0.7
1511	1508	germacrene-A	0.2	0.5
1517	1510	γ -cadinene	0.2	0.5
1521	1522	δ -cadinene	0.8	2.0
1540	1537	α -cadinene	0.0	0.1
1553	1548	elemol	3.6	7.7
1582	1579	germacrene-D-4-ol	0.4	3.6
1588	1582	caryophyllene oxide	0.1	0.1

Continued

1637	1630	γ -eudesmol	0.4	0.5
1647	1638	epi α -cadinol	0.0	0.6
1649	1640	epi α -muurolol	0.6	0.5
1651	1649	β -eudesmol	0.1	0.2
1661	1652	α -cadinol	1.3	2.1
1680	1680	Elemol acetate	0.2	0.4
1716	1722	fanesol 2Z 6E	0.1	0.3
2791	-	NI	5.1	4.0
2789	-	NI	6.6	2.0
		Total	98.8	98.2
		Monoterpene hydrocarbons	1.7	1.0
		Oxygenated monoterpenes	75.3	65.5
		Sesquiterpene hydrocarbons	3.3	9.2
		Oxygenated sesquiterpenes	18.5	22.0
		Others	0.1	0.5

NI: non-identified; *superimposition of peaks with approx. 5% iso-isopulegol.

- 75.3%), oxygenated sesquiterpenes (18.5% - 22.0%), monoterpene hydrocarbons (1% - 2%) and other compounds (0.5% - 1.0%).

Table 3 lists 48 constituents of leaf or inflorescence of *C. nardus* essential oil (individual content > 0.1%). Sixteen components represent more than 80% of the oil composition: limonene, linalool, isopulegol, citronellal, isopulegol, citronellol, citral, geraniol, eugenol, citronellyl acetate, geranyl acetate, β -elemene, germacrene-D, δ -cadinene, elemol, and α -cadinol. This major constituent profile, consistent with our previous results [5], is also consistent with the following list compiled by [2] based on literature data: limonene, linalool, isopulegol, citronellol, citronellal, citral, geraniol, eugenol, citronellyl acetate, geranyl acetate, and elemol.

3.3. Citronella Chemotype of *C. flexuosus* and *C. nardus*

According to the literature, “true” lemongrass (Indian *C. flexuosus*, source of citral) presents morphological and chemical variability. The citronella chemotype, which refers to *C. nardus*, was observed in Southeast Asia in the plantations of *C. flexuosus* mainly set up for the citral chemotype [2] [7].

To highlight similarities and differences among these three essential oils, radar plots were used (**Table 4**, **Figure 1** and **Figure 2**).

Close examination of representative radar plots of these oils built with 14 main constituents shows that: 1) **Figure 1** and **Figure 2** are totally different, with a very

Table 4. Summary of major constituent compositions of essential oils of inflorescences of *Cymbopogon flexuosus* var. *albescens*, *Cymbopogon nardus* L. Rendle var. *nardus* and *Cymbopogon flexuosus* var. *flexuosus*.

Constituents		<i>C. flexuosus</i> var. <i>albescens</i>	<i>C. nardus</i> var. <i>nardus</i>	<i>C. flexuosus</i> var. <i>flexuosus</i>
Limonene	1	2.0	1.0	0.0
Isopulegol	2	0.3	0.9	0.0
Citronellal	3	8.3	17.0	0.2
Citronellol	4	6.7	11.7	0.0
Neral	5	8.7	2.0	34.4
Geraniol	6	33.0	23.5	0.0
Geranial	7	13.4	3.0	50.6
Citronellyl acetate	8	1.5	2.2	0.0
Geranyl acetate	9	7.5	2.1	1.1
β -Caryophyllene	10	0.3	0.3	1.4
Elemene	11	4.5	2.3	0.0
Germacrenes A/D	12	0.2	0.5	0.0
Elemol	13	0.3	7.7	0.2
α -Cadinol/Cadinene	14	1.1	2.7	0.0
Citral (geranial + neral)		22.1	5.0	85.0

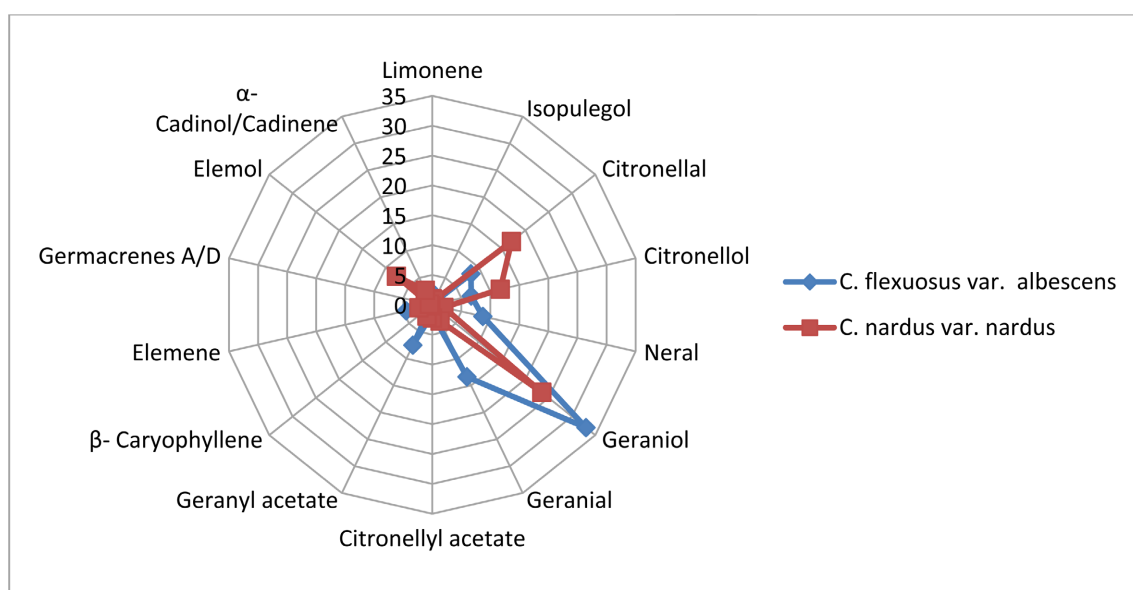


Figure 1. Radar plots of the inflorescence essential oils of *C. flexuosus* var. *albescens* and *C. nardus* var. *nardus*.

high citral content (neral: 34.4%; geranial: 50.6%) for 2, 2) the two radar plots in **Figure 1** are qualitatively similar for the major constituents, 3) *C. nardus* differs from *C. flexuosus* only by its citral content; *C. flexuosus* var. *albescens* contains

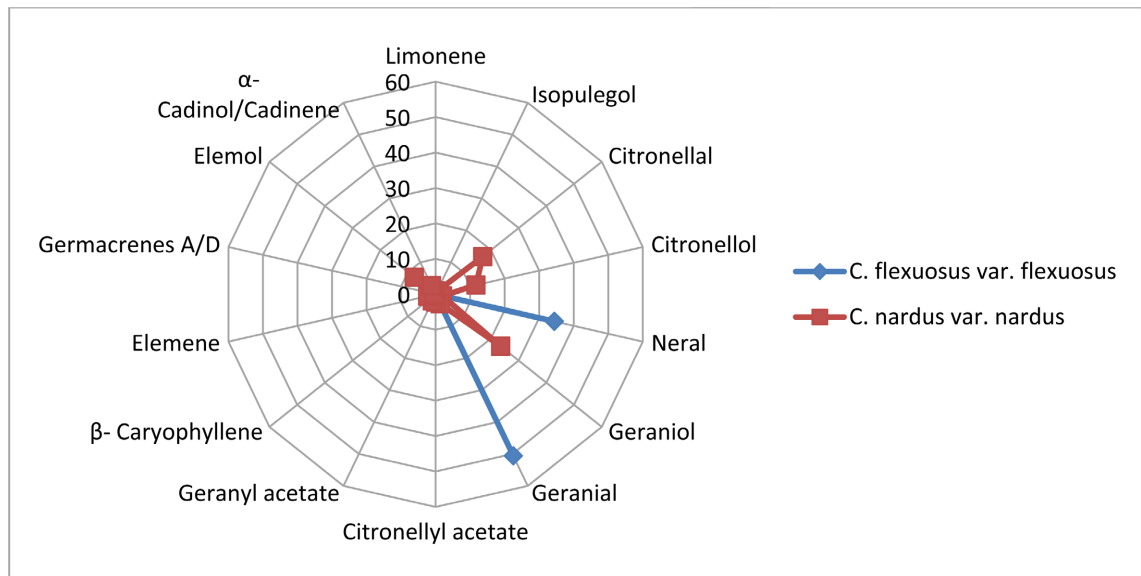


Figure 2. Radar plots of the inflorescence essential oils of *C. flexuosus var. flexuosus* and *C. nardus var. nardus*.

four times more citral (22.1%) than *C. nardus* (5.0%), which could be a marker for distinguishing between the two oils, 4) the “other” constituents, not considered for the radar plots are almost 10 times more abundant in *C. nardus* than in *C. flexuosus*, and 5) the difference between **Figure 1** and **Figure 2** illustrates the difference in composition between the two chemotypes of *C. flexuosus* (citronella and lemongrass).

The essential oils of the inflorescences of *C. flexuosus var. albascens* and *C. nardus* present the same chemotype according to their major constituent profiles; however, the citral content and the complexity of the chemical profile are markers that could if necessary be used to distinguish between their two oils.

4. Conclusion

The essential oil of inflorescences of *C. flexuosus var. albascens* extracted by steam distillation with a yield of 0.4% is mainly composed of geraniol (33.0%), citral (22.1%), citronellal (8.3%), geranyl acetate (7.5%), citronellol (6.7%), β -elemene (4.5%), limonene (2.0%), citronellyl acetate (1.5), and 6-methyl-heptan-5-en-2-one (1.4%). Its major constituent profile is totally different from that of *C. flexuosus var. flexuosus* (citral chemotype), and indicates a citronella chemotype (*C. nardus var. nardus*). It differs, however, from the *C. nardus* profile by its high citral content, and its less complex chromatogram.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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