Advance Journal of Food Science and Technology 2(6): 312-317, 2010
ISSN: 2042-4876
© Maxwell Scientific Organization, 2010
Submitted date: August 07, 2010 Accepted date: September 24, 2010 Published date: November 30, 2010

Characterisation of a Giant Lemon Grass Acclimatised in the Congo-Brazzaville

[1]A.N. Loumouamou, [1]E. Biassala, [1]Th. Silou, [1]P. Ntondele-Nsansi, [2]J. Diamouangana, [3]J.M. Nzikou, [4]J.C. Chalchat and [4]G. Figuérédo
[1]Equipe Pluridisciplinaire en Alimentation et Nutrition (EPRAN), Pôle d'Excellence en Alimentation et Nutrition, Faculté des Sciences, BP 389, Brazzaville, Congo
[2]Centre d'Etudes de Ressources Végétales (CERVE), Brazzaville, Congo
[3]ENSP-UMNG, Laboratory of Food Physicochemistry and Biotechnology, Laboratoire de Chimie des Hétérocycles et des Glucides, Chimie des Huiles Essentielles, Campus des Cézeaux, Université Blaise Pascal de Clermont, 63177 Aubière cedex, France
[4]LEXVA Analytique, 460 rue du Montant, 63540 Romagnat, France

Abstract: The aim of this study to investigate the essential oil of the giant variety of lemon grass (Poaceae) obtained from farmers in Congo-Kinshasa and tested in Congo-Brazzaville. Chemical analysis, by GC and GC-MS, of the essential oil from different parts of the plant, extracted at different stages of growth, revealed the very high stability of the citral chemotype (>80%); giving it the status of interesting species for the production of citral oil. However, it could not be identified to any of the oil-yielding grasses already described in the literature. Like *Cymbopogon citratus* (DC) Stapf it produces an essential oil containing more than 80% citral, but displays morphological characteristics of vigorous grasses. The botanical description of the plant and the chemical composition of its oil identify it to *Cymbopogon densiflorus* (Steud) Stapf.

Key words: Chemotype, citral, Congo-Brazzaville, *Cymbopogon densiflorus* (Steud) Stapf, essential oil chemical composition, lemon grass

INTRODUCTION

A comparative study conducted in Congo-Brazzaville on *Cymbopogon nardus* (citronellal-rich essential oil) and *Cymbopogon citratus* (DC) Stapf (citral-rich essential oil) showed that *Cymbopogon citratus* (DC) Stapf was difficult to grow on a large scale because of agronomic constraints and a very low biomass yield (Loubaki, 2003). One possible alternative was a grass obtained from farmers in Congo-Kinshasa that was perfectly adapted to the soil and climate of Congo-Brazzaville. We studied this grass with a view to developing it as a crop to produce citral-rich essential oil, for which there is a strong demand for food flavouring and local soap-making. This oil is also of medicinal interest (Menut *et al*., 2000; Jirovetz *et al*., 2007).We undertook an evaluation of its essential oil production potential.

The plant material, introduced in Congo-Brazzaville and acclimatised for 5 years was grown and monitored for 18 months for the purpose of identification. The chemical analysis of the essential oil from its leaves, extracted at different stages of growth, confirmed the very high stability of the citral chemotype (>80%) in which it ressembled *Cymbopogon citratus* (DC) Stapf (Loubaki, 2003) and *Cymbopogon flexuosus* (Singh, 1994) and even *Elionurus muticus* (Spreng.) Kunth (Chagonda *et al*., 2000). However, its morphology was closer to that of vigorous Poaceae species, in particular *Cymbopogon densiflorus* (Steud) Stapf ex *Cymbopogon giganteus* Chiov.

Three hypotheses were advanced: the species under study could be:

- A new hitherto undescribed chemotype of *Cymbopogon giganteus* Chiov
- A hybrid resulting from a natural crossing of *Cymbopogon citratus* (DC) Stapf with *Cymbopogon giganteus* Chiov
- An essential oil-yielding grass belonging to a genus other than *Cymbopogon*, e.g., *Elionurus*

Here we report a study of the chemical composition of the essential oils extracted from different parts of this plant, and at different growth stages, for the purpose of botanical identification.

MATERIALS AND METHODS

Plant material: The plant material studied was obtained from farmers in Congo-Kinshasa (DR Congo). It was

Corresponding Author: Dr. A.N. Loumouamou, Equipe Pluridisciplinaire en Alimentation et Nutrition (EPRAN), Pôle d'Excellence en Alimentation et Nutrition, Faculté des Sciences, BP 389, Brazzaville, Congo

Fig. 1: Plants aged 18 months

acclimatised in a nursery with three rotations over 5 years in Congo-Brazzaville according to the scheme shown in Fig. 1. Offshoots from the same acclimatised clump were used to make up an experimental bed of 16 plants (March 2005). Test samples were taken from different characteristic parts of the plant (leaves, flowers, stems and roots) and at different growth stages (initial cuttings, offshoots and adult plant). The following variables were measured in the course of the experiment: length (cm) and width (cm) of the leaf and height (m) of the plant, three times per plant. Flowering tips were examined under a magnifying glass.

Essential oil extraction: The steam distillation set-up used consisted of a 2 L steam generator surmounted by a 2 L glass reactor containing the plant material, connected to a cooling system to condense the distillate. A volume of 250 mL of water was placed in the lower vessel and 50 g of plant material in the upper vessel. The apparatus was heated and the essential oil was carried away by the steam. The distillate was collected from the condenser and the essential oil was separated by decantation. The extraction took 3 h and was repeated three times.

Analyses: GC analyses was performed on a Hewlett-Packard 6890 equipped with a split/splitness injector (280ºC, split ratio 1:10) using DB-5 column (30 m x 0.25 mm, df: 0.25 mm). The temperature program was 50ºC (5 min) rising to 300ºC at rate of 5ºC/min. Injector and detector temperature was 280ºC. Helium was used as carrier gas at a flow rate 1 mL/min. The injection of the sample consisted of 1.0 L of oil diluted to 10% v/v with acetone.

GC/MS was performed on a Hewlett-Packard 5973/6890 system operating in EI mode (70 eV), equipped with a split/splitness injector (280ºC, split ratio 1:20), using using DB-5 column (30 m x 0.25 mm, df: 0.25 mm). The temperature program was 50ºC (5 min) rising to 300ºC at rate of 5ºC/min. Injector and detector temperature was 280ºC. Helium was used as carrier gas at a flow rate 1 mL/min.

The identification was carried out by calculating retention indices and comparing mass spectra with those in data banks (Adams, 1995; McLafferty and Stauffer, 1989).

RESULTS AND DISCUSSION

To identify the species studied the planted cuttings were monitored for 18 months. The first measurement, made on leaves 4 months after planting of cuttings, gave lengths in the range 17-20 cm and widths between 1.2 and 1.7 cm.

A second measurement was made at 7 months, giving lengths in the range 40-50 cm and widths of 1.6-2.0 cm. At 12 months the plant had reached a height of about 4 m and become robust.

An overall assessment of the plant's morphology was made at 18 months (Fig. 1). The results obtained are grouped in Table 1. The variability of the morphological

Table 1: Morphological features of plants at 18 months, three measures per plant

	Leaves		Plant
Plant number	Length in cm (CV%)	Width in cm (CV%)	in m (CV%)
1	*176.0 (2.4)	1.9 (8.5)	3.6 (0.1)
2	164.3 (2.9)	2.0 (5.0)	4.1 (0.0)
3	162.7 (3.3)	2.0 (0.0)	4.3 (2.3)
4	180.0 (0.4)	1.7 (0.0)	4.1 (2.4)
5	180.0 (8.2)	1.6 (6.2)	3.9 (2.6)
Mean (Ectype)**	172.6 (6.0)	1.8 (11.0)	4.0 (5.0)

*: Mean of 3 measures; **: Mean of 15 measures

Table 2: Composition (%) of oils extracted from different parts of the plant (18 months) (flowers, leaves, stems and roots)

Constituants	RI	Leaves	Flowers	Stems	Roots
non identified	839	0.12	-	0.12	0.2
6-methyl-5-hepten-2-one	986	1.05	0.52	1.14	0.18
linalool	1097	0.30	0.26	0.05	0.11
trans-sabinol	1142	0.47	-	0.33	-
non identified	1150	0.26	-	-	-
citronellal	1153	0.30	0.15	0.33	0.12
β-pinene oxide	1156	2.08	-	1.50	0.49
rosefuran epoxide	1177	0.21	0.26	0.22	0.07
ethyl-3(2-furyl)-propanoate	1181	2.80	-	2.20	0.95
neral	1238	36.24	34.4	33.61	15.21
geranial	1267	48.88	50.6	49.4	21.54
(Z)-α-damascone	1359	0.37	-	0.45	0.64
non identified	1371	0.24	-	0.48	0.32
geranyl acetate	1381	0.71	1.05	0.70	0.59
β-elemene	1491	0.89	-	0.18	0.89
β-caryophyllene	1418	0.57	1.44	0.83	5.84
γ-cadinene	1514	0.46	0.24	0.63	1.52
trans-calamenene	1529	0.16	-	0.30	0.56
elemol	1550	-	0.21	0.72	3.22
caryophyllene oxide	1581	0.61	-	0.45	2.79
non identified	1600	-	-	-	0.24
humulene epoxide II	1608	-	-	-	0.18
non identified	1621	-	-	-	0.26
non identified	1628	-	-	-	0.32
γ-eudesmol	1630	-	-	-	2.54
β-eudesmol	1651	-	-	-	9.38
Z,Z-Farnesol	1718	-	-	-	0.50
E,E-Farnesol	1725	-	-	-	0.27
non identified	1836	0.54	-	-	-
hexadecanoic acide	1987	-	0.17	-	7.08
Total		97.26	89.34	93.64	76. 01
Citral (%)		83.12	85.00	83.01	36.75

characteristics can be approached through the coefficients of variation associated with the values in Table 1. This coefficient generally ranged from 2-3% for leaves on the same plant to 6% for the whole population studied, peaking at 9 and 11% for the width and 3 and 5% for the height. These values of variability are extremely low compared with what is usually observed in living populations. A coefficient of variation lower than 20% is considered to reflect a homogeneous population, one in the range 30-50% a moderately variable population and a value higher than 50% a widely variable population. The very high stability observed here does not therefore support the hypothesis of a hybrid in the course of morphological stabilisation.

Paniculate inflorescences, with sessile spikelets (3-7 mm), appeared one year after planting of the cuttings. Examination under magnification showed:

- Pairs of heterogamous twin spikelets.
- Sessile spikelets 4-5 mm long, shortly ciliate callus, truncate lower glume and deep, narrow median axilla.

All these characteristics were compared and found to be concordant with those of *Cymbopogon giganteus* Chiov (Van der Zon, 1992).

Table 2 gives the composition of oils extracted from the different parts of the plant aged 18 months. Neral and geranial, which are isomers of citral, together formed more than 80% of the oil from the above-ground parts. The citral was accompanied by three constituents with individual levels of 1-2.5%. The chromatogram was thus very simple (Fig. 2). The essential oils from the roots were also rich in citral (37%), but its chromatogram was more complex at the high retention end, with some ten constituents at levels in the range 1-10% (Fig. 2).

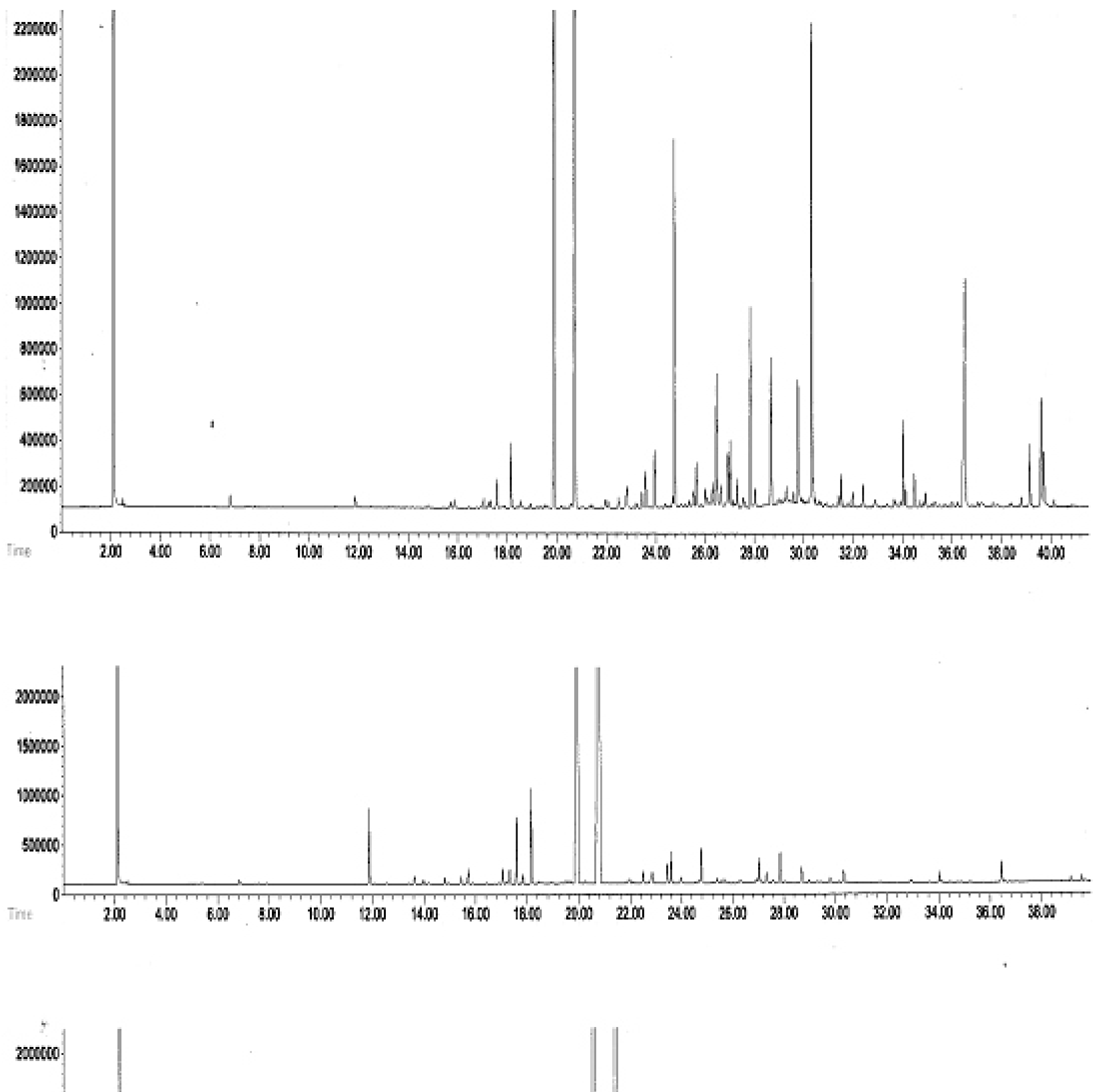

Fig. 2: Chromatograms of oils extracted from leaves (l), stems (s) and roots (r) of *Cymbopogon densiflorus* (Steud)

This pattern, which probably reflects the specialisation of the above- and below-ground parts, had already been made for other Poaceae, in particular *Elyonurus henseii* Schum (Silou *et al.*, 2006).

The essential oils from three plants aged 6.5 months after planting of cuttings were extracted and analysed. The oil content was about 0.2-0.3% and the average composition of the three oils analysed is presented in Table 3.

The shoots from the first full cut, aged 6 months, showed oil content of the same order of magnitude. The average chemical composition of the oils obtained is given in Table 3. The composition of the essential oil of leaves from the adult plant in Table 2 is included for comparison in Table 3.

The essential oils extracted from the above-ground parts of *Cymbopogon* studied here, comprised about 80% citral, with a neral/geranial ratio of about 1.3. The ketone

Table 3: Compared composition (%) of essential oils from leaves of three African *Cymbopogon* species from different locations

	C. giganteus			*C. citratus*			*Cymbopogon* sp. (a)		
Constituants	Mali (b)	Bénin (c)	Ivory Coast (d)	Bénin (e)	Congo (f)	Mali (b)	*Nongrafted plants (7 months)	*New shoots (6 months)	*Adult plant (18 months)
myrcene	-	0.1	-	12.1	1.0	18.1	-	-	-
6-methylhept-5ene-2-one	-	-	-	1.0	10.1	2.4	3.0	2.0	1.1
limonene	0.5	18.4	12.5	-	-	-	-	-	-
verbanol (cis/trans)	-		-	-	-	-	4.9	0.7	0.6
trans-p-mentha-2.8-dien-1-ol	13.3	22.5	18.4	-	-	-	-	-	-
cis-p-mentha-2.8-dien-1-ol	8.2	8.6	8.7	-	-	-	-	-	-
trans-pmentha-1(7).8-dien-2-ol	24.0	16.2	15.7	-	-	-	-	-	-
cis-pmentha-1(7).8-dien-2-ol	16.0	4.4	16.0	-	-	-	-	-	-
linalool	-	-	-	-	0.7	1.2	0.5	0.5	0.5
neral	-	-	-	33.7	30.1	23.6	35.5	35.2	36.2
geranial	-	-	-	45.3	44.5	30.5	48.7.	45.4	48.9
geraniol	-	-	-	2.5	4.1	6.2	-	-	-
geranyle acetate	-	-	-		1.7		0.6	0.4	-
caryophyllene	-	-	-	-	0.2	-	1.3	1.9	1.2
Total	62.0	80.2	71.4	94.6	92.4	82.0	94.5	86.1	88.5
p-menthadiene	61.5	61.7	58.9	-	-	-	-	-	-
citral	-	-	-	79.0	74.6	54.1	84.2	80.6	85.1

(a) this work, (b) Sidibe *et al.* (2001), (c) Ayedoun *et al.* (1999), (d) Boti *et al.* (2006), (e) Molangui (1996), (f) Silou *et al.* (2002), *: mean of three plants taken at random

6-methylhept-5-en-2-one and pinene oxide were minor constituents (1-2%) alongside citral.

This composition recalls that of *Cymbopogon citratus* (DC) Stapf, summarised by Sidibe *et al.* (2001), Molangui (1996) and Silou *et al.* (2002) on the basis of a compilation of literature results. Their findings were as follows:

- Oils from Zaire, Somalia and Ghana contained about 70% citral.
- Oils from India, Rwanda and Madagascar contained even more citral (80%), with a geranial/neral ratio slightly in favour of geranial. The other constituents were present in proportions of a few percent.
- Oils from Tunisia, Russia, Brazil, Burkina and Cameroon contained myrcene in appreciable amounts (15-20%).
- Oil from Madagascar contained 5-10% of 6-methylhept-5-en-2-one, against about 1% in the oils from other sources.
- Oils from Turkey, Nigeria and Qatar contained twice as much geranial as neral.
- Oil from Ethiopia stood out, with geraniol (40%), citral (13%), alpha-oxobisabolol (12%) and borneol (5%).

The essential oils obtained in this work fit perfectly into this scheme. They contained more than 80% citral (with a neral/geranial ratio of about 1.3), along with 6-methylhept-5-en-2-one and pinene oxide (with amounts in the range 1-2%). These oils are thus similar to those from India, Rwanda and Madagascar.

Likewise for *Cymbopogon flexuosus*, whose essential oil is composed of more than 80% citral. We may also mention the essential oil extracted from leaves, which although of a different nature was very similar in composition, with a lower proportion of citral (50-70%), and some geranyl acetate (2-19%). We looked at the composition of the essential oils of *Cymbopogon giganteus* Chiov, given its morphological closeness to the *Cymbopogon* studied here.

Sidibe *et al.* (2001), in a comparative study of the oils of *Cymbopogon citratus* and *Cymbopogon giganteus* Chiov from Mali, found oils that were totally different from those of *Cymbopogon giganteus*, which are composed essentially of *p*-menthadienols. These findings corroborate earlier results with *Cymbopogon giganteus* Chiov from Bénin (Ayedoun, 1999) and support those obtained subsequently for essential oil from Côte d'Ivoire by Boti *et al.* (2006). The massive presence of *p*-menthadienols brings these oils close to those of *Elyonorus henseii* Schum, which we studied previously (Silou *et al.*, 2006).

CONCLUSION

The hypothesis that the *Cymbopogon* sp studied here was a variety of *Cymbopogon giganteus* Chiov or this *Cymbopogon* was an hybrid resulting from a natural crossing of *Cymbopogon citratus* (DC) Stapf with *Cymbopogon giganteus* Chiov are thus refuted. Finally, descriptive systematics argues decisively in favour of *Cymbopogon densiflorus* (Steud) Stapf, and so we can consider the species studied as a citral chemotype of *Cymbopogon densiflorus* (Steud) Stapf.

ACKNOWLEDGMENT

The authors thank the members of the Laboratory of Chemistry of Heterocycles and Carbohydrate Chemistry of Essential Oils, University Blaise Pascal, Clermont Ferrand for their collaboration in experimental work. Our thanks also to the authorities of Marien Ngouabi University who promote this collaboration by providing us with necessary funding.

REFERENCES

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Ayedoun, M.A., K.D. Sohounhloué, C. Menut, G. Lamaty and J.M. Bessière, 1999. Chemical composition of essential oils of two species of Cymbopogon of Benin industrially exploitable. Bioresour. Energ. Environ. Dev., 8: 4-6.

Boti, J.B., A. Muselli, F. Tomi, G. Koukoua, T. Yao Nguessam, J. Costa and J. Casanova, 2006. Combined analysis of *Cymbopogon giganteus* Chiov. leaf from Ivory coast, C.R. Chimie, 9: 164 -168.

Chagonda, L.S., C. Makanda and J.C. Chalchat, 2000. The essential oils of wild and cultivated *validus* (Stapf) Stapf ex Burtt Davy and *Elionurus muticus* (Spreng.) Kunth from Zimbabwe. Flav. Frag. J., 15: 100-104.

Jirovetz, L., G. Buchbauer, G. Eller, M.B. Ngassoum and P.M. Maponmetsem, 2007. Composition and antimicrobial activity of *Cymbopogon giganteus* (Hochst.) Chiov. Essential flower, leaf and stem oils from Cameroon. J. Essent. Oil Res., 19: 485-489.

Loubaki, L., 2003. Contribution to the optimization of production and extraction of essential oils of *Cymbopogan citratus* and *Cymbopogon nardus* in peasant culture. Thèse de doctorat, Université, Marien Ngouabi, Brazzaville, pp: 114.

McLafferty, F.W. and D.B. Stauffer, 1989. The Wiley/NBS Registry of Mass Spectral Data. John Wiley and Sons, New York.

Menut, C., J.M. Bessiere, D. Samate, A.K. Djibo, G. Buchbauer and B. Schopper, 2000. Aromatic plants of tropical West Africa. Xl. chemical composition, antioxidant and antiradical properties of the essential oils of three *Cymbopogon* species from Burkina Faso. J. Essent. Oil Res., 12: 207-212.

Molangui, T., 1996. Study of essential oils from aromatic plants of sub-saharan Africa. Thèse de Doctorat, Université de Montpellier II, pp: 180.

Sidibe, L., J.C. Chalchat, R.P. Garry and M. Hamara, 2001. Aromatic plants of Mali: chemical composition of two cymbopogons: *Cymbopogon citratus* L, *Cymbopogon giganteus* Chiov. L. J. Ess. Oils Res., 13: 110-113.

Silou, T., L. Loubaki, G. Figuérédo and J.C. Chalchat, 2006. Study of essential oil composition of *Elyonurus hensii* Schum from Congo. J. Essent. Oil Res., 18: 518-520.

Silou, T., B. Mabiala, L. Loubaki and J.C. Chalchat, 2002. Impact of conservation of traitments on the content and composition of essential oils of *Cymbopogan citrates*. Ann. Fals., Exp. Chim., 95(961): 369-380.

Singh A.K. and A.A. Naqvi, 1994. Effect of hay storage on oild yeild and qualité in three *cymbopogon* species (*C. winterianus*, *C. martini* and *C. Floxuosus*) during different harvesting seasons. J. Essent. Oil Res., 6: 289-294.

Van der Zon, A.P.M., 1992. Grasses of Cameroon. Wageningen Agr. University, 2: 92-101.