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Chemical composition and biological activities of essential oils from the leaves of *Cymbopogon giganteus* Chiov. and *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) from Benin

Guy Alain ALITONOU[1], Félicien AVLESSI[1], Fidele TCHOBO[1], Jean-Pierre NOUDOGBESSI[1], Aretas TONOUHEWA[1], Boniface YEHOUENOU[1], Chantal MENUT[2] and Dominique K. SOHOUNHLOUE [1*]

[1]*Laboratoire d'Etude et de Recherche en Chimie Appliquée. BP : 2009 Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Bénin.*

[2]*Institut des Biomolécules Max Mousseron, IBMM UMR 5247 CNRS-UM1-UM2, ENSCM, 8 rue de l'Ecole Normale, 34296, Montpellier cedex 5, France.*

[*] *Corresponding author, E-mail: alainguy20022002@yahoo.fr*

ABSTRACT

The chemical composition of essential oils obtained from the leaves of *Cymbopogon giganteus* Chiov. and *Cymbopogon schoenanthus* (L.) Spreng, two Poaceae growing wild in Benin were analyzed by GC and GC/MS. The main constituents of *Cymbopogon giganteus* were *cis*-p-mentha-1(7),8-dien-2-ol (19.4%), *trans*-p-mentha-2,8-dien-1-ol (16.4%) and limonene (13.7%). The major components identified in the oil of *Cymbopogon schoenanthus* were piperitone (68.4%), and δ-2-carene (11.5%). The antimicrobial activity of the essential oil of *Cymbopogon giganteus* was found to be moderate on *Staphylococcus aureus* ATCC 25923 with MIC equal to 0.32 ± 0.02 mg/mL and *Escherichia coli* ATCC 25922 with MIC equal to 0.64 ± 0.34 mg/mL. This same oil induced the death of 57.84% of ticks at 8µL. Therefore, essential oil of *Cymbopogon schoenanthus* had a low antimicrobial activity on *Staphylococcus aureus* ATCC 25923 with MIC equal to 2.63 ± 0.16 mg/mL and *Escherichia coli* ATCC 25922 with MIC equal to 2.63 ± 0.16 mg/mL.
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Keywords: Essential oils, *Cymbopogon giganteus* Chiov., *Cymbopogon schoenanthus* (L.) Spreng, antimicrobial and acaricidal activities.

INTRODUCTION

Cymbopogon giganteus and *Cymbopogon schoenanthus* are West African endemic species of plants belonging to Poaceae family. *Cymbopogon giganteus* is used against mental illness and broncho-pulmonary affections (Alitonou et al., 2006), pain from scorpions bite (Fortin et al., 1990). The same plants are used for febrifuge, pulmonary, antiicteric disinfectants and antimalarial properties (Menut et al., 2000), and also against bilharziose, jaundice, cold, conjunctivitis, migraine, dermatoses, rheumatic pains, childhood coughs and hepatitises (Alitonou, 2006). This species showed strong effect against chloroquine resistant *Plasmodium* (Kimbi and Fagbenro-Beyioku, 1996). *Cymbopogon schoenanthus*

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is used for the treatment of madness in Africa and in Ethiopia (Akoegninou et al., 2006).

Studies were already carried out on *Cymbopogon giganteus* species. Only the more recent studies, especially relating to essential oils of the leaves, will be cited here. Several chemotypes of the essential oil of *Cymbopogon giganteus* have been reported according to the major compounds and their distribution: limonene (17.3%), *trans*-p-mentha-1(7),8-dien-2-ol (17%), *cis*-p-mentha-1(7),8-dien-2-ol (16.5%), *trans*-p-mentha-2,8-dièn-1-ol (13.8%) and *cis*-p-mentha-2,8-dien-1-ol (9.3%). The *cis*-isopiperitenol (4.5%) and carvone (4.1%) are also present at considerable amounts (Menut et al., 2000) as well as *trans* and *cis*-p-mentha-1(7),8-dien-2-ols (24.0-35.2% and 16.0-24.0%), *trans* and *cis*-p-mentha-2,8-dien-1-ols (13.3-16.2% and 8.2-10.2%). We note the presence of limonene with variable proportions (0.5-13.2%), p-methylacetophenone (3.2-6.0%) and carvone (0.1-2.9%) (Sidibe et al., 2001); *trans*-p-mentha-2,8-dien-1-ol (20.7%), *trans*-p-mentha-1(7),8-dien-2-ol (19.6%), *cis*-p-mentha-1(7),8-dièn-2-ol (19.0%), *cis*-p-mentha-2,8-dièn-1-ol (9.2%) and limonene (5.1%) (Kanko et al., 2004); *trans*-p-1(7),8-menthadien-2-ols (22.3%), *cis*-p-1(7),8-menthadien-2-ols (19.9%), *trans*-p-2,8-menthadien-1-ols (14.3%) and *cis*-p-2,8-menthadien-1-ols (10.1%) (Alitonou et al., 2006); limonène (23%), *cis*-*p*-mentha-2,8-dien-1-ol (14.3%) and *trans*-*p*-mentha-2,8-dien-1-ol (5.6%) (Nyamador et al., 2010); p-menthadienols (44%) and limonene (42%) (Bassolé et al., 2011). The reports have shown that *Cymbopogon schoenanthus* is rich in piperitone (68%) and carene-2 (16.48%) (Koba et al., 2003); *cis*-p-menth-2-en-1-ol (18.6%), *trans*-p-ment-2-en-1-ol (9.5%), elemol (7.4%), *cis*-piperitol (7.2%) and limonene (7.0%) (Bouchikhi et al., 2010).

These oils also have some biological activities: antimicrobial and antifungal (Koba et al., 2003; Bassolé et al., 2011) ; insecticide (Ketoh et al., 2006; Bouchikhi et al., 2010; Nyamador et al., 2010), larvicide (Ketoh et al., 2006); anti-inflammatory and antiradical (Menut et al., 2000; Sahouo et al., 2003; Alitonou et al., 2006).

We have previously reported the chemical composition of the volatile constituents of two species of *Cymbopogon* species from Benin. This study was carried out in order to evaluate the chemical composition and investigate on the antimicrobial and acaricide properties of essential oils of *Cymbopogon giganteus* and *Cymbopogon schoenanthus* samples collected in two different areas of Benin.

MATERIALS AND METHODS

Plants material

Fresh leaves of *Cymbopogon giganteus* and *Cymbopogon schoenanthus* were collected in two areas of Benin, at Houin-topka (Mono) (Sample *C. g.*) and Djougou (Donga) (Sample *C. s.*) in May 2012. Voucher specimens [AA6419/HNB] and [AA6420/HNB] respectively were deposited in the Herbarium of the University of Abomey-Calavi, Department of Vegetal Biology.

Essential oils isolation

The essential oils were obtained from the air-dried leaves by hydrodistillation for 2 h rs using a Clevenger-type apparatus. The oil was collected, dried over anhydrous sodium sulphate and stored in sealed vials below 10 °C until required for analyses.

GC and GC-MS analyses

Quantitative and qualitative analyses of the essential oils were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

GC/FID analyses were performed using a Varian CP-3380 GC equipped with a DB1 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary

column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50 °- 200 °C at 5 °C/min, injector temperature 220 °C, detector temperature 250 °C, carrier gas N_2 at a flow rate of 0.5 mL.min^{-1}. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes (C_9-C_{20}).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70 ° to 200 °C at 10 °C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow rate of 0.6 mL.min^{-1}, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of standard samples on the DB1 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data (Adams, 2007; Joulain et König, 1998).

Antibacterial activity

Essential oil emulsion

Two milliliters of Mueller Hinton broth and 0.02 g/L (w/v) of phenol red were added 40 µL of essential oil and 2 drops of Tween 80, introduced in an hemolyse test tube and homogenized.

Preparation of bacteria suspensions

Bacteria preparations were prepared from the stocks of the three bacteria tested. A pure colony of each stock was suspended in 5 mL of Mueller Hinton Broth. After incubation at 37 °C for 2 hours, we obtained a turbidity of 10^6 cfu/mL corresponding to the scale 2 of McFarland standard.

Determination of Minimum Inhibitory Concentrations (MIC)

The method used the one described by Yehouenou et al. (2010a, b). 100 µL of bubble Mueller Hinton broth containing phenol red at 0.02 g/L were introduced in each well of 96 wells microplate. 100 µL of essential oil emulsion (initial solution) were added in the wells of the first column except that of the second line and a serial two fold dilution was performed to obtain final concentration range. 100 µL of Mueller Hinton not containing phenol red were introduced in the first well of the first columns and a serial two fold dilution was performed as before. All the wells of the second column received 100 µL of bacteria suspension except the first and second lines, the negative and positive control respectively. The microplate was covered with parafilm and incubated at 37 °C for approximately 18 hours.

Determination of Minimum Bactericidal Concentrations (MBCs)

MBCs were appreciated by method proposed by Oussou et al. (2004), also used by Kpadonou et al. (2012). Each well of the microtiter-plate received 50 µL of mixture of essential oil and the strain. The strain was isolated on sterile MHA (Mueller Hinton Agar) cultured in Petri dishes. These plates were incubated at 37 °C for 24 h. The MBC is the lowest concentration of essential oil at which 99.9% of the microorganisms are killed. The tests were carried out in triplicate.

Acaricide activity

The harvest of the ticks was carried out as described by Pamo et al. (2003). The ticks were brought to the laboratory, in plastic Petri dish perforated by four small air pockets surroundings of 1mm of diameter (for airing) from where they were identified with the binocular magnifying glass thanks to the key

of Walker et al. (2002) and selected according to their size (4.2 ± 0.4) mm and weight (0.05 ± 0.01) g for their use for the tests. The sensitivity test consisted in putting a definite number of adult ticks (10) in different Petri dish. To varied amount of essential oil were added a variable essential oil amount (1µL, 2µ, 4µL, 6µL and 8µL). A conrol was performed under the same experimental conditions and the petri dish did not receive essential oil under the same experimental conditions or Petri dish did not receive any essential oil amount. The number of ticks dead was counted after 6h. The experiment was carried out 3 times. The death rate was calculated by using the expression of Abbott (1925).

Mo = mortality recorded in the treated batches (%); Me = mortality recorded at the witnesses (%); Mc = corrected mortality (%).

$$Mc = \frac{Mo - Me}{100 - Me} \times 100$$

Statistical analysis

Data were subjected to analysis of variance (ANOVA). They were expressed as the mean ± standard error of mean of triplicate measurements; standard deviations did not exceed 5 %.

RESULTS AND DISCUSSION

Chemical composition of essential oils obtained from fresh leaves of two Poaceae from Benin

Hydrodistillation of the air-dried leaves of *Cymbopogon giganteus* and *Cymbopogon schoenanthus* gave oil yield of 0.91% (w/w) and 3.49% (w/w) respectively. The compounds identified in the essential oils of *Cymbopogon giganteus* and *Cymbopogon schoenanthus* are listed in Table 1. A total of 27 and 30 compounds representing 95.3% and 98.3% of the oils were identified in the essential oils of *Cymbopogon giganteus* and *Cymbopogon schoenanthus* respectively. The major constituents of *Cymbopogon giganteus* oil were *cis*-p-mentha-1(7),8-dien-2-ol (19.4%), *trans*-p-mentha-2,8-dien-1-ol (16.4%) and limonene (13.7%). This composition is similar to those obtained in previously works (Sidibé et al., 2001; Sahouo et al., 2003; Alitonou et al., 2006). The main constituents in *Cymbopogon schoenanthus* essential oil were piperitone (68.4%), δ-2-carene (11.5%). All of samples were characterized by high percentage of oxygenated monoterpenes.

Antibacterial activity

Two microbial stocks were used in the present study. The Minimum Inhibitory Concentration (MIC) values were determined for all. The essential oil of the leaves of *C. giganteus* showed a very interesting antimicrobial activity against *Staphylococcus aureus* ATCC 25923 (MIC = 0.32 ± 0.02 mg/mL) and *Escherichia coli* ATCC 25922 (MIC = 0.64 ± 0.34 mg/mL). *C. schoenanthus* showed an average activity against the same microbial agent with the MIC = 2.63 ± 0.16 mg/mL for *Staphylococcus aureus*, MIC = (2.63 ± 0.16) mg/mL respectively (Table 2).

The Minimum Bactericidal Concentration (MBC) values were determined for the essential oil of *C. giganteus* on *Escherichia coli* ATCC 25922 with MBC equal to 2.56 ± 0.15 mg/mL and on *Staphylococcus aureus* ATCC 25923 with MBC equal to 1.32 ± 0.08 mg/mL (Table 2).

The essential oil of *C. giganteus* was more active than that of *C. schoenanthus* on the two microbial strains used. It should be also noted that the oil of *C. giganteus* was bactericidal on *E. coli* with an antibiotic capacity (MBC/MIC) = 4 and bacteriostatic on *S. aureus* with an antibiotic capacity (MBC/MIC) > 4 (Kpadonou et al., 2012).

The antimicrobial activities have been mainly explained through the presence of

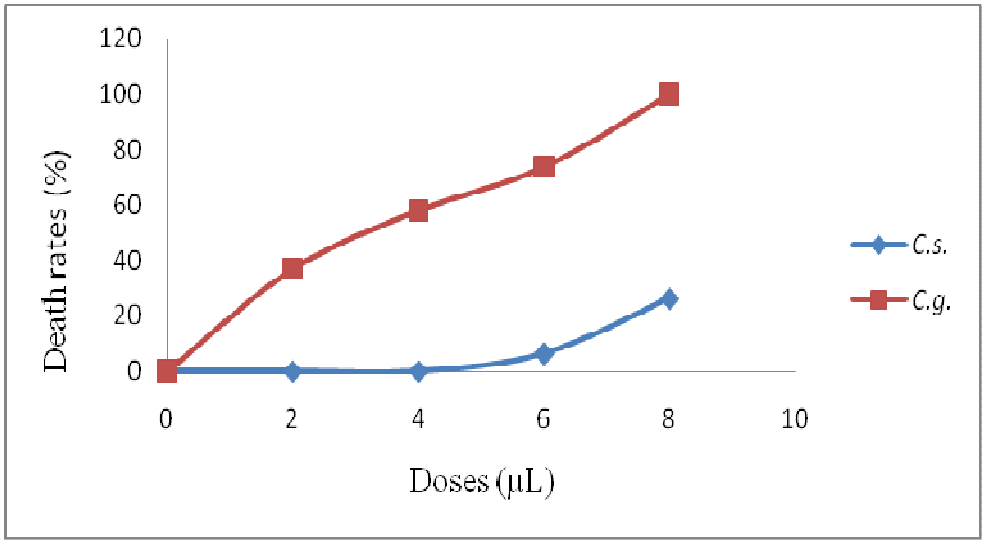
oxygenated sesquiterpenes and monoterpenes. The synergistic effect of essential oil components is a promising field that could lead to the optimization of a given bioactivity.

On the contrary *C. schoenanthus* displayed a low antimicrobial activity against the two strains relating to the absence of its antibiotic capacity against these latters. This fact may be due to the essential oil composition.

Acaricidal activity

The analysis of the results shows a variability of the death rate of *Amblyomma variegatum*, according to the essential oil amount of *C. giganteus*. After six hours, the death rate is high, what shows that the essential oil of *C. giganteus* has an action on these parasites. This activity was observed starting from the amount of 2 µL, became interesting (> 50%) starting from amount of 4 µL by inducing the death of 57.84% of ticks, and reached the maximum at the strongest amount of 8 µL. We can conclude that the essential oil of *C. giganteus* has an insecticidal activity on *Amblyomma variegatum* ticks. Our results confirm those obtained by Nyamador et al. (2010) in Togo.

On the other hand, with the essential oil of *C. schoenanthus*, we observed no effect on *Amblyomma variegatum* for the amounts of 2, 4, and 6 µL while a small percentage of death rate was noted for the maximum quantity of 8 µL. Our results are in contradiction with those obtained by Bouchiki et al. (2010) in Tunisia on two harmful insects. This contradiction can be allotted to the physiology of the insects or to the chemical composition of essential oil. The essential oil of *C. schoenanthus* of Tunisia, rich in para-menth-2-en-1-ol and was devoid of piperitone whereas our own was mainly constituted of piperitone (Figure 1).



C. g.: Cymbopogon giganteus; C.s.: Cymbopogon schoenanthus.

Figure 1: Evolution of the death rates according to the essential oils amount of *C. giganteus* and *C. schoenanthus*.

Table 1: Chemical composition of essential oils of leaves of *Cymbopogon schoenanthus* and *Cymbopogon giganteus* from Benin.

RI	Component	Samples	
		***C.s.* (%)**	***C.g.* (%)**
839	4-hydroxy-4-methyl-pentan-2-one	0.1	-
990	dehydro-1,8-cineole	0.1	-
1000	**δ-2-carene**	**11.5**	**-**
1002	Myrcene	**-**	0.1
1006	α-phellandrene	0.2	
1021	p-cymene	0.1	0.4
1029	**limonene** + β-phellandrene	2.2	**13.7**
1036	(Z)-β-ocimene	0.1	**-**
1047	(E)-β-ocimene	0.1	-
1081	p-cymenene	-	0.1
1089	fenchone	0.1	-
1092	Nonanal	-	0.1
1105	1,3,8-p-menthatriene	-	0.1
1118	***trans*-p-mentha-2,8-dien-1-ol**	-	**16.4**
1126	*cis*-menth-2-en-1-ol	0.9	-
1132	***cis*-p-mentha-2,8-dien-1-ol**	-	**9.5**
1138	*trans*-epoxylimonene	-	0.1
1141	Tetrahydroacetophenone	-	0.4
1145	*trans*-menth-2-en-1-ol	0.6	-
1155	4-isopropenylcyclohex-2-enone	-	1.2
1170	p-methylacetophenone	-	0.3
1174	mentha-1,5-dien-8-ol	1.3	**-**
1189	**3,9-epoxymentha-1,8(10)-diene**	-	**6.4**
	***trans*-p-mentha-1(7),8-dien-2-ol**	-	**0.3**
1193	***cis*-dihydrocarvone**	-	**6.4**
1198	α-terpineol	1.3	**-**
1199	***trans*-isopiperitenol**	-	**7.1**
1201	*trans*-dihydrocarvone	-	4.6
1212	*trans*-piperitol	0.3	-
1215	*cis*-isopiperitenol	-	5.9
1217	*trans*-carveol	-	0.1
1221	nerol	0.2	**-**
1226	p-mentha-1(7),8-dien-2-one	-	0.1
1229	***cis*-p-mentha-1(7),8-dien-2-ol**	-	**19.4**
1237	*cis*-carveol	-	2.9
1244	carvone	-	0.5
1247	carvotanacetone	0.1	-
1250	eucarvone	0.3	-
1265	**piperitone**	**68.4**	-
1267	hexanoate d'isoamyle	**-**	0.2
1270	isopiperitenone	**-**	0.2

1272	perillaldehyde	-	1.8
1392	β-elemene	0.3	-
1424	β-caryophyllene	0.1	-
1468	octanoate d'isoamyle	-	0.4
1485	germacrene-D	0.1	-
1493	β-selinene	0.1	-
1512	germacrene-A + cuparene	0.1	-
1516	γ-cadinene	0.1	-
1520	δ-cadinene	0.2	-
1552	elemol	3.9	-
1587	oxyde de caryophyllene	0.4	-
1646	γ-eudesmol	0.3	-
1646	epi-α-cadinol	0.2	-
1662	**α-eudesmol**	**4.6**	-
Monoterpenes hydrocarbons		**2.6**	**14.4**
Oxygenated monoterpenes		**82.7**	**80.6**
Sesquiterpenes hydrocarbons		**1.2**	-
Oxygenated sesquiterpenes		**10.4**	-
Others compounds		**0.1**	**0.7**
Aromatics compounds and of derivatives		**-**	**0.7**
Total		**98.3**	**95.3**

- : not determined

RI*, Retention index relative to n-alkanes (C_9-C_{20}) on a DB1 capillary column (100% dimethylpolysiloxane);
Identification methods:

- GC, identification based on retention times of authentic compounds
- MS, identification based on computer matching of the mass spectra of peaks with NBS75K.L, NIST98.L libraries and published data (Adams, 2007; Joulain et König, 1998).
- RI*, tentative identification based on comparison of retention index of the compounds with published data (Adams, 2007; Joulain et König, 1998).

C.s. : *Cymbopogon schoenanthus*
C.g. : *Cymbopogon giganteus*

Table 2: Antimicrobial activities of essential oils of the leaves of *C. giganteus* and *C. schoenanthus*.

Microbial stocks	**Minimum Inhibitory Concentration (MIC) (mg/mL)**		**Minimum Bactericide Concentration (MBC) (mg/mL)**	
	C. giganteus	***C. schoenanthus***	***C. giganteus***	***C. schoenanthus***
Escherichia coli ATCC 25922	0.64 ± 0.34	2.63 ± 0.16	2.56 ± 0.15	-
Staphylococcus aureus ATCC 25923	0.32 ± 0.02	2.63 ± 0.16	1.32 ± 0.08	-

- : not determined ; *C. giganteus : Cymbopogon giganteus ; C. schoenanthus : Cymbopogon schoenanthus.*

Conclusion

The aim of this work was to study the chemical composition, antimicrobial and acaricide activities of the essential of *C. giganteus* and *C. schoenanthus* from Benin. The oils obtained from leaves by hydrodistillation were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The total compounds accounted for about 95.3 % for the oil of *C. giganteus* and 98.3% for the oil of *C. schoenanthus*. The essential oils extracts reveal a very important *in vitro* activity on the studied strains, confirmed by Minimum Inhibitory Concentration (MIC) ranging from 0.32 to 0.64 mg/mL for *C. giganteus* and 2.63 mg/mL for *C. schoenanthus*. The oils *C. giganteus* could be recommended like active principle in formulations (creams, lotions, etc…) against the ectoparasites of the domestic ruminants.

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