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Chemical composition and diuretic potential of the essential oil of *Cymbopogon densiflorus* (Steud.) Stapf. (Poaceae) in the mouse

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ABSTRACT

The present study focused on the diuretic activity of the essential oil (EO) of *Cymbopogon densiflorus* (Steud.) Stapf and the citral (majoritaire compound). The analysis of the chemical composition of this oil by gas chromatography coupled with mass spectrometry (GC/MS) made it possible to identify several molecules. It emerges from this study that only EO at 150 mg/kg causes significant diuretic activity (V.U.E = 168.39%) such as furosemide; the latter being modest (V.U.E = 143.62%) for citral at a dose of 75 mg/kg. In treated mice, removal of sodium was significant and potassium was spared compared to mice treated with distilled water. The diuretic effect of EO of *C. densiflorus* leaves demonstrated in the present study is a mode of action of this plant that justifies its use in traditional medicine against high blood pressure.

Keywords: *Cymbopogon densiflorus*, essential oil, citral, diuretic, diuretic index.

INTRODUCTION

Diuretics are substances that inhibit the renal reabsorption of sodium and cause urinary excretion of water and sodium. They are used against edema and represent one of the most prescribed classes of drugs in first intention in hypertensive patients. They alone are able to control about 20% of essential HTA, currently recognized as a real public health problem in the world with regard to

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epidemiological data (Boutin, 2015). However, many of the diuretics currently used in clinical practice are only accessible to a privileged class of populations because of their high costs. Others, however, have been associated with a number of adverse effects, including electrolyte imbalance, metabolic alterations, onset of diabetes and impaired sexual function (Gupta and Neyses, 2005 ; Wile, 2012). Therefore plant extracts (less expensive), are presented as natural alternatives "safe" to conventional diuretics. In the Republic of Congo, several traditional plant-based preparations are used by the local population for their alleged diuretic properties, including a giant citronella species acclimated. The botanical description of this species and the chemical composition of its oil identify it to *C. densiflorus*. 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%). The essential oil of the leaves consisted mainly of 36.24% of neral and 48.88% of geranial (Loumouamou et al., 2010). Although much research is devoted to essential oils of the genus *Cymbopogon*, little information is available about their diuretic activities and their constituents. The purpose of this article was to evaluate the diuretic potential of the essential oil of the fresh leaves of *C. densiflorus* and one of its major constituents.

MATERIALS AND METHODS

Plant material

The leaves of *Cymbopogon densiflorus* were identified by Professor Jean-Marie Moutsambote at the Center for the Study of Vegetable Resources (C.E.R.VE) of Brazzaville compared to dried herbarium specimens N◦ 15233.

Animal material

Female albino swiss mice (*Mus musculus* Linnaeus, 1758) of body weighing between 20 and 30 g and aged 14 ± 2 weeks were used. These animals were provided by the animal facility of the Faculty of Science and Technology of Marien Ngouabi University (Brazzaville, Congo). They were kept under a photoperiodic cycle of 12 h of light and 12 h of dark at room temperature of 28 ± 1 °C and had free access to standard food and drinking water.

Drugs and chemicals

Furosemide and sodium chloride were purchased from Sigma-Aldrich, S.L. (USA), deionized water was used *in all experimental procedures.* Diethyl ether and citral were of analytical grade and purchased from Merck (Darmstadt, Germany).

Extraction procedure

Fresh leaves of *C. densiflorus* (500 g) were extracted by hydrodistillation, using a Clevenger type apparatus for 5 h. The essential oil was extracted with diethyl ether and dried over anhydrous sodium sulfate. The essential oil solution (1% (v/v)) in diethyl ether was then analyzed by GC-MS.

Analysis condition of the essential oil

The essential oil was analyzed with a Hewlett Packard (HP) 6890 gas chromatograph coupled with a HP MD5973 quadrupole mass spectrometer. Essential oil was injected using the split mode with a split ratio of 50:1. Helium was the carrier gas at a flow rate of 1.3 mL. min^{-1}. The injector temperature was 250 °C. Compounds were separated on a DB-5 ms capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Oven temperature program started at 40 °C (held for 1 min), heated at 10 °C/min up to 130 °C, followed by a 3 °C.min^{-1} increase rate up to 250 °C. Electron impact (EI) ion source was set to 150 °C. The electron energy was 70 eV and mass spectra were collected in the full scan mode (m/z 30-300). The compounds were identified by comparison of their mass spectra with those of standards available in

commercial mass spectral libraries like the NIST'05 database. The identification of most of the molecules was confirmed by comparison of their experimental Kovat's retention index (IK) with those provided by Adams'book (2001). The retention time of n-alkanes to calculate retention index were obtained with a commercial mixture of C_8-C_{20} in n-hexane. Relative amounts of individual components were calculated on the basis of their GC peak areas.

Evaluation of diuretic activity

The Basic diuresis was measured by administering to the mice of the water distilled at the rate of 50 ml/kg. Diuretic activity was evaluated according to the method reported by Sanogo and al. (2009)**.** The mice were divided into six groups (n = 5) and were deprived of food (but not water) for 12 h prior to testing. Each mouse received orally 50 ml/kg of body weight of isotonic saline (NaCl, 1.8%) to impose a uniform water loading. Each group received primarily a saline solution of 1.8% (50 ml/kg oral administration). Group I, as a negative control, received further distilled water (50 ml/kg oral administration); group II additionally received the reference diuretic, furosemide at 20 mg/kg administered orally. Groups III and IV served as a test group and received the essential oil leaves of *C. densiflorus* orally at doses of 75 and 150 mg/kg, respectively ; groups V and VI received citral at doses of 75 and 150 mg/kg, respectively. After these treatments, the 5 mice of the same group were placed in a metabolic cage. Then, for each group, the urine was collected and the following parameters were noted: the delay or time of elimination of the first drop of urine after placing the animals in the metabolic cage, the volume of urine excreted by hour and then after six (6) hours of experimentation. Volumetric Urinary Excretion (EUV) was calculated by the formula:

$$EUV = \frac{VE}{VA} \times 100 \quad (1)$$

EUV: Volumetric Urinary Excretion (%);
VE: Volume of Excreted Urine (mL);
VA: Volume of 1.8% NaCl (mL).

From the VUE values, the diuretic activity of the various products administered was estimated according to the Kau et al, (1984) scale recorded in Table 1.

Determination of Na^+, K^+ and Cl^- ions

The urinary Na^+ and K^+ concentrations were measured using a Micro Touch Biochemistry Analyzer spectrophotometer. The concentrations of Cl^- ions in the urine were determined by flame photometry. The Na^+/K^+ ratio, diuretic index and ionic quotient were deduced.

Table 1: Estimation of diuretic activity (Kau et al., 1984).

VUE	Interpretation
< 80 %	Anti diuretic
80 – 110 %	No activity
110 - 130 %	Low activity
130 – 150 %	Modest activity
> 150 %	Important activity

VUE: Volumetric Urinary Excretion.

RESULTS

Chemical study

The essential oil of leaves of *C. densiflorus* was obtained with a yield of 0.22 ± 0.02% (w/w). The compounds identified are listed in Table 2. Of the 16 volatile compounds identified in the essential oil of fresh leaves, citral alone accounts for 85.98% of the total extract.

Kinetics of urinary excretion and diuretic activity in mice treated with the essential oil of leaves of *C. densiflorus* and Citral

Kinetics of urinary excretion

Table 3 exhibits the volume of urine collected every hour from mice treated with distilled water, *C. densiflorus* essential oil and citral. The essential oil (EO) of *C. densiflorus* (150 mg/kg) causes, like furosemide, short urine elimination times respectively of 18 and 10 min against 24 min in the control mice. The essential oil at the dose of 150 mg/kg causes a maximum elimination of urine from the first hour. In addition, the essential oil of *C. densiflorus* (150 mg/kg) and citral (75 mg/kg) cause the elimination of urine only during the first 5 hours, such as furosemide (Table 3).

Diuretic activity

Table 4 shows that the essential oil of *C. densiflorus* (150 mg/kg) and Citral (75 mg/kg) cause after six hours the elimination of the respective cumulated urinary volumes of; 8.1 and 8.1 mL versus 4.6 mL in control mice. Volumetric urinary excretion is greater than 150% as for furosemide at 20 mg/kg (190.11%) than for the essential oil of C. densiflorus at 150 mg/kg (168.39%). With citral (75 mg/kg) the value of volumetric urinary excretion is (143.62%).

Effects of the essential oil of *C. densiflorus* and citral on the excretion of electrolytes in mice

Table 5 shows that in the mice treated with the different products, the diuretic indexes are greater than 1; except with citral (150 mg/kg). The Na^+/K^+ ratios are very high for the EO at 150 mg/kg (9.82) and the citral at 150 mg/kg (14.81) against (5.98) for the control mice. Moreover, it can be seen from Table5 that the ionic quotients in the mice treated with EO (75 and 150 mg/kg) are not between 0.8 and 1, as for furosemide.

Table 2: Chemical constituents of essential oils of *C. densiflorus.*

Pics	RI	Compounds	Peak area (%)
1	986	6-methyl-5-hepten-2-one	2.09
2	1095	linalool	0.60
3	1142	trans-sabinol	tr
4	1153	citronellal	0.90
5	1156	β-pinene oxide	3.01
6	1177	rosefuran epoxide	0.21
7	1181	ethyl-3(2-furyl)-propanoate	3.00
8	1237	neral	37.00
9	1264	geranial	48.98
10	1359	z-α-damascone	0.37
11	1381	geranyl acetate	0.71
12	1396	β-elemene	0.89
13	1423	β-caryophyllene	0.67
14	1515	γ-cadinene	0.56
15	1529	trans-calamenene	tr
16	1581	Caryophyllene oxide	0.91
Total identified compounds:			99.90
Citral			85.98

RI = retention index on DB-5ms ; tr = trace.

Table 3: Kinetics of urinary excretion in mice treated with the essential oil of leaves of *C. densiflorus* and citral.

treatments	Time (min)	Volume of urine eliminated in mL / h					
		1h	2h	3h	4h	5h	6h
NaCl 1,8 % + distilled water(1 mL/100 g)	24	1,8	1	0,2	0	0	0
NaCl 1,8 % + Furosemide (20 mg/kg)	10	6	2,8	0,5	0,5	1,2	0
NaCl 1,8 % + EO (75 mg/kg)	48	2	1,5	0,6	0,1	0	0,6
NaCl 1,8 % + EO (150 mg/kg)	18	3,8	2,2	0,2	1,8	0,1	0
NaCl 1,8 % + Citral (75 mg/kg)	59	0,6	3,4	3	1	0,1	0
NaCl 1,8 % + Citral (150 mg/kg)	48	2,2	0	0	0	0	0

EO: Essential oil.

Table 4: Diuretic activity of the essential oil of leaves of *C. densiflorus* and citral in mice.

Treatments	U.V.E (mL) in 6 h	V.U.E (%)	Conclusion
NaCl 1.8% +distilled water (1 mL/100 g)	4.6	84.09	No activity
NaCl 1.8 % + Furosemide (20 mg/kg)	10	190.11	Important activity
NaCl 1.8% + EO (75 mg/kg)	4.8	92.66	No activity
NaCl 1.8% + EO (150 mg/kg)	8.1	168.39	Important activity
NaCl 1.8 % + Citral (75 mg/kg)	8.1	143.62	Modest activity
NaCl 1.8 % + Citral (150 mg/kg)	2.2	38.13	Anti diuretic

EO: Essential oil, U.V.E: Urinary volume excreted, VU.E: Volumetric urinary excretion.

Table 5: Data on diuretic index and urinary excretion of electrolytes in mice treated with *C. densiflorus* essential oil and citral.

Treatments	Diuretic index	Ratio Na^+/K^+	Ionic quotient
NaCl 1,8 % + distilled water (1 ml/100 g)	1	5,98	1,12
NaCl 1,8 % + Furosemide (20 mg/kg)	2,17	6,16	1,03
NaCl 1,8 % + EO (75 mg/kg)	1,04	6,53	1,15
NaCl 1,8 % + EO (150 mg/kg)	1,76	9,82	1,13
NaCl 1,8 % + Citral (75 mg/kg)	1,76	14,81	0,95
NaCl 1,8 % + Citral (150 mg/kg)	0,48	2,98	0,94

EO: Essential oil.

DISCUSSION

The average yield of essential oils of this variety is comparable to those reported by other authors (Loumouamou et al., 2010; Nguimale, 2016). The results of the chemical analysis of the essential oil of the leaves confirm those published by Loumouamou et al. (2010), which revealed the very high stability of the citral chemotype for this specimen. The EO of C. densiflorus (150 mg/kg) causes, like furosemide, the elimination of the first drop of urine earlier compared to the control mice. Both products therefore have short diuretic action times. This result suggests that leaf EO of *C. densiflorus* has a fleeting and rapid diuretic effect in mice such as furosemide (Amonkan et al., 2013). Similarly, like furosemide, the essential oil (EO) at a dose of 150 mg/kg causes maximum elimination of urine from the 1st hour. In addition, EO at 150 mg/kg and citral at 75 mg/kg cause the elimination of urine only during the first 5 hours, such as furosemide. Previous work has shown that furosemide at doses between 10 and 20 mg/kg exerts its diuretic effect during the first five hours (Sanogo et al., 2009, Parasuraman and Raveendran, 2012). Also, in the mice treated with EO at 150 mg/kg, the volumetric urinary excretion is greater than 150% as in the mice that received the furosemide (20 mg/kg). These results suggest that this oil (150 mg/kg) has, like furosemide (20 mg/kg), a significant diuretic activity, according to the Kau et al. (1984) scale. Several plant extracts or medicinal recipes have also shown important diuretic effects. This is the case of *Trema orientalis* and *Lippia multiflora* (Akinda, 2013), the infused *Portulaca oleracea* (Diallo et al., 2001 and 2010), the Nitrokoudang recipe (Sanogo et al., 2009), of *Ziziphus mauritiana* (Ba, 2005) and *Spondias mombin* (Guindo, 2005). Regarding the Na^+ / K^+ ratio, the results show that they are very high for EO at 150 mg/kg 9.82 and citral at 150 mg/kg 14.81 against 5.98 for mice witnesses. These results suggest that this oil would cause the elimination of sodium ions more than potassium ions. Like other test products used and furosemide, this oil could be considered a good diuretic, sparing potassium ions. Indeed, it is reported that a good diuretic is one that causes a strong elimination of sodium and spares potassium (Dembélé, 2009). Diuretic indices greater than 1 in the mice that received the test products at different doses (except citral 150 mg/kg) or furosemide 20 mg/kg indicate that these products would cause diuresis more important than distilled water. In sum, these results suggest that, of all the test products used, it is probably the EO of *C. densiflorus* that acts according to the same mechanism as furosemide. Indeed, furosemide causes increased sodium excretion with water by inhibiting the co-transporter $Na^+ / K^+ / 2Cl^-$ responsible for the reabsorption of sodium and water at the level of the ascending limb of the Henle (Amonkan, 2013). This increased elimination of sodium and water would be beneficial in the treatment of high blood pressure and edema. In the mice given the EO, at the two doses, the ionic quotient is not between 0.8 and 1 as in the control mice. It is reported that substances that do not cause the ionic quotient between 0.8 and 1 inhibit carbonic anhydrase (Netsanet et al., 2017). However, the inhibition of carbonic anhydrase leads to the inhibition of the concomitant reabsorption of bicarbonates, sodium and chlorine, at the level of the proximal tubule. By this mechanism, these substances also have a diuretic effect, although low (Thomas, 2000). Thus, it is possible to suggest that the EO of *C. densiflorus* could, unlike furosemide and the other extracts of *C. densiflorus* studied, exert its diuretic effect by a second mechanism: the inhibition of carbonic anhydrase. This second mechanism could explain the important diuretic activity observed with this oil in the present study compared to the other extracts studied of this plant.

Conclusion

These results suggest that both substances (EO and Citral) tested, it is probably the OE of the leaves of *C. densiflorus* which acts like the furosemide, reference molecule reputed powerful diuretic. A study evaluating the antihypertensive effects of these extracts is worthwhile.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MB and MAEN carried out the extraction and analysis of the essential oil. JMM realized the botanical identification of the studied species. AAA, CE, AWEO, NOA and BSWL performed the biological activity, data processing, data analysis and writing the article.

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