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Chemical composition and biological activities of essential oil from lemongrass (*Cymbopogon citratus* [D.C.] Stapf.) leaves grown in Amazonian Ecuador

Composición química y actividades biológicas del aceite esencial de hojas de hierba luisa (*Cymbopogon citratus* [D.C.] Stapf.) cultivado en el Ecuador amazónico

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ABSTRACT:

Cymbopogon citratus (D.C.) Stapf., commonly known as lemongrass or “hierba luisa”, is widely cultivated in many tropical and subtropical regions due to its aromatic and medicinal properties. The essential oil from leaves has many pharmacological activities

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reported. The present paper reports the chemical composition and the antioxidant and antimicrobial activities of the essential oil of leaves from plants grown in the Amazonian Ecuador. The essential oil was obtained by steam distillation. Analyses of the essential oil were performed by gas chromatography with flame ionization and mass selective detectors. Seventy compounds were identified in the essential oil. Oxygenated compounds were the most represented class of volatiles (86%), including neral (33.2 %) and geranial (39.8 %) as major compounds. Monoterpene hydrocarbons were the second class (10.7 %) with myrcene (9.6%) as predominant. Antioxidant properties of the essential oil were determined by ABTS and FRAP methods. The essential oil had a low radical-scavenging activity and it showed ferric reducing capacity, while it has antimicrobial activity against *Staphylococcus aureus* and *Salmonella Enteritidis*, followed by *E.coli* and very low against *Bacillus subtilis*, *Aspergillus niger* and *Penicillium citrinum*.

KEYWORDS: *Cymbopogon citratus*, essential oil, composition, antioxidant activity, antimicrobial activity.

RESUMEN:

Cymbopogon citratus (D.C.) Stapf., comúnmente conocido como caña santa o hierba luisa, es ampliamente cultivado en muchas regiones tropicales y subtropicales debido a sus propiedades medicinales y aromáticas. Se han reportado múltiples actividades farmacológicas del aceite esencial de las hojas. En este trabajo se estudió la composición química, así como las actividades antioxidante y antimicrobiana del aceite esencial de hojas de plantas cultivadas en el Ecuador amazónico. El aceite esencial se obtuvo por destilación por arrastre con vapor. Los análisis se hicieron por cromatografía de gases con detectores de llama de hidrógeno y selectivo de masas. Se identificaron 70 compuestos en el aceite esencial. Los compuestos oxigenados fueron la clase química más representativa con 86%. Entre ellos, el neral (33,2 %) y geranial (39,8 %) fueron los más abundantes. Los hidrocarburos monoterpénicos fueron la segunda clase química (10,7 %) con el mirceno (9,6%) como predominante. La capacidad antioxidante fue evaluada en el aceite esencial mediante los métodos del ABTS y FRAP. El aceite esencial presentó baja actividad secuestradora del radical ABTS y mostró capacidad reductora del férrico, mientras que tuvo actividad antibacteriana contra *Staphylococcus aureus*, *Salmonella Enteritidis* seguido de *Escherichia coli* y en menor medida contra *Bacillus subtilis*, *Aspergillus niger* y *Penicillium citrinum*.

PALABRAS CLAVE: *Cymbopogon citratus*, aceite esencial, composición, actividad antioxidante, actividad antimicrobiana.

INTRODUCTION

Cymbopogon citratus (D.C.) Stapf., commonly known as lemongrass, belongs to family Poaceae and is widely cultivated in many tropical and subtropical regions due to its aromatic and medicinal properties. In Ecuador, this grass with lemon scented property is commonly named "hierba luisa". The essential oil (EO) obtained from the leaves is widely used in perfumery, food and pharmaceutical industries due to its high citral (a mixture of isomers neral and geranial) content which are responsible of the strong lemony aroma and the antimicrobial activity, including the inhibition of pathogenic and spoilage microorganisms (Menut et al., 2000; Appendini & Hotchkiss, 2002; Raybaudi-Massilia, Mosqueda-Melgar & Martín-Belloso, 2006; Tzortzakis & Economakis, 2007; Raybaudi-Massilia et al., 2009; Naik et al., 2010; Matasyoh et al., 2011; Leite et al., 2016; Athayde et al., 2016; Ekpenyong & Akpan, 2017).

In Ecuador, *C. citratus* is commercially grown in the Amazonian region, where its leaves are used as a food seasoning and for preparation of tea. However, only one study has been done about the chemical composition of its EO and biological activities (Sacchetti et al., 2005). Therefore, the present study was done to analyze the chemical composition and biological activities of the EO from lemongrass (*Cymbopogon citratus* [D.C.] Stapf.) leaves grown in Amazonian Ecuador.

MATERIALS AND METHODS

Materials and isolation of essential oil

Leaves of lemongrass were collected by Fundacion Chankuap' (Macas, Ecuador) in May 2017 from wild trees on the outskirts of the Wasak'entsa reserve in eastern Ecuador and positively identified by the National

Herbary of Pontificia Universidad Católica del Ecuador (voucher nr. HERUTEQ1056). Fresh leaves (300 g) were steam distilled for 6 h in a pilot- scale distiller. EO yield was 0.3 % v/m.

Gas chromatography

Analyses of the EO was performed by gas chromatography with a flame ionization detector (GC-FID) on a Konik 4000A (Konik, Barcelona) equipped with a 30 m x 0.25 mm i.d x 0.25 mm DB-5ms (J & W Scientific, Folsom, CA, USA) column. The GC parameters were: oven temperature program, 70 oC (2 min), 70 - 240 oC (4 oC/min) and 240 oC (5 min); carrier gas hydrogen flow rate 1 mL/min; injector and detector temperatures 250 oC, Samples (1 µL) were injected using split ratio 1:100, and previously diluted in n-pentane (1:6 v/v). The constituents were quantified after the areas of each detected compound were normalized and expressed as a percentage area.

The EO oil was also examined by gas chromatography-mass spectrometry (GC-MS) using a QP-2010 Ultra (Shimadzu, Japan) with a 30 m x 0.25 mm i.d. x 0.25 mm BP-5 (SGE Analytical Science Pty. Ltd., Victoria, Australia) column. Temperature program and Helium carrier gas flow rate same as in GC-FID. EIMS: electron energy, 70 eV; ion source and connecting parts temperature, 250 oC. Acquisition was performed in scanning mode (mass range m/z 35-400 u). Compounds were identified using their linear retention indices and mass spectra. Linear retention indices, calculated using linear interpolation relative to retention times of C8-C24 of n-alkanes, were compared with those standards and data from the literature (Adams, 2001)). Mass spectra were compared with corresponding reference standard data reported in the literature (Re et al., 1999) and mass spectra from NIST 05, Wiley 6, NBS 75 k, and in-house Flavorlib libraries. In many cases, the essential oil was subject to co-chromatography with authentic compounds.

ABTS radical cation decolorization assay

The spectrophotometric analysis of ABTS scavenging activity was determined according to an established method (Re et al., 1999). Briefly, the pre-formed radical cation of ABTS was generated by reacting ABTS solution (7 mmol/L) with 2.45 mmol/L potassium persulfate (K₂S₂O₈). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.1 units at 750 nm. An aliquot of 100 µL of methanolic dilution of EO was added to 1 mL of ABTS free radical cation solution. The absorbance, after 10 min, was measured spectrophotometrically at 750 nm. Methanolic solutions of known concentration of Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid, a water-soluble vitamin E analog) in the range of 50 to 700 µmol/L were used as calibration curve. The results were expressed as millimol Trolox per 100 mL EO.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP of the EO was measured by the method earlier reported (Benzie, 1996). Briefly, acetate buffer (300 mmol/L, pH=3.6), TPTZ (2,4,6-tripyridyl-s-triazine; Sigma) 10 mmol/L in 40 mmol/L HCl and FeCl₃·6H₂O (20 mmol/L) were mixed in the ratio of 10:1:1 to obtain the working FRAP reagent. The EO (90 µL) was mixed with 900 µL of FRAP reagent. A solution of ascorbic acid was used as standard and Trolox, a stable antioxidant was used as positive control. The mixtures were incubated at room temperature for four minutes and the absorbance was measured at 593 nm. The FRAP was expressed in units of ascorbic acid equivalent.

Minimal inhibitory concentration

For determination of minimal inhibitory concentration (MIC), 5; 0.5; 0.05 and 0.005 $\mu\text{L/mL}$ of the EO were prepared with dimethyl sulfoxide. One milliliter of each EO dilution was placed in different test tubes followed by addition of 1 mL of 24h-broth culture of the microorganism. The test tubes were all sealed with sterile corks and subsequently incubated at 32 oC for bacteria and 25 oC for fungus during 48 h. After incubation the tubes were observed for clearance or turbidity. The tube with highest degree of clearance was taken as the MIC. Three independent tests were performed for each sample. This procedure was separately carried out for the six test microorganisms: *Bacillus subtilis* ATCC 6633 (G+), *Staphylococcus aureus* ATCC 25923 (G+), *Escherichia coli* ATCC 25922 (G-), *Salmonella Enteritidis* ATCC 13036(G-), *Aspergillus niger* ATCC 16404, *Pennicilium citrinum* ATCC 9849.

RESULTS AND DISCUSSION

A total of 70 volatile compounds were identified in the EO from *C. citrates* leaves (99.6 % of the total composition) (Table 1). As can be seen, oxygenated monoterpenes were the most represented class of volatiles with 86 %. Among them, neral and geranial were the most abundant. Monoterpene hydrocarbons were found as the second major chemical class (10.7%) with myrcene being the main component.

TABLE 1
Chemical composition (%) of the essential oil from *C. citratus* leaves.

Compound	LRI	Area (%)	Compound	LRI	Area (%)
3-Methyl-2-buten-1-ol	774	tr	<i>exo</i> -Isocitral	1144	0.3
Hexanal	802	tr	<i>trans</i> -Chrysanthemal	1150	0.3
(<i>Z</i>)-Salvene	856	tr	Citronellal	1154	0.3
(<i>E</i>)-Salvene	870	tr	(<i>Z</i>)-Isocitral	1166	1.3
Heptanal	902	tr	<i>p</i> -Mentha-1,5-dien-8-ol	1170	tr
Santolina triene	908	tr	Rosefuran epoxide	1175	tr
α -Thujene	930	tr	(<i>E</i>)-Isocitral	1179	2.2
Benzaldehyde	962	tr	<i>p</i> -Cymen-8-ol	1183	tr
Sabinene	975	tr	α -Terpineol	1186	0.1
β -Pinene	980	tr	<i>trans-p</i> -Mentha-1(7),8-dien-2-ol	1189	0.2
6-Methyl-5-hepten-2-ol	991	1.9	Decanal	1203	tr
Myrcene	998	9.6	<i>trans</i> -Carveol	1217	0.1
<i>p</i> -Cymene	1025	tr	β -Citronellol	1226	0.6
Limonene	1028	0.5	Neral	1240	33.2
1,8-Cineole	1031	tr	Geraniol	1255	4.2
(<i>Z</i>)- β -Ocimene	1036	0.4	Geranial	1267	39.8
Phenylacetaldehyde	1044	tr	(2 <i>E</i> ,5 <i>E</i>)-3,7-Dimethylocta-2,5-dien-1,7-diol	1273	0.2
(<i>E</i>)- β -Ocimene	1051	0.3	Undecan-2-one	122	0.3
Bergamal	1056	tr	Neric acid	1330	0.1
γ -Terpinene	1061	tr	Geranic acid	1355	0.5
Acetophenone	1065	tr	6,7-Epoxy-geraniol	1369	0.2
Octan-1-ol	1069	tr	Geranyl acetate	1382	0.8
<i>cis</i> -Linalool oxide (furanoid)	1075	tr	(<i>E</i>)-Caryophyllene	1417	tr
<i>trans</i> -Linalool oxide (furanoid)	1085	tr	<i>trans</i> - α -Bergamotene	1431	0.1
Nonan-2-one	1090	tr	α -Guaiene	1440	tr
6,7-Epoxymyrcene	1093	0.1	(<i>Z</i>)- β -Farnesene	1442	tr
Rosefuran	1095	0.1	α -Humulene	1453	tr
Linalool	1098	1.3	Tridecan-2-one	1496	0.3
Perillene	1102	0.3	(<i>Z</i>)- α -Bisabolene	1505	tr
6-Methyl-3,5-heptadien-2-one	1106	tr	γ -Xadivene	1514	tr
<i>trans-p</i> -Mentha-2,8-dien-1-ol	1123	tr	(<i>E</i>)-Nerolidol	1563	tr
<i>trans</i> -Rose oxide	1126	tr	Caryophyllene oxide	1582	0.1
<i>allo</i> -Ocimene	1130	tr	Selin-11-en-4- α -ol	1660	0.1
(<i>Z</i>)-Myroxide	1135	tr	(<i>E,E</i>)-Farnesol	1726	tr
<i>cis-p</i> -Mentha-2,8-dien-1-ol	1139	tr	Geranyl linalool	2016	0.2

Identity A identification based on the linear retention times (LRI) and mass spectra of pure compounds; B: identification based on LRI and mass spectra comparison with databases or literature data. tr: traces (< 0.1%).

The bioactivity of any plant species is attributed to its chemical compounds. If these constituents are secondary metabolites, they are often essential in many processes affecting plant growth, development, and environmental interaction. Many studies show that the chemical composition of EOs varies considerably according to several factors, including geobotanical conditions of the environment, cultivation method, plant age, photoperiod, harvest period, among others (Figueiredo et al., 2008). Nevertheless, our results are closely related with the chemical composition reported for the previously analyzed EO from the same region, where major constituents were geranial (41.3 %), neral (32.3 %) and myrcene (15.5 %) (Sacchetti et al., 2005) and for the EO from Burkina Faso were geranial (44.6 %), neral (33.0 %) and myrcene (10.7 %) (Menut et al., 2000). Other studies only found the citral isomers as major compounds: geranial (40.8 %) and neral (31.8 %) from plants grow in Manchester (UK) Tzortzakis & Economakis, (2007), geranial (31.7 %) and neral (46.2 %) (Leite et al., 2016) or geranial (29.2 %) and neral (27.0 %) (Athayde et al., 2016), both from Brazil.

Antioxidant properties of the EO were determined by two complementary methods: radical- scavenging capacity of the oil (ABTS) and ferric-reducing antioxidant power (FRAP) assay. The reducing effect on the radical cation ABTS was 26.8mmol/100 mL for the EO (Table 2). In the second assay, the FRAP were between 263.4 ± 13.3 to 404.3 ± 16.4 $\mu\text{mol/L}$ of ascorbic acid equivalents by different concentrations. The chemical methods to evaluate the antioxidant capacity measure the combined effects of many substances present in the sample, which their antioxidant function is due to different mechanisms: elimination of free radicals generated in the assay (ABTS method) and the transfer of electrons in reactions of redox type (FRAP method). This explains the differences observed between the methods used, according to the results of the present study. Nevertheless, the weak activity is attributed to the presence of citral isomers (Baschieri et al., 2017). Unlike phenols, oxygenated monoterpenes exhibit less antioxidant activity (Sell, 2010), which would explain the lower antioxidant activity observed with respect to other EOs like oregano. It must be pointed out that EO from the present work performed worse than the EO from Ecuadorian- grown plants (Sacchetti et al., 2005) or of the same botanical source but of different geographical origin (Menut et al., 2000).

TABLE 2
Antioxidant effectiveness of the essential oil.

Table 2. Antioxidant effectiveness of the essential oil ¹ .			
Sample	ABTS	FRAP	
	$\mu\text{mol/L}/100 \text{ mL}$	Concentration (mg/mL)	($\mu\text{mol/L}$ of ascorbic acid equivalents)
Essential oil	36.8 ± 0.2	4	404.3 ± 16.4
		2	413.4 ± 6.7
		1	399.0 ± 15.4
		0.5	263.4 ± 13.3
Trolox	nt ²	0.05	303.4 ± 8.8

¹Antioxidant effectiveness expressed as ABTS and FRAP assays. Values represent an average of three determinations with standard deviation. ²nt: not tested.

¹ Antioxidant effectiveness expressed as ABTS and FRAP assays. Values represent an average of three determinations with standard deviation. ²nt: not tested

The MIC of the EO was ranged between 0.05-5 $\mu\text{L/mL}$ (Table 3). The essential oil showed better activity against *St. aureus* and *S. Enteritidis* followed by *E. coli*. The activity was low against *B. subtilis*, *A. niger* and *P. citrinum*. In general, these results are in accordance with those reported for this EO (Raybudi-Massilia, Mosqueda-Melgar & Martín-Belloso, 2006; Tzortzakis & Economakis, 2007; Naik et al., 2010; Matasyoh et al., 2011; Leite et al., 2016; Athayde et al., 2016; Ekpenyong & Akpan, 2017) and they are attributed to the presence of citral isomers as responsible for showing antimicrobial activity against specific pathogens (Ekpenyong & Akpan, 2017; Abe et al., 2003).

TABLE 3
Minimal inhibitory concentrations of the essential oil against tested microorganisms.

Microorganism	MIC ($\mu\text{L/mL}$)
<i>Bacillus subtilis</i> ATCC 6633	5.0
<i>Staphylococcus aureus</i> ATCC 25923	0.005
<i>Escherichia coli</i> ATCC 25922	0.5
<i>Salmonella Enteritidis</i> ATCC 13036	0.05
<i>Aspergillus niger</i> ATCC 16404	5.0
<i>Penicillium citrinum</i> ATCC 9849	5.0

CONCLUSIONS

The EO composition of *Cymbopogon citratus* leaves show the presence of 70 volatile constituents, of which the most prominent were nerol (33.2 %) and geranial (39.8 %). The EO had a low antioxidant activity by using the ABTS assay and it showed ferric reducing antioxidant power, while it has antimicrobial activity against *St. aureus* and *S. enteritidis* followed by *E. coli*. The activity was low against *B. subtilis*, *A. niger* and *P. citrinum*.

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