Rev. CENIC Cienc. Quím.; 49: 1-11. 2018. e-ISSN: 1221-2450

SHORT COMMUNICATION

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

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

**Jorge A. Pino[a,*]; Flor M. Fon-Fay[b]; Julio C. Pérez[c]; Ana S. Falco[a]; José L. Rodríguez[a]; Ivones Hernández[d]; Idania Rodeiro[d]; Miguel D. Fernández[d]**

[a]Food Industry Research Institute, Cuba. E-mail: jpino@iiia.edu.cu
[b]Universidad Técnica Estatal de Quevedo, Ecuador.
[c]Toxicology National Center, Cuba;
[d]Marine Sciences Institute, Cuba.

**Received**: 6 February 2018; **Accepted**: 11 April 2018

## 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 aromaticand medicinal properties. The essential oil from leaves has many pharmacological activities 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 *Pennicilium citrinum*.

**Keywords**: *Cympopogon 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*y *Salmonella Enteritidis* seguido de *Escherichia coli* y en menor medida contra *Bacillus subtilis*, *Aspergillus niger*y *Penicilium citrinum*.

**Palabras clave**: *Cympopogon 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 aromaticand 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 (*Cympopogon 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 °C (2 min), 70 - 240 °C (4 °C/min) and 240 °C (5 min); carrier gas hydrogen flow rate 1 mL/min; injector and detector temperatures 250 °C, 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 °C. 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 $C_8$-$C_{24}$ 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 anestablished 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 ($K_2S_2O_8$). 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 $FeCl_3 \cdot 6H_2O$ (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 µL/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 | *exo*-Isocitral | 1144 | 0.3 |
| Hexanal | 802 | tr | *trans*-Chrysanthemal | 1150 | 0.3 |
| (*Z*)-Salvene | 856 | tr | Citronellal | 1154 | 0.3 |
| (*E*)-Salvene | 870 | tr | (*Z*)-Isocitral | 1166 | 1.3 |
| Heptanal | 902 | tr | *p*-Mentha-1,5-dien-8-ol | 1170 | tr |
| Santolina triene | 908 | tr | Rosefuran epoxide | 1175 | tr |
| α-Thujene | 930 | tr | (*E*)-Isocitral | 1179 | 2.2 |
| Benzaldehyde | 962 | tr | *p*-Cymen-8-ol | 1183 | tr |
| Sabinene | 975 | tr | α-Terpineol | 1186 | 0.1 |
| β-Pinene | 980 | tr | *trans*-*p*-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 | *trans*-Carveol | 1217 | 0.1 |
| *p*-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 |
| (*Z*)-β-Ocimene | 1036 | 0.4 | Geranial | 1267 | 39.8 |
| Phenylacetaldehyde | 1044 | tr | (2*E*,5*E*)-3,7-Dimethylocta-2,5-dien-1,7-diol | 1273 | 0.2 |
| (*E*)-β-Ocimene | 1051 | 0.3 | Undecan-2-one | 122 | 0.3 |
| Bergamal | 1056 | tr | Neric acid | 1330 | 0.1 |
| γ–Τερπινενε | 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 |
| *cis*-Linalool oxide (furanoid) | 1075 | tr | (*E*)-Caryophyllene | 1417 | tr |

| | | | | | |
|---|---|---|---|---|---|
| *trans*-Linalool oxide (furanoid) | 1085 | tr | *trans*-α-Bergamotene | 1431 | 0.1 |
| Nonan-2-one | 1090 | tr | α-Guaiene | 1440 | tr |
| 6,7-Epoxymyrcene | 1093 | 0.1 | (*Z*)-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 | (*Z*)-α-Bisabolene | 1505 | tr |
| 6-Methyl-3,5-heptadien-2-one | 1106 | tr | γ–Χαδινενε | 1514 | tr |
| *trans-p*-Mentha-2,8-dien-1-ol | 1123 | tr | (*E*)-Nerolidol | 1563 | tr |
| *trans*-Rose oxide | 1126 | tr | Caryophyllene oxide | 1582 | 0.1 |
| *allo*-Ocimene | 1130 | tr | Selin-11-en-4-α-ol | 1660 | 0.1 |
| (*Z*)-Myroxide | 1135 | tr | (*E,E*)-Farnesol | 1726 | tr |
| *cis-p*-Mentha-2,8-dien-1-ol | 1139 | tr | Geranyl linalool | 2016 | 0.2 |

**Note**: 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 plantgrowth, 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 μmol/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[1].

| Sample | ABTS | FRAP | |
|---|---|---|---|
| | (mmol/100 mL) | Concentration (mg/mL) | (µmol/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 |

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

The MIC of the EO was ranged between 0.05-5 µL/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 (µL/mL) |
|---|---|
| *Bacillus subtilis* ATCC 6633 | 5.0 |
| *Staphylococcus aureus* ATCC 25923 | 0.005 |
| *Escherichia coli* ATCC 25922 | 0.5 |
| *Salmonella Enteritidis* ATCC 13036 | 0.05 |
| *Aspergillus niger* ATCC 16404 | 5.0 |
| *Pennicilium citrinum* ATCC 9849 | 5.0 |

## CONCLUSIONS

The EO composition of *Cympopogon citratus* leaves show the presence of 70 volatile constituents, of which the most prominent were neral (33.2 %) and geranial (39.8 %). The EO had a low antioxidant activity by using the ABTSassay 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*.

## REFERENCES

Abe, S., Sato, Y., Inoue, S., Ishibashi, H., Maruyama, N., Takizawa, T., ... & Yamaguchi, H. (2003). Anti-Candida albicans activity of essential oils including Lemongrass (Cymbopogon citratus) oil and its component, citral. *Nippon Ishinkin Gakkai Zasshi*, *44*(4), 285-291.

Adams RP. (2001). Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Carol Stream, IL: Allured Publishing Co.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*(2), 113-126.

Athayde, A. J. A. A., De Oliveira, P. D. L., Guerra, I. C. D., Da Conceicao, M. L., De Lima, M. A. B., Arcanjo, N. M. O., ... & de Souza, E. L. (2016). A coating composed of chitosan and Cymbopogon citratus (Dc. Ex Nees) essential oil to control Rhizopus soft rot and quality in tomato fruit stored at room temperature. *The Journal of Horticultural Science and Biotechnology*, *91*(6), 582-591.

Baschieri, A., Ajvazi, M. D., Tonfack, J. L. F., Valgimigli, L., & Amorati, R. (2017). Explaining the antioxidant activity of some common non-phenolic components of essential oils. *Food Chemistry*, *232*, 656-663.

Benzie, I. F. (1996). An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA). *Clinical biochemistry*, *29*(2), 111-116.

Ekpenyong, C. E., & Akpan, E. E. (2017). Use of Cymbopogon citratus essential oil in food preservation: Recent advances and future perspectives. *Critical reviews in food science and nutrition*, *57*(12), 2541-2559.

Figueiredo, A. C., Barroso, J. G., Pedro, L. G., & Scheffer, J. J. (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour and Fragrance journal*, *23*(4), 213-226.

Leite, C. J. B., de Sousa, J. P., da Costa Medeiros, J. A., da Conceição, M. L., dos Santos Falcão-Silva, V., & de Souza, E. L. (2016). Inactivation of Escherichia coli, Listeria monocytogenes, and Salmonella Enteritidis by Cymbopogon citratus DC Stapf. essential oil in pineapple juice. *Journal of food protection*, *79*(2), 213-219.

Matasyoh, J. C., Wagara, I. N., & Nakavuma, J. L. (2011). Chemical composition of Cymbopogon citratus essential oil and its effect on mycotoxigenic Aspergillus species. *African Journal of Food Science*, *5*(3), 138-142.

Menut, C., Bessiere, J. M., Samate, D., Djibo, A. K., Buchbauer, G., & Schopper, B. (2000). Aromatic plants of tropical west Africa. XI. chemical composition, antioxidant and antiradical properties of the essential oils of three Cymbopogon species from Burkina Faso. *Journal of Essential Oil Research*, *12*(2), 207-212.

Naik, M. I., Fomda, B. A., Jaykumar, E., & Bhat, J. A. (2010). Antibacterial activity of lemongrass (Cymbopogon citratus) oil against some selected pathogenic bacterias. *Asian Pacific Journal of Tropical Medicine*, *3*(7), 535-538.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., & Martin-Belloso, O. (2006). Antimicrobial activity of essential oils on Salmonella enteritidis, Escherichia coli, and Listeria innocua in fruit juices. *Journal of Food Protection*, *69*(7), 1579-1586.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. *Comprehensive reviews in food science and food safety*, *8*(3), 157-180.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food chemistry*, *91*(4), 621-632.

Sell C. (2010). Chemistry of essential oils. In K. Başer & G. Buchbauer (eds.), *Handbook of Essential Oils. Science, Technology, and Applications* (pp. 121-43). CRC Press: Boca Raton.

Tzortzakis, N. G., & Economakis, C. D. (2007). Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens. *Innovative Food Science & Emerging Technologies*, *8*(2), 253-258.