Physicochemical and Antioxidant Properties of Cymbopogon citratus Essential Oil

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Abstract

In this study, the essential oil (EO) of lemongrass (*Cymbopogon citratus*) or "lemon tea" leaves was studied. The EO was obtained by the steaming ($0.75 \pm 0.05\%$) and distillation ($1.5 \pm 0.07\%$), assisted by microwaves, methods. The EO had a refractive index of 1.483 ± 0.001 ($20 \,^{\circ}$ C) and a density of $0.873 \pm 0.005 \,\text{g/mL}$ ($27 \,^{\circ}$ C). Color parameters of the oil corroborate the yellow hue observed by the naked eye. The Gas chromatography-Mass spectrometry (GC-MS), Fourier Transform Infrared (FT-IR) Spectrometry, and Nuclear Magnetic Resonance (NMR) techniques permitted to characterize the EO and revealed the chemical structure of the major component: citral (neral and geranial). The phenolic compounds content was $149.2 \pm 6.0 \,\text{mg}$ Gallic acid equivalents (GAE) per 100 mL of oil and the antioxidant activity was $44.06 \pm 0.20 \,\text{mg}$ Trolox (T) per 100 mL of essential oil.

Keywords: essential oil, Cymbopogon citratus, distillation, antioxidant activity, NMR, GC-MS, FT-IR

1. Introduction

Essential oils are defined as volatile substances of a complex mixture of chemical components (terpenes, monoterpenes, terpenoids, alcohols, aldehydes, and ketones) which evaporate when contact with air and are biosynthesized by plants (Parikh & Desai, 2011; Acevedo, Navarro, & Monroy, 2013). They can be obtained from different parts of plants and are generally recognized as safe (GRAS). Attention is now given to natural antimicrobial substances of plant origin since they could be a rich source of bioactive compounds (Burt, 2004; Silva, Gutierrez, Weisheimer, & Schapoval, 2008; García et al., 2008; Bakkali et al., 2008) and they might replace synthetic additives. The essential oils of basil (Zivanovic, Chi, & Draughon, 2005), garlic, cinnamon, lemongrass (Pranoto, Salokhe, & Rakshit, 2005; Xing et al., 2011; Maqbool et al., 2011; Azarakhsh et al., 2013), oregano (Dos Santos et al., 2012; Vatavali et al., 2013) and rosemary (Ponce, Roura, Del Valle, & Moreira, 2008) have been added as active chemical compounds in edible coatings.

The scientific name of lemongrass is *Cymbopogon citratus*. The *Cymbopogon* word derives from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the arrangement of the spike of the flower. The word *citratus* derives from the old Latin, meaning lemon-scented leaves (Shah et al., 2011). The common name of *Cymbopogon citratus* in Mexico is "zacate limón" (lemongrass) or "té limón". It is a perennial tropical grass; is resistant to different temperatures and can grow in warm, semi-warm and temperate climates. It is from 60 to 120 centimeters high, its leaves are green, long and slats and have pleasant aroma and taste. This grass is native to India (Parikh & Desai, 2011). Because of its pleasant flavor, in Mexico is consumed as infusion in water or milk just because the herbs intake is a custom in the Mexican population (Juárez-Rosete et al., 2013). The essential oil of *Cymbopogon citratus* has shown to have anti-inflammatory, analgesic and antipyretic properties, (Gbenou et al., 2013) besides having antimicrobial effects (Hammer, Carson, & Riley, 1999; Pranoto, Salokhe, & Rakshit, 2005; Adukwu, Allen, & Phillips, 2012). Tzortzakis & Economakis (2007) reported that the essential oil of lemongrass inhibited the growth of *Botrytis cinerea*. Later Raybaudi-Massilia, Rojas-Graü, Mosqueda-Melgar, and Martín-Belloso (2008) reported that the oil of lemongrass could suppress the growth of mesophiles and psychrophiles in fresh-cut apples.

The aim of this study was to obtain and characterize the essential oil of Cymbopogon citratus.

2. Materials and Methods

2.1 Plant Material

Fresh Cymbopogon citratus leaves were acquired in the Puebla City Central Market, Puebla, Mexico.

2.2 Essential Oil Extraction

Fresh *Cymbopogon citratus* leaves were dried at room temperature for one week; leaves were extended on trays, turning them three times daily for ventilation, accelerate drying, and preventing the growth of microorganisms. The essential oil of *Cymbopogon citratus* was obtained by two methods. *Hydrodistillation*: 50 g of ground plant material was extracted for about 60 minutes (Baizabal, 2010) using a Clevenger apparatus. *Microwave assisted extraction*: 50 g of ground leaves and 200 mL of distilled water were placed in a 500 mL spherical flask; the distillation was performed for about 30 min. The distillation apparatus was placed inside of a conventional Daewoo DC (model KOR 6LYB) microwave oven. A power of 600 W was used. The yield of oil was calculated by the following equation:

Yield (%) = (Oil (mL))/(Plant (g)) \times 100

2.3 Physicochemical Properties

2.3.1 Refraction Index

To measure the refractive index of oil, an Atago ND R5000 refractometer (Osaka, Japan) was used according to the 921.08 AOAC (2000) method.

2.3.2 Density

It was assessed according to the 985.19 AOAC (2000) method using a 2 mL pycnometer.

2.3.3 Color

The CIELAB color parameters were measured using a Colorgard 05 colorimeter (Gardner Laboratory, USA) in the transmittance mode. The equipment calibration was performed with the black mosaic and the calibration parameters: $L^* = 100$, $a^* = 0$, and $b^* = 0$. A cell of quartz and a volume of 3 mL of oil was used.

2.4 Gas Chromatography-Mass Spectroscopy

It was performed using an Agilent Technologies 6850 gas chromatograph (Santa Clara, CA, USA) coupled to an Agilent Technologies 5975 mass selective detector (Santa Clara, CA, USA). An Agilent capillary column (HP-5 ms nonpolar 5 % phenyl methyl polysiloxane) of 30 m, 0.25 mm in diameter and 0.25 microns in thickness was used. Helium, at a flow rate of 1.1 mL/min, was used as the carrier gas. The oven temperature of the gas chromatograph was maintained at 300 °C. The temperature in the column was started at 60 °C and maintained for 2 min, then increased, until reaching 250 °C, at a rate of 10 °C/min. Therefore, the final temperature was maintained for 10 min. One microliter of oil was injected into the column using a split injection of 10:1. The injector temperature was 250 °C (Conde-Hernández & Guerrero-Beltrán, 2014). The components were identified by their fragmentation patterns of mass spectra compared with data stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 2010).

2.5 Fourier Transform Infrared (FT-IR) Spectrometry

Spectrometry of the EO of *Cymbopogon citratus* was carried out with a Spectrum One FT-IR Spectrometer, (PerkinElmer[®], Waltham, Massachusetts, USA). An EO sample was placed directly on the surface of the ATR top plate at room temperature; measurements were performed in the IR region at 4000-650 cm⁻¹. Four scans were performed at a resolution of 4.00 cm⁻¹ at a scan speed of 0.20 cm/s. The crystal used was diamond SSNS. An air spectrum was used as reference (Wany et al., 2014).

2.6 Nuclear Magnetic Resonance (NMR)

NMR spectroscopic measurements were performed using a Varian Gemini 200 spectrometer (Varian Associates Inc., Palo Alto, CA, USA) (operating at 200 MHz for hydrogen) using deuterated chloroform solution containing tetramethyl silane (TMS) as internal standard (Fortuna et al., 2011).

2.7 Phenolic Compounds

The Folin & Ciocalteu method (Singleton, & Rossi, 1965) was used for determining phenolic compounds with some modifications. Fifty microliters of EO were diluted with 950 μ L of ethanol. From this dilution, 500 μ L were taken and added with 3 mL of distilled water and 250 μ L of Folin-Ciocalteu reagent. The reaction was

stopped adding 750 μ L of 20 % Na₂CO₃. Finally, the solution was brought to 5 mL with distilled water. The reaction was left in the dark for 2 hours. Samples were filtered through Whatman paper No. 4 before recording absorbances. A blank was prepared with 4 mL of distilled water, 250 μ L of Folin-Ciocalteu reagent, and 750 μ L of 20% Na₂CO₃. The absorbance was recorded at 765 nm using a UNICO[®] 2800H UV–visible spectrophotometer (Shanghai, China). Phenolic compounds were expressed in mg equivalent of Gallic acid per 100 mL of oil. The measurements were performed in triplicate. The phenolics concentration was calculated from a standard curve of Gallic acid (0-0.0672 mg): Abs = (15.03/mg)*[mg]+0.0886 (R² = 0.973). From a standard solution of Gallic acid (0.186 mg/mL), nine tubes were prepared to obtain concentrations of 0 to 400 μ L.

2.8 Antioxidant Capacity (AC)

The method used to determine antioxidant capacity of the essential oil was adapted from Brand-Williams, Cuvelier and Berset (1995) with some modifications. *Radical solution*: it was prepared dissolving 3.94 mg of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Toluca, Edomex, Mexico) in 100 mL of ethanol. *AC*: Fifty μ L of EO was mixed with 950 μ L of ethanol, 2 mL of DPPH radical solution, made up to 4 mL with ethanol, and thoroughly mixed. The mixture was left to react in the dark for 30 minutes. The absorbance of samples (*A_s*) was recorded at 517 nm in a UNICO® 2800H UV–visible spectrophotometer (Shanghai, China). A blank (*A_b*) was prepared using the same procedure substituting only the sample for ethanol. The percentage of inhibition (*I*) was calculated as follow:

$I = ((A_b - A_s)/A_b) * 100$

The antioxidant capacity (mg T/100 mL of EO) was calculated from a standard curve of Trolox (0-0.028 mg): I = (2699.2/mg)*[mg]-4.3749 (R² = 0.989). To prepare the calibration curve a standard solution of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97 %) was prepared (0.35 mg/mL) in ethanol. Eight tubes were used to obtain concentrations in the range 0 to 0.028 mg of Trolox.

2.9 Statistic Analysis

An analysis of variance and Tukey test were performed to state differences between means using the Minitab 17 software (LEAD Technologies Inc., NJ). A P value of 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Essential Oil Yield

Yields of 0.752 ± 0.05 and $1.5 \pm 0.07\%$ (V/W) of EO were obtained by the steam and microwave assisted distillation methods, respectively. Differences (p < 0.05) within means of yields were observed. Very probably the core of ground plant placed in the spherical flask was efficiently heated due to microwaves. As observed in other works, when using a simple distillation procedure, sometimes the steam do not reach the center of the ground plant; therefore, the yield of the oil extraction is not optimized. The moisture content of the dried *C. citratus* was 9.14 \pm 0.70%.

3.2 Physical Properties

The values of refractive index, color, and density are shown in Table 1. These values are close to those reported by Tovar et al. (2011) ($\rho = 0.885$ g/mL; IR = 1.482), Paviani, Pergher, and Dariva (2006) ($\rho = 0.848$ g/mL), Essien, Essien, Ita, & Ebong (2008) ($\rho = 0.888$ g/mL; IR = 1.477), Ibrahim, Ibo and Adejare (2010) ($\rho = 0.866$ g/mL; IR = 1.472), and Monteiro et al. (2011) ($\rho = 0.949$ g/mL; IR = 1.332) who reported similar refractive index and specific gravity values for essential oils of lemongrass, lemongrass, citron (*Citrus medica* L.), ginger, and allspice (*Pimenta dioica*), respectively, obtained by hydrodistillation. Color parameters indicate a yellow hue (68.81 ± 0.36) which was observed in the EO. Tovar et al. (2011) reported high values for *L** (98.59 to 101.63) but similar values for *a** (-2.45), and *b** (6.28) color parameters for the *Cymbopogon citratus* EO.

Characteristic		Value
Refraction index (20 °C)		1.483 ± 0.001
Density (g/mL) (27 °C)		0.873 ± 0.005
Color parameters	L^*	97.04 ± 0.25
	a*	-2.44 ± 0.04
	b^*	6.29 ± 0.12

Table 1. Refractive index, density, and color parameters of dried *C. citratus* EO obtained by distillation assisted by microwaves

3.3 Chemical Compounds by GC-MS

Tables 2 and 3 list the main components of the EO of C. citratus revealed by the GC-MS technique.

Table 2. Chemical compounds of essential oil of dried C. citratus obtained by steam distillation

Chemical	Area (%)
Cis-linalool oxide	0.7
Linalool	0.64
2-hydroxy-1, 1, 10-trimethyl-6,9-decalin epidioxi	8.09
Oxirane methanol, 3-methil-3-(4-methyl-3-pentenyl)	28.4
Dihydronopol	2.52
Neral	19.35
Geranial	15.97
Geranic acid	5.76
Neric acid	9.15
2-tridecanone	1.21
7-Methyl-z-tetradecen-1-ol- acetate	2.37
1-allyl-2-hydroxy-6-methyl-cyclohexanecarboxylic acid	1.1
9-methyl-z10-tetradecen-1-ol-acetate	4
Cholestan-3-ol, 2-methylene $(3\beta, 5\alpha)$	0.3
Ethyl-linoleate	0.43

Table 3. Chemical compounds of essential oil of dried C. citratus obtained by distillation assisted by microwaves

Chemical	Area (%)
3-Methyl-2-butenal	0.16
Nerol	1
Limonene	0.46
Citronellal	0.21
2-cyclohexen-1-one	0.33
Cis-linalool oxide	0.4
Linalool	0.86
Neral	29
Geranial	22.63
Methyl acetate	2.56
Oxirane carboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	25.29
Cis-pulegone oxide	3.25
Neric acid	9.19
Carotol	0.88
7-methyl-z-tetradecen-1-ol-acetate	0.25

The predominant component of the essential oil of *C. citratus* obtained by the two distillation methods of extraction was citral (mixture of geranial and neral aldehydes) (Figure 1). These results agree with those reported by Mahanta et al. (2007) and Negrelle and Gomes (2007). Adukwu, Allen, and Phillips (2012) and Andrade et al. (2012) informed that citral was the main component in *C. flexuosusu* and *C. nardus*, respectively. The highest percentage (51.63%) of citral in the EO obtained by the microwave assisted distillation method is close to that reported by León-Anzueto et al. (2011) (59.7 to 64.0%) in *C. citratus* (DC.) Stapf. Chanthai, Prachakoll, Ruangviriyachai, and Luthria (2012) reported that the plant has a content of citral of 65 to 80%. Mohamed Hanaa, Sallam, El-Leithy, and Aly (2012) reported concentrations of geranial (31.53%, 39.86% and 37.24%), neral (30.08%, 34.52% and 31.28%) and myrcene (16.61%, 14.49% and 15.42%) in essential oils extracted from leaves of *Cymbopogon citratus*, dried in the sun, in the shade, and in oven, respectively. The differences in type of components reported by other researchers is probably due to the climatic conditions where the plant was grown. The quality of *Cymbopogon citratus* is determined by the content of citral. It has been observed that citral has an inhibitory effect on bacteria and fungi (Adinarayana et al., 2012; Marques et al., 2013).



Figure 1. Chemical structures of geranial and neral

3.4 Infrared Spectrum

As mentioned above, the main component of the *C. citratus* EO is citral. In the IR spectrum (Figure 2) of EO of *C. citratus*, the functional groups of citral were observed. In the vibrations at 2968 cm⁻¹, a predominant asymmetric stretching of -CH₃ is observed corresponding to an alkyl saturated aliphatic group and at 2915 and 2857 cm⁻¹, symmetric and asymmetric stretching of -CH₂ are observed. The intense band observed at 1671 cm⁻¹ is due to vibrations of C=C (cis and trans), confirming the presence of conjugated double bonds (C = C-CHO) in citral which are common in acyclic monoterpenes. The peak at 1632 cm⁻¹ indicates stretching of C=O of the aldehyde group. At the 1442 cm⁻¹ peak, bending of the -CH₂ group is observed. At 1377 cm⁻¹ bending of -CH₃ group is observed. From 1194 to 1120 cm⁻¹, stretching of -C-O and vibrations of the -CH skeleton are observed. At 841 cm⁻¹, substitution in 1,3 and 1,4 are observed. Similar peaks were reported by Wany et al. (2014) for geranial from other species of *Cymbopogon winterianus* (citronella grass).



Figure 2. Infrared spectrum of Cymbopogon citratus essential oil

The IR spectrum of *C. citratus* EO is consistent with data obtained from gas chromatography coupled to mass spectroscopy, IR shows principally the citral spectrum (Mahanta et al., 2007; Marques et al., 2013) which is the main component in the mixture of chemicals in this oil. In addition, the IR spectrum shows absorption at 3334 cm⁻¹, characteristic of the -OH group which is also in agreement with results of the GC-MS analysis where the presence of nerol and other alcohols in small proportions were observed.

3.5 Nuclear Magnetic Resonance (NMR)

The NMR analysis allowed the characterization and structural elucidation of the main component in the essential oil of *C. citratus*. The NMR spectra of hydrogen (1 H) (Figure 3) and carbon-13 (C 13) (Figure 4) clearly show that the EO of *C. citratus* is composed of citral (Restrepo, Vinasco, Jaramillo, & Colmenares, 2009), where the two typical signals of the group aldehyde correspond to geranial and neral in proportions of 61 and 39%, respectively.





Figure 4. 13 C NMR spectrum of Cymbopogon citratus essential oil

3.6 Phenolic Compounds

Fruits and vegetables containing phenolic compounds may have high antioxidant activity, which means that they could have positive effects on the preservation of foods quality and human health (Becker, Nissen, & Skibsted, 2004). Some studies have shown that phenolic compounds having antioxidant activity may retard aging, prevent degenerative diseases such as cancer, cardiovascular disorders, and brain dysfunctions (Ames, Shigenaga, & Hagen, 1993). In this study, a phenolic content of $149.2 \pm 6.0 \text{ mg GAE per 100 mL of oil (obtained by distillation assisted by microwaves) was obtained. Koh, Mohd, Mokhtar, & Iqbal (2012) reported a phenolic content of <math>30.74 \pm 1.13 \text{ mg GAE per g of sample in ethanol extracts of$ *Cymbopogon citratus*. Mirghani, Liyana, and Parveen (2012) reported 184.69 mg of GAE per 100 mL of EO of*Cymbopogon citratus*lives. Several factors can affect the content of phenolic compounds in plants: preparation (drying time, temperature, among others) and growing conditions of the plant as well as the extracting method and the technique of analysis (Moraes-de-Souza et al., 2008).

3.7 Antioxidant Capacity

The essential oil of *C. citratus* had an antioxidant capacity of 44.06 ± 0.20 mg Trolox per 100 mL of essential oil (obtained by distillation assisted by microwaves), equivalent to 55.57% of inhibition. Selim (2011) reported values of 40.63 to 63.75% inhibition for *Cymbopogon proximus* (Stapf). Mirghani, Liyana, and Parveen (2012) reported higher values of inhibition (78.89%) when diluting the EO at 1:2 oil:methanol proportion. The differences in the antioxidant capacity values, reported by different researchers, can be attributed to factors such as climate, soil composition, and season as well as part, age and stage of growing of the plant (Angioni et al., 2006) in addition to the EO or extract concentration (Mirghani, Liyana, & Parveen, 2012). The antioxidant activity of the EO of *C. citratus* could be of great interest in the food industry to be used as a natural additive for flavoring.

4. Conclusions

The distillation, assisted by microwaves, was the best method for extracting the EO. The physicochemical properties of the EO of *C. citratus* were determined. The EO of *C. citratus* had high citral (a mixture of geranial and neral aldehydes) concentration which was corroborated by different instrumental techniques (GC-MS, FT-IR, and NMR). The EO showed high phenolics content (149.20 mg GAE per 100 mL) and antioxidant capacity (44.06 mg Trolox per 100 mL of essential oil).

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