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Chemical composition and antioxidant activity of *Lippia alba* essential oil obtained by supercritical CO₂ and hydrodistillation

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Lippia alba essential oils were obtained by hydrodistillation (HD) and supercritical fluid (SFE) extraction methods. These were analyzed by gas chromatography-mass spectrometry-flame ionization detector (GC-FID-MS). Antioxidant activity was tested by DPPH and ABTS methods, and total soluble phenolics (TSP) were also determined. While in the SFE extract 14 compounds were identified (mostly: myrcenone 3.4%, α -terpineol 1.0% and β -caryophyllene 2.3%), in the HD extract 17 compounds were identified (mostly: eucalyptol 15.6%, myrcenone 9.3% and Z-ocimenone 5.8%). The results showed that *L. alba* essential oils obtained by SFE was IC₅₀=17.35 mg/mL and by HD was IC₅₀ = 12.45 mg/mL in DPPH assay. *L. alba* has an excellent eucalyptol content.

Key words: Antioxidants, bioactive compounds, supercritical fluid, green technologies.

INTRODUCTION

The Verbenaceae family consists of 76 genera with over 2000 species widely distributed across almost all the planet. The genus *Lippia* includes about 200 species that are abundantly present in Central America, South America and Africa. *L. alba* produces aromatic leaves and flowers and aerial portions of the plant are routinely used in native medicines. Some of the medicinal properties of *L. alba* or other *Lippia* species have been attributed to the presence of biologically active volatile

components found in the essential oil of the plant (Hennebelle et al., 2006). The composition of the essential oil is variable and depends on the characteristics of the geographic location, climate and soil. Based on the identity of the major constituents found in essential oil samples from around the world, it is proposed that at least seven chemotypes exist (Table 1) (Hennebelle et al., 2006). Although the essential oil composition of *L. alba* samples from many regions has

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Table 1. Characteristics of the seven chemotypes of *Lippia alba* essential oil.

Chemotype	Major compounds
I	Citral, linalool, and β -Caryophyllene
II	Ocimenone and myrcenone
III	Limonene, carvone, dihydrocarvone, piperitone, piperitenone and lippione.
IV	Myrcene
V	α -Terpinene
VI	Camphor and Eucalyptol
VII	Estragole

been reported, there are presently no reports for the analysis of plants harvested in Mexico (García-Abarrio et al., 2014; Linde et al., 2016; Braga et al., 2005). In Mexico, *L. alba* is an aromatic shrub or tree, up to 2 m tall; this plant is popularly known by the name of mints leaves (Willman et al., 2000). The leaves and flowers of this plant are commonly prepared as an infusion or decoction that is employed as folk remedies for the treatment of diarrhea and stomach ache (Pascual et al., 2001).

Aromatic and medicinal plants have been extensively studied for their antioxidant activity among other functional properties. This is mainly due to an obvious consumer preference for natural ingredients combined with concerns about toxic effects of synthetic antioxidants (Agnaniet et al., 2005; Wojdylo et al., 2007). Essential oils are composed of many chemical compounds; therefore some of them might be regarded as valuable components and might exhibit antioxidant potency (Kamaliroosta et al., 2012).

With the exception of the work by Stashenko et al. (2004), *L. alba* essential oil extracts have been principally obtained by a singular method, either hydrodistillation or microwave radiation-assisted hydrodistillation (Bahl et al., 2000; Lorenzo et al., 2001; Fischer et al., 2004; Mesa-Arango et al., 2009; Escobar et al., 2010). The Supercritical Fluid Extraction (SFE) systems extract chemical compounds normally using supercritical carbon dioxide instead of an organic solvent, as compared to the hydrodistillation method. The supercritical fluid state occurs when a fluid is above its critical temperature (T_c) and critical pressure (P_c), when it is between the typical gas and liquid state (Oliveira et al., 2016; Zermane et al., 2014; Bagheri et al., 2014).

Stashenko et al. (2004) compared different methods of extraction in terms of composition. Extracts were obtained through hydrodistillation, microwave radiation-assisted hydrodistillation, distillation solvent extraction and supercritical fluid extraction and their results showed that the method of extraction affected quantitatively the essential oil composition. In other work, Duran et al. (2007) found that extraction time directly impacted the composition of *L. alba* essential oil extracts prepared by microwave radiation-assisted hydrodistillation. The

influence of extraction method on the resulting essential oil composition has also been reported for clove buds, patchouli, and *Mirtus communis* L. (Wenqiang et al., 2007; Donelian et al., 2009; Ghasem et al., 2011). Thus, it appears essential to use more than a single extraction technique when characterizing a plant's volatile secondary metabolite composition (Stashenko et al., 2004).

Given the above, this work aimed to obtain fractions of essential oil of *L. alba* by supercritical CO₂ and hydrodistillation, and to evaluate their chemical composition and antioxidant activity.

MATERIALS AND METHODS

Plant material

L. alba leaves were collected in Sinaloa, Mexico (24°45'56.8"N, 107°39'03.6"W), and plants were confirmed and authenticated by the botanical researcher Rito Vega-Aviña from the herbarium "Jesus Gonzalez-Ortega" located at the Agricultural School from the Sinaloa State University [*Lippia alba* (Mill.) N. E. Br. ex Britton & Wilson, ACJA 85 (UAS), AEJA 43 (UAS); PTJL 86 (UAS); VAR 7248 (UAS)]. The leaves (old and young) were separated from the plant manually and combined into a single lot. *L. alba* leaves were dried in an oven at 40°C for 12 h. The dried leaves were ground with a grinder to produce a fine powder that was stored at room temperature in polyethylene bags.

Chemicals

Cinnamon essential oil, butylated hydroxytoluene (BHT), carvacrol, limonene, carvone, geraniol, camphor, citral, p-cymene, thymol eucalyptol, β -caryophyllene, cinnamaldehyde, benzyl alcohol, estragole, myrcene, α -terpineol, caryophyllene oxide, linalool, eugenol and α -terpinene were purchased from Sigma Aldrich (St Louis, Mo, USA). Origanum oil was purchased from LKT Laboratories. Oregano, lemongrass and chinese cinnamon oils were purchased from Lhasa Karnak Herb company (Berkeley, Ca, USA). Piperitone was purchased from TCI America (Portland, Or, USA). Dichloromethane (HPLC grade) was purchased from Acros (New Jersey, USA) and methanol (HPLC grade) was purchased from Fisher Scientific (New Jersey, USA). Kovats standard (C7-C30) was purchased from Supelco (Bellefonte, PA, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from MP Biomedicals (Aurora, Ohio, USA).

Hydrodistillation

Dried leaves (10 g of powder) were submitted to hydrodistillation for 2 h, using a distilling flask, a vigrex column, a condenser and a receiving vessel. The essential oil layer and aqueous layers were separated and the oil collected. The oil was weighed using an analytical balance (Mettler-Toledo, Columbus, OH, USA) and stored in a glass vial at 4°C protected from light.

Supercritical fluid extraction

Extraction of *L. alba* essential oil was prepared using a supercritical fluid equipment (Model SFT-150, Supercritical Fluid Technologies, Inc., Newark, DE, USA). The flow rate of supercritical CO₂ was 5 mL/min. The extraction vessel (100 mL) was charged with 10 g of plant powder. The conditions of extraction were 47°C and 3800 Psi. The extract was collected in a glass vial. In order to improve the collection efficiency, the collected vial was placed in an ice bath during the dynamic extraction. The extract weight was measured using an analytical balance and stored in a glass vial at 4°C protected from the light.

GC and GC/MS analyses

GC analyses were performed using a Hewlett-Packard 6890 series gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector (FID) and a DB-1 column (60 m × 0.2 mm i.d. × 0.25 µm of film thickness) (Agilent Technologies, Wilmington, DE, USA). Oven temperature was programmed at 45°C (2 min), and then increased to 270°C (20 min) at a rate of 4.5°C/min. Injector temperature was 250°C and detector temperature was 270°C. Helium was used as carrier gas with a linear velocity of 32 mL/min. The samples (1 µL) were injected using the splitless mode.

The GC/MS analyses were carried out on two separate systems. The first system consisted of a HP-589066 with a DB-Wax column (60 m × 0.25 mm i.d. × 0.25 µm of film thickness) coupled to a HP-5972 MSD. The GC oven temperature was programmed from 35°C hold for 5 min to 230°C hold for 40 min at 4.5°C/min. Injector temperature was 250°C. The temperature of the ionization chamber was 285°C. The second system was a HP-6890 series (Wilmington, DE, USA) with a DB-1 column (60 m × 0.2 mm i.d. × 0.25 µm of film thickness) coupled to a HP-5972 MSD (Agilent Technologies, Wilmington, DE, USA). The GC oven temperature was programmed from 45°C hold for 5 min to 270°C hold for 20 min at 4.5°C/min. Injector temperature was 250°C. The temperature of the ionization chamber was 285°C. The samples were diluted in dichloromethane and 1 µL of the solution were injected using the splitless mode. The compounds of the extracts were identified by Kovats retention indices and comparing their mass spectra with those in the NIST 2011 mass spectra library and with authentic standards.

Antioxidant activity

DPPH radical assay

The radical scavenging capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the Agnani et al. (2005) method with some modifications. Cinnamon, oregano, organum, Chinese cinnamon and *L. alba* (HD) extracts and standard samples were dissolved in methanol at different concentrations. Supercritical fluid extraction of *L. alba* extracts was dissolved in methanol, centrifuged (1300 rpm, 5 min at 5°C) and then filter (13 mm and 0.22 µm porosity, PVDF. Millex-GV, Millipore). DPPH was dissolved in methanol to give a 100 µM solution. To 1.2 mL of the methanolic solution of DPPH were added

100 µL of a methanolic solution of the antioxidant compounds (standards and essential oils) at different concentrations. The control is represented by the DPPH methanolic solution containing 100 µL of methanol. After a 30 min incubation period at room temperature the decrease in absorption at 517 nm was measured with a SpectraMax Plus 384 spectrophotometer (Molecular Device, USA). Antioxidant activity was reported as IC₅₀ (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%) and was calculated using SoftMax® Pro software Version 5.0.1 (Molecular Devices Corp., USA). BHT was used as a positive control. The assay was carried out in triplicate and results are reported as averages.

ABTS radical cation decolorization assay (TEAC)

The antioxidant activities of essential oils were determined by the method of Re et al. (1999) with some modifications. Cinnamon, oregano, organum, Chinese cinnamon and *L. alba* (HD) oils were dissolved in methanol. Supercritical fluid extraction of *L. alba* essential oil was dissolved in methanol, centrifuged (1300 rpm, 5 min at 5°C) and then filtered (13 mm and 0.22 µm porosity, PVDF. Millex-GV, Millipore). ABTS was dissolved in water (HPLC grade) to a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. For the determination of antioxidant activity the ABTS radical solution was diluted with methanol to an absorbance of 0.70 ± 0.01 at 734 nm. To 20 µL of the sample was added 400 µL of diluted ABTS radical solution. After incubation for 6 min at 30°C, the absorbance was measured with a SpectraMax Plus 384 spectrophotometer at 734 nm. Ascorbic acid (0.05 mg/mL) and BHT (0.125 mg/mL) were used as a positive control and cinnamic acid (1 mg/mL) as a negative control. The activities of essential oils were estimated within the range of Trolox calibration curve and results were expressed as µmol Trolox equivalent per gram (Tr eq µmol/g). The assay was carried out in triplicate and results are reported as averages.

Total soluble phenolics

The Folin-Ciocalteu method for the colorimetric estimation of total polyphenols was adapted to a 96-well plate format according to Breksa et al. (2010). Cinnamon, oregano, organum, Chinese cinnamon and *L. alba* (HD) oils were dissolved in methanol. Supercritical fluid extraction of *L. alba* essential oil was dissolved in methanol, centrifuged (1300 rpm, 5 min at 5°C) and then filtered (13 mm and 0.22 µm porosity, PVDF. Millex-GV, Millipore). Standards (100 µL) were mixed with water (1500 µL) in a 2 mL polypropylene plate. Trolox (250 µg/mL) and BHT (250 µg/mL) were used as positive controls and cinnamic acid (1.0 mg/mL) was used as a negative control. Samples were mixed with water 100:1500 (sample: H₂O). Controls were diluted with water in the same way as samples. All dilutions were mixed with Folin Ciocalteu's phenol reagent (1 N, 100 µL). After a brief incubation at room temperature (5 min), saturated sodium carbonate (300 µL, 75 g/L) was added. Solutions were mixed and incubation continued at room temperature. After 2 h, 300 µL were transferred to a well of a 96-well plate and the absorbance measured at 765 nm with a molecular Devices Spectromax 384-Plus plate reader (Sunnyvale, CA). Quantification was based on the standard curve generated with 50, 100, 200, 300, 400 and 500 mg/L of gallic acid. Samples with absorbance values greater than the 500 mg/L standard were diluted and reanalyzed. Values were reported as µmol gallic acid equivalents (GAE) per gram material ± SD and represent the average of three independent analyses.

Table 2. Chemical composition of *L. alba* essential oil obtained by hydrodistillation and SFE.

Number of peak	Compound	RI (DB-1)	HD	SFE
1	Octen-3-ol	968	1.6	n.d.
2	Myrcene	980	n.d.	n.d.
3	Benzyl alcohol	1010	n.d.	n.d.
4	p-Cymene	1017	n.d.	n.d.
5	Limonene	1023	n.d.	n.d.
6	Eucalyptol	1024	15.6	n.d.
7	γ -Terpinene	1053	n.d.	n.d.
8	Trans-Linalool oxide	1060	Tr	n.d.
9	Linalool	1086	6.2	0.4
10	Myrcenone	1123	9.3	3.4
11	Camphor	1124	n.d.	n.d.
12	Terpine-4-ol	1164	0.5	0.1
13	α -Terpineol	1175	7.0	1.0
14	Estragole	1177	n.d.	n.d.
15	(E)-Tagetone	1130	0.5	Tr
16	(Z)-Tagetone	1151	0.7	Tr
17	(Z)-Ocimenone	1206	5.8	0.8
18	Neral	1217	n.d.	n.d.
19	Carvone	1218	n.d.	n.d.
20	Piperitone	1229	n.d.	n.d.
21	Cinnamaldehyde	1232	n.d.	n.d.
22	Geraniol	1235	n.d.	n.d.
23	Geranial	1245	n.d.	n.d.
24	Thymol	1268	n.d.	n.d.
25	Bornyl acetate	1274	n.d.	n.d.
26	Carvacrol	1278	n.d.	n.d.
27	Eugenol	1333	n.d.	n.d.
28	α -Copaene	1382	0.9	0.4
29	Geranyl acetate	1362	n.d.	n.d.
32	β -Caryophyllene	1426	0.9	2.3
33	α -Humulene	1458	Tr	0.4
34	Allo-Aromadendrene	1463	1.1	0.5
35	Germacrene D	1487	0.7	8.2
36	Spathulenol	1576	1.7	0.9
37	Caryophyllene oxide	1584	8.0	1.5

RI, Retention index obtained using a DB-1 column. HD, Hydrodistillation; SFE, supercritical fluid extraction; n.d., no detected; Tr, Traces.

RESULTS AND DISCUSSION

Chemical composition

Table 2 shows the relative proportion of the compounds identified in both samples (SFE and HD) using a DB-1 column. Also in order to confirm the identity of the compounds a DB-wax column was used (data not show) but it is important to highlight that the retention index value for the ketone: myrcenone was 1588, based on the fact that no previous reports were found using this type of column.

L. alba is probably the most studied species in the

Lippia genus however, in spite of the diversity and medicinal properties of this plant, in Mexico no previous reports have reported about the chemical compositions of *L. alba* essential oil (Hennebelle et al., 2008). As show in Table 2 the major compounds in *L. alba* essential oil obtained by SFE were myrcenone (3.4%), α -terpineol (1.0%), β -caryophyllene (2.3%), germacrene-D (8.2%) and caryophyllene oxide (1.5%), while by hydrodistillation the major compounds were eucalyptol (15.6%), myrcenone (9.3%) and (Z)-ocimenone (5.8%) (Table 2). Based on these results and in accordance with Hennebelle et al. (2006) chemotypes classification (Table 1), *L. alba* essential oil could correspond to chemotype II (ocimenone

Table 3. Main compounds in essential oils.

Sample	Main compounds
<i>L. alba</i> oil (HD)	Eucalyptol (15.6%), myrcenone (9.3%), caryophyllene oxide (8.0%), α -terpineol (7.0%), linalool (6.2%) and (Z)-ocimene (5.8%),
<i>L. alba</i> oil (SFE)	Myrcenone (3.4%), α -terpineol (1.0%), β -caryophyllene (2.3%), germacrene-D (8.2%) and caryophyllene oxide (1.5%)
Cinnamon oil	Cinnamaldehyde (71.63%), eugenol (7.40%), limonene (3.82%), benzyl alcohol (3.30%), 3-phenylpropanol (2.56%) and linalool (1.25%)
Chinese cinnamon oil	Cinnamaldehyde (81.74%), cinnamaldehyde,o-methoxy (7.58%), cinnamyl ester (2.71%), coumarin (2.10%) and benzaldehyde (1.28%)
Lemongrass oil	Geranial (46.52%), neral (31.35%), geraniol (5.14%), β -caryophyllene (2.91%), limonene (2.42%), geranyl acetate (1.79%), caryophyllene oxide (1.47%) and linalool (1.15%)
Oregano oil	Carvacrol (66.68%), p-cymene (13.87%), γ -terpinene (6.63%), thymol (3.99%), α -pinene (1.33%), linalool (2.86%) and β -myrcene (1.20%)
Origanum oil	Carvacrol (63.52%), linalool (16.91%), p-cymene (6.45%), γ -terpinene (3.68%), β -myrcene (1.46%) and thymol (0.56%)

% identified by GC-FID (DB-1 column) and GC-MS (DB-1 column).

and myrcenone) since both compounds are present in the sample composition of *L. alba* extract obtained by hydrodistillation.

In the SFE prepared sample the sesquiterpenes including β -caryophyllene (2.3%), α -humulene (0.4%) and germacrene-D (8.2%), were at a greater proportion than found in the hydrodistillation sample. A similar result was observed by Stashenko et al. (2004) who found more sesquiterpenes in the supercritical CO₂ extract of *L. alba* than in extracts obtained with other extraction techniques. Eucalyptol was reported in *Lippia alba* essential oil as the main compound in samples obtained from Uruguay while was differed from that reported from Colombia where the main compounds were carvone (51%) and limonene (32.60%) carvone (31.8-52.6%) and geraniol (15-21.5%) (Mesa-Arango et al., 2009; Dellacassa et al., 1990). In Brazil *L. alba* essential oil was rich in geranial (12.9%) and myrcene (15%) (Oliveira et al., 2006). While in Guatemala, Senatore and Rigano (2001) reported as the main compounds limonene (44%) and piperitone (31%). Fischer et al. (2004) analyzed 16 *L. alba* populations from all over Guatemala. They reported myrcenone (37.8-58%) as the main compound in 14 samples and neral (17.6-18.9%) and geranial (24.7-27%) as the main compounds in two samples collected from two different geographic locations. The variability in the chemical composition of *L. alba* essential oil was attributed to different factors such as season of harvest, collection site, soil composition, water hydric stress, extreme temperatures and sunlight (Nogueira et al., 2007; Olivero-Verbel et al., 2010; Blanco et al., 2007). However, other works have suggested that the composition of the essential oil of *L. alba* may not only be due to climatic or environmental conditions. Pandelo et al. (2012) analyzed

three chemotypes of *L. alba* cultivated at the same conditions and were collected at the same time. No changes were observed in the main compounds of the samples. The authors concluded that these results are a consequence of genetic variation among the chemotypes. Also, it was observed that developmental stage such as flowering and vegetative growth affect the total oil production but not the quality of oil in leaves of *L. alba*, reinforcing the suggestion of genetic control.

In order to compare the chemical composition of *L. alba*, essential oil commercial oils were tested in this work. As show in Table 3 six main compounds were identified in cinnamon oil, representing 89.96% of the total oil. The most abundant components were cinnamaldehyde (71.63%) and eugenol (7.4%). Cinnamaldehyde is one of the main aroma-active compounds in cinnamon essential oil (Schmidt et al., 2006). The results found in this work are in accordance with those of Tomaino et al. (2005) who reported cinnamaldehyde (45.84%) and eugenol (49.09%) as the main compounds in cinnamon oil. The major compounds in Chinese cinnamon oil were cinnamaldehyde (81.74%) and cinnamaldehyde,o-methoxy (7.58%) but eugenol was not present in the sample analyzed. The composition of the essential oil of Chinese cinnamon oil (*Cinnamomum cassia*) have been reported that trans-cinnamaldehyde and cinnamaldehyde,o-methoxy as the main compounds and eugenol content was no present in the sample evaluated (Geng et al., 2011). The major compounds in lemongrass were geranial (46.52%) and neral (31.35%), representing already of 80% of the total oil. These results are similar with previous reports. Sacchetti et al. (2005) reported geranial (41.30%), neral (32.30%) and geraniol (3.35%) as the main compounds. Katsukawa et al. (2010)

Table 4. Table 4. Antioxidant activities (DPPH and ABTS) and soluble phenolics (TSP) of essential oils and essential oil of *Lippia alba* obtained by hydrodistillation and CO₂ supercritical extraction.

Sample	DPPH (IC ₅₀ mg/mL)	TSP (GA Eq μ mol/g)	ABTS (Tr eq μ mol/g)
BHT (control, 0.125 mg/mL)	0.21 $\pm$ 0.01	2718.3 $\pm$ 210.8	1042.0 $\pm$ 75.4
Ascorbic acid (control, 0.050 mg/mL)	NA	NA	5803.4 $\pm$ 92.4
Cinnamic acid (control, 1.0 mg/mL)	NA	NA	No activity
Trolox (control, 0.250 mg/mL)	NA	1220.0 $\pm$ 27.6	NA
<i>Lippia alba</i> oil (HD)	12.45 $\pm$ 0.57	137.5 $\pm$ 1.6	15.6 $\pm$ 0.1
<i>Lippia alba</i> oil (SFE)	17.35 $\pm$ 0.92	84.0 $\pm$ 4.7	55.2 $\pm$ 1.6
Cinnamon oil	0.62 $\pm$ 0.02	448.8 $\pm$ 16.1	6322.1 $\pm$ 143.5
Chinese cinnamon oil	No activity	141.8 $\pm$ 7.9	74.5 $\pm$ 1.7
Lemongrass oil	33.68 $\pm$ 1.99	16.9 $\pm$ 0.1	48.3 $\pm$ 1.1
Oregano oil	3.06 $\pm$ 0.21	1501.0 $\pm$ 39.4	33204.0 $\pm$ 810.4
Origanum oil	3.72 $\pm$ 0.21	1368.4 $\pm$ 47.5	28158.7 $\pm$ 684.7

HD, Hydrodistillation; SFE, supercritical fluid extraction; NA = not analyzed.

reported citral (mixture of geranial (31.43%) and neral (26.03%) as the main compound in lemongrass essential oil. The most abundant components in oregano were carvacrol (66.68%) and p-cymene (13.87%) and for origanum, carvacrol (63.52%) and linalool (16.91%) (Table 1). In both essential oils, carvacrol was the main compound identified. It is well known that in *Origanum* species carvacrol is the main constituents (Hussain et al., 2011). However, thymol concentrations found in this work were lower than that reported previously (Puertas-Mejia et al., 2002).

Antioxidant activity

The chemical complexity of essential oils, often a mixture of dozens of compounds with different functional groups, polarity and chemical behaviour, could lead to scattered results depending on the test employed (Sacchetti et al., 2005). DPPH and ABTS (TEAC) assays are useful for determining the activity of both hydrophilic and lipophilic species (Sacchetti et al., 2005; Katsukawa et al., 2010; Hussain et al., 2011; Puertas-Mejia et al., 2002). The radical scavenging activity of essential oils, assessed by the antioxidant concentration required for 50% reduction in DPPH radical concentration in 30 min (IC₅₀), decreased in the following order: cinnamon oil > oregano oil > origanum oil > *L. alba* oil (obtained by hydrodistillation) > *L. alba* (obtained by SFE) > lemongrass oil; chinese cinnamon oil showed no antioxidant activity (Table 1). It was found that the essential oils analyzed showed very different antioxidant activity (Table 4). The radical scavenging activity (IC₅₀) of standard compounds decreased in the following order: eugenol > carvacrol > thymol. The other standards tested showed low radical scavenging activity (IC₅₀ = >20 mg/mL) (Table 5). Table 4 also shows the antioxidant activities of essential oils tested by ABTS assay. The highest antioxidant activity

Table 5. Antioxidant activities of references compounds.

Standards compounds	DPPH (IC ₅₀ mg/mL)
BHT (control)	0.21 $\pm$ 0.01
Carvacrol	1.85 $\pm$ 0.14
Eugenol	0.041 $\pm$ 0.001
Thymol	2.10 $\pm$ 0.11
α -Terpineol	> 20
Benzyl alcohol	> 20
β -Caryophyllene	> 20
Camphor	> 20
Carvone	> 20
Caryophyllene oxide	> 20
Cinnamaldehyde	> 20
Citral	> 20
Eucalyptol	> 20
Geraniol	> 20
Estragole	> 20
γ -Terpinene	> 20
Limonene	> 20
Linalool	> 20
Myrcene	> 20
p-Cymene	> 20
Piperitone	> 20

decreased in the following order: oregano > origanum > cinnamon > Chinese cinnamon > *L. alba* (SFE) > lemongrass > *L. alba* (HD). The antioxidant activity tested was different between both methods. Antioxidant activities of essential oils from aromatic plants are mainly attributed to their chemical structure (Saleh et al., 2010). Politeo et al. (2006) suggested that the antioxidant activity may be attributed either to high percentage of the main constituents but also to the presence of other constituents in small quantities or to synergy among

them. Respectively the antiradical scavenging activity of *L. alba* (HD) was modest in comparison to the others oils with an IC_{50} = 12.45 mg/mL and 15.6 Trolox eq μ mol/g in DPPH and TEAC assay. In this case it was difficult to attributed the antioxidant activity to the main compounds since eucalyptol showed a low antiradical activity, whereas can be attributed to high percentage of the others main constituents but also to the presence of other compounds in small quantities or to synergy among them (Politeo et al., 2006). Puertas-Mejia et al. (2002) reported that *L. alba* essential oil samples from Colombia showed a low IC_{50} =0.28 kg oil/mmol DPPH and also displayed lower activity against the ABTS radical (14.4 mmol Trolox/kg oil).

Cinnamon essential oil demonstrated the highest inhibitory activity (IC_{50} =0.62 mg/mL) compared to other essential oils tested and this is attributed to the hydrogen-donating capacity of the phenolic component eugenol and this compound was present in significant concentration in the essential oil tested and also presented the highest inhibitory activity with an IC_{50} =0.041 mg/mL (Schmidt et al., 2006; Tomaino et al., 2005) while in ABTS assay, cinnamon oil showed lower activity than oregano and origanum oils. It is of note that Chinese cinnamon oil showed no antioxidant activity by DPPH method and this can be attributed to the absence of eugenol in its composition but showed antioxidant activity by ABTS assay. Mantle et al. (1998) reported that cinnamon oil showed the highest antioxidant activity against ABTS assay but showed no significant evidence of antioxidant activity against hydroxyl and superoxide radicals. Suggesting that the variation in antioxidant capacity of oils extracts depends on the particularly assay method employed to determine antioxidant status. This result can be attributed to the fact that ABTS radicals involve electron transfers process while DPPH radical involve H atoms transfers (Kaviarasan et al., 2007). On the other hand, oregano and origanum essential oil exhibited DPPH radical scavenging activity and this can be related to their chemical compositions since both samples are rich in carvacrol and this compound showed radical scavenging activity with an IC_{50} =1.85 mg/mL. Also, thymol was present in the samples, this compound showed a radical scavenging activity similar than carvacrol with an IC_{50} =2.10 mg/mL. Sahin et al. (2004) reported that DPPH radical scavenging activity of *Origanum vulgare* spp. essential oil was low and this was related to its chemical composition because the percentage of carvacrol were low (0.057%) in the composition. Oregano and origanum essential oils showed better activity by ABTS assay; 33204 and 28158.7 Trolox eq μ mol/g, respectively, than positive control (Table 4). Puertas-Mejia et al. (2002) found that total antioxidant activity of *O. vulgare* L. essential oil against ABTS radical was 25.1 Trolox eq mmol/kg oil while Karakaya et al. (2011) reported that oregano essential oil had a strong inhibitory effect on ABTS

radical cation oxidation with a value of 2.69 Trolox eq μ mol/ μ L of oil.

The radical scavenging of lemongrass essential oil was lower than the other essential oils. The low antioxidant activity can be attributed to the main compounds composition since the standard compounds present in the essential oil were tested and also these compounds showed low activity (Table 5). Also, the antioxidant activity tested by ABTS was low (48.3 Trolox eq μ mol/g). Sacchetti et al. (2005) reported an intermediate inhibition with 60% of radical scavenging activity percentage. These results demonstrate the difficulties in comparing data on antioxidant effectiveness obtained by different assays. Therefore, an approach with multiple assays in screening work is highly advisable (Sacchetti et al., 2005).

Total soluble phenolic

The presence of phenolic compounds in essential oil has been investigated in terms of antioxidant activity. This activity is mainly due to their redox potential, which can play an important role in adsorbing and neutralizing free radicals and quenching reactive oxygen species (Kähkönen et al., 1999). The total soluble phenolic (TSP) were measured by Folin-Ciocalteu reagents in terms of the acid gallic equivalent. TSP content of essential oils decreased in the following order: oregano> origanum> cinnamon>chinese cinnamon>*L. alba* (HD)>*L. alba* (SFE)>lemongrass (Table 4). Generally, a positive correlation between the phenolic content and antioxidant capacity is reported. It has been shown that the antioxidant activity of extracts is roughly connected to their phenolic composition and strongly depends upon their phenolic structures (Chaillou and Nazareno, 2006). It is of note that oregano and origanum had the highest TSP content; 1501 and 1368 GA Eqv μ mol/g, respectively. In this case both essential oils were rich in carvacrol, a phenolic compound. On the other hand, Chinese cinnamon (141.8 GA eq μ mol/g) showed less activity but greater than lemongrass. Lemongrass showed lower phenolic content (16.9 GA eq μ mol/g); these results are in contradiction with previous reports. Mirghani et al. (2012) reported a high phenolic concentration of 2100.7 mg/L GA eq. No data was found concerning the total soluble phenolic content of *L. alba* (SFE and HD) essential oil in previous reports. It does appear that this is the first report. It is of note that the essential oil tested did not seem to depend on TSP since antioxidant activity by DPPH and ABTS assay in all samples were very different between both methods.

Conclusions

The chemical composition and antioxidant activity of the

extracts obtained by SFE and hydrodistillation methods were different. SFE extracts was characterized by the presence of sesquiterpenes compounds. *L. alba* plants from Mexico can be classified in chemotype II (ocimene and myrcene). Results provided here show that *L. alba* essential oil has a good eucalyptol content, which makes it an important natural source for the nutraceutical industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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