ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:4 (2016) 485-496

records of natural products

Composition of Essential Oils and Ethanol Extracts of the Leaves of *Lippia* Species: Identification, Quantitation and Antioxidant Capacity

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(Received April 23, 2015; Revised July 19, 2015; Accepted July 19, 2015)

Abstract: The principal components of essential oils, obtained by steam-hydrodistillation from the fresh leaves of five species of the genus *Lippia*, namely *Lippia gracilis* AV, *Lippia sidoides* Mart, *Lippia alba* carvoneifera, *Lippia alba* citralifera and *Lippia alba* myrceneifera and ethanol extracts, were evaluated. The greater antioxidant capacity (IC_{50} = 980 µg/mL; $p < 0.05$), assessed by the HPLC-based hypoxanthine/xanthine oxidase assay, was determined in the essential oil obtained from *Lippia alba* carvoneifera, while the remainder were comparatively inactive (IC_{50} > 2000 µg/mL). Major compounds in the oils were, thymol (*Lippia sidoides* Mart, 13.1 g/k), carvone (*Lippia alba* carvoneifera, 9.6 g/kg), geranial (*Lippia alba* citraleifera, 4.61 g/kg; *Lippia alba* myrceneifera, 1.6 g/kg), neral (*Lippia alba* citraleifera, 3.4 g/kg; *Lippia alba* myrceneifera, 1.16 g/k), carvacrol (*Lippia gracilis* AV, 5.36 g/kg) and limonene (*Lippia alba* carvoneifera, 5.14 g/kg). Of these, carvone (IC_{50} = 330 µM) along with thymol (1.88 units), and carvacrol (1.74) units, were highly active in the hypoxanthine/xanthine oxidase, and ORAC assays respectively. The following compounds namely **1**: calceolarioside E, **2**: acteoside, **3**: isoacteoside, **4**: luteolin, **5**: 5,7,3′,4′-tetrahydroxy-3,6-dimethoxy flavone (spinacetin), **6**: naringenin, **7**: apigenin, **8**: 6-methoxy apigenin (hispidulin), **9**: 5,7,3′-trihydroxy-3,6,4′-trimethoxy flavone, **10**: 5,7,4′-trihydroxy-3,6-dimethoxy flavone, **11**: naringenin-4′-methyl ether, **12**: 5,7-dihydroxy-3,6,4′-trimethoxy flavone (santin), **13**: 5,7-dihydroxy-6,4′-dimethoxy-flavone (pectolinaringenin) and **14**: 5-hydroxy-3,7,4′-trimethoxy flavone were identified in the ethanol extracts. Of the leaf ethanol extracts, strong antioxidant capacity was only evident in that of *Lippia alba* carvoneifera (IC_{50} = 1.23 mg/mL). Overall, the data indicates that use of the leaves of *Lippia* species in food preparations, should be beneficial to health.

Keywords: Antioxidant assays; carvone; HPLC; HPLC-ESI-MS; hypoxanthine; mass spectrometry; xanthine oxidase.

1. Introduction

The most widely used synthetic antioxidants used historically, in the preservation of foodstuffs such as butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate and tertiary butyl hydroquinone are suspected to cause or promote negative health effects [1, 2]. Indeed they have been

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replaced in Japan since 1996, by the natural secondary plant metabolite ellagic acid. For this reason there is a growing interest in replacing synthetic compounds with natural secondary plant metabolites as potential antioxidants. A range of plants has been studied in recent years, as potential sources of antioxidants. Among these, many essential oils of aromatic plants and spices have been shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups. Therefore a systematic examination of antioxidant properties of various plant extracts is extremely important to validate their use as preservatives in both the food and the pharmaceutical industries, and as potential health beneficial agents.

A number of studies, on the components of essential oils from various aromatic plants, have already shown, e.g., that oils obtained from the genus *Oreganum*, rich in thymol and carvacrol, have considerable antioxidant, antiinflammatory and anticholinesterase activities [3-5].

The genus *Lippia* (Verbenaceae), comprises a large number of medical and economically important species, including several which are used in traditional Brazilian medicine [6, 7]. In general, the genus appears to present consistent chemical composition profiles, pharmacologic activities, and folk uses. In most cases, the leaves and flowers are used, commonly prepared as infusions or decoctions, and administered orally. The most common use of *Lippia* species, is for the treatment of respiratory disorders, such as a remedy for colds, influenza, bronchitis, coughs and asthma [8-11].

L. alba (Mill.) N.E. Brown, *L. dulcis* Trevir., *L. geminata* H.B.K., *L. graveolens* H.B.K., *L. javanica* (N.L. Burm.) Spreng., *L. nodiflora* (L.) Michx. and *L. triphylla* L'Hèr. are used as analgesic, antiinflammatory and/or antipyretic remedies [8, 11-16]. In addition, the leaves from the majority of these species, are utilized to season food preparations [8], and furthermore, the leaves of *L. citriodora* (Ort.) H.B.K. are used for flavouring beverages [17].

Essential oils, include antioxidants such as monoterpenoid and phenolic components. In particular, the antioxidant capacity of some phenolic compounds has invoked the promotion of their use as natural food additives [18].

However, there is a dearth of available published data, addressing in a single study, the antioxidant capacity of essential oils, and solvent extracts of *Lippia* species [19-21] in terms of health protective effects.

2. Materials and Methods

2.1. Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), $Fe_2Cl_3.6H_2O$, DMSO, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide and fluorescein were obtained from Sigma/Aldrich (Deisenhofen Germany); carvone, cavracol, geranial, neral, limonene, thymol, acteoside (verbascoside) luteolin, apigenin and naringenin from Extrasynthese, (Lyon Nord, Genay, France); ethanol, *n*-hexane, $FeSO_4.7H_2O$, methanol, 2,4,6,-tripyridyl-s-triazine complex (TPTZ), and acetic acid from Fluka/Riedel de Haen (Seelze, Germany); methanol, hypoxanthine, xanthine, xanthine oxidase, and salicylic acid from Merck (Darmstadt, Germany) and K_2HPO_4 and KH_2PO_4 from Serva (Heidelberg, Germany). All solutions were made up in either double distilled water or methanol.

2.2. Plant material

Leaves from adult plants of five species of the genus *Lippia* namely *Lippia alba* carvoneifera, *Lippia alba* citralifera, *Lippia alba* myrceneifera, *Lippia gracilis* AV and *Lippia sidoides* Mart were collected from the Medicinal Plant Gardens of the Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil and identified by Prof. J.W. Matos. Voucher specimens are deposited at the Herbarium Prisco Bezerra of the UFC, Fortaleza, Brazil.

2.3. Steam-hydrodistillation

The fresh leaves of the *Lippia* species (1 kg) were extracted (1 h.), by steam-hydrodistillation immediately after collection, using a modified Clevenger apparatus, to yield the essential oils as described by Trevisan et al. [22], validated by Pimentel et al. [23].

2.4. Ethanol extracts

The fresh leaves of the *Lippia* species (30 g) were dried to constant weight, after which 5.0 g were extracted with ethanol (400 mL) for 5 days, at room temperature. The suspensions were filtered and evaporated to dryness under reduced pressure. The dried samples were suspended in methanol (10.0 mL) prior to HPLC analyses.

2.5. Gas-chromatography coupled with mass spectrometry (GC-MS)

Analyses were performed using a Hewlett Packard (HP) 5971 mass selective detector coupled to a HP 5890 gas chromatograph. Sample volumes of 1 μL were injected into the gas chromatograph. Separation of analytes was achieved using a HP dimethylpolysiloxane DB-1 fused silica capillary column, (30 m × 0.25 mm I.D., 0.10 μm film thickness). Helium was used as carrier gas with linear velocity of 1 mL/minute. The oven temperature program was; initial temperature 35°C, 35-180 °C at 4 °C/min., followed by 180-250 °C at 10 °C/minute. The GC injector temperature was 250 °C; the transfer line temperature was held at 280 °C. The mass spectrometer parameters for EI mode were: ion source temperature: 200 °C; electron energy: 70 eV; filament current: 34.6 μA; electron multiplier voltage: 1200 V. Individual components were identified by spectrometric analyses.

2.6. Analytical high performance liquid chromatography (HPLC)

Analytical HPLC was conducted on a Hewlett-Packard (HP) 1090 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a reverse-phase C18 column (250 mm × 4 mm i.d., 5 μm; Latek, Eppelheim, Germany). Dried ethanol extracts of *Lippia* species were dissolved in methanol (5.0 mL) and, when necessary, further diluted prior to injection (10 μL) into the HPLC. The mobile phase consisted of 2% acetic acid in double distilled water (solvent A) and acetonitrile (solvent B) with the following gradient profile: 95% A for 2 min; reduced to 75% A over 8 min; to 60% A over 10 min; to 50% A over 10 min; to 0% A over 5 min; continuing at 0% A until completion of the run. The flow rate of the mobile phase was 1.0 mL/min. Phenolic compounds in the eluant were detected at 278 and 340 nm with a diode-array UV detector (HP 1040M). Standard curves of acteoside (verbascoside), luteolin, apigenin and naringenin were conducted in the range 50-4000 μM.

2.7. High performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS)

HPLC-ESI-MS was conducted on an Agilent 1100 HPLC, coupled to an Agilent single-quadrupole, mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Chromatographic separation of ethanolic extracts was conducted using a column of the same type, and dimensions as for analytical HPLC (Latek, Eppelheim, Germany). The mobile phase consisted of 2% acetic acid in double distilled water (solvent A) and acetonitrile (solvent B) with the following gradient profile: initially 95% A for 10 min; to 90% A over 1 min; to 60% A over 9 min; to 80% A over 10 min; to 60% A over 10 min; to 0% A over 5 min and continuing at 0% A until completion of the run. Phenolic compounds were detected by their UV absorbance (A) at 278 and 340 nm at room temperature. Mass spectra in negative-ion mode were generated under the following conditions: fragmentor voltage = 100 V, capillary voltage = 2500 V, nebulizer pressure = 30 psi, drying gas temperature = 350 °C, mass range = 100 - 1500 D.

2.8. Hypoxanthine-xanthine oxidase HPLC-based assay

The assay used to determine the antioxidant capacity of essential oils obtained from the *Lippia* species was based on the methods of Trevisan et al., [22] and Owen et al. [24, 25]. The oils were dispersed in the assay buffer, at a concentration of 2.0 μL/mL, and diluted appropriately (in duplicate) in assay buffer to a final volume of 1.0 mL, giving a range of 0.04 - 1.0 μL/mL, and 10 μL of xanthine oxidase (18 mU), in 3.2 M NH_4SO_4 were added to initiate the reactions.

Samples for determination of the antioxidant capacity of *Lippia* ethanol extracts were prepared by adding 0 - 500 μL of methanol solutions (5.0 mg/mL) of the extracts to 15 mL centrifuge tubes (in

duplicate), and removing the solvent under a stream of nitrogen. In each case, the dried sample material was suspended in the assay buffer (1.0 mL), prior to addition of xanthine oxidase.

The tubes were incubated at 37 °C for 3 h., at which time reaction was complete. Reaction mixtures (20 µL) were analyzed by analytical HPLC, using the mobile phase and conditions described above.

The concentrations of individual phenolic compounds, and the hydroxylation of hypoxanthine, were monitored at λ = 278 nm, whereas the hydroxylation of salicylic acid, was monitored at 325 nm utilising analytical HPLC. The quantitation, of end-products of enzyme (uric acid) or free radical reactions (2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid) were performed using appropriate standard curves against concentration, measured at the same wavelength.

2.9. DPPH, FRAP and ORAC assays

The DPPH, FRAP and ORAC assays were conducted as previously described by Pfundstein et al. [26]. Details are given in the Supporting information file.

2.10. Statistics

For the antioxidant assay described above, the volume of oil (µL/mL, µM), and concentration of extracts (µg/mL), producing a 50% inhibition of oxidation (IC_{50}), were determined using the Table curve program (Jandel Sciencific, Chicago, IL). Differences in antioxidant capacities were evaluated by ANOVA.

3. Results and Discussion

3.1. Characteristics of the essential oils

The amount of raw essential oil obtained from the *Lippia* species was variable (Table 1). The greater yields were from *Lippia alba* carvoneifera and *Lippia sidoides* Mart. (both 2.0 %), and the least from *Lippia alba* myrceneifera (0.5 %). The principal components detected and identified by GC-MS was also variable. Major compounds in the oils were identified as thymol (*Lippia sidoides* Mart, 13.1 g/k, 65.5%), carvone (*Lippia alba* carvoneifera, 9.6 g/kg, 48%) geranial (*Lippia alba* citraleifera, 4.61 g/kg, 38.4%; *Lippia alba* myrceneifera, 1.6 g/kg 31.9%), neral (*Lippia alba* citraleifera, 3.4 g/kg, 28.3%; *Lippia alba* myrceneifera, 1.16 g/kg, 23.2%), carvacrol (*Lippia gracilis* AV, 5.36 g/kg, 31.5%) and limonene (*Lippia alba* carvoneifera, 5.14 g/kg, 25.7%).

Table 1. Yield of essential oils from the fresh leaves (1 kg) of *Lippia* species after steam-hydrodistillation.

Lippia species	Yield (%)	*Voucher Nº
Lippia alba carvoneifera	2.0	24149
Lippia gracilis AV	1.7	13308
Lippia alba citraleifera	1.2	24151
Lippia sidoides Mart	2.0	84749
Lippia alba myrceneifera	0.5	24151

*Voucher specimens are deposited in the Herbarium, Prisco Bezerra at the UFC, Fortaleza, Brazil

3.2. Antioxidant capacity of the raw essential oils

The greater antioxidant capacity (IC_{50} = 980 µg/mL; $p < 0.05$), assessed by the HPLC-based hypoxanthine/xanthine oxidase assay, was determined in the raw essential oil obtained from *Lippia alba* carvoneifera, while the remainder were relatively inactive (IC_{50} > 2000 µg/mL). The concentration dependent scavenging of reactive oxygen species by the oils is depicted in Figure. 1. None of the oils inhibited xanthine oxidase, unlike that reported for *Ocimum* [22].

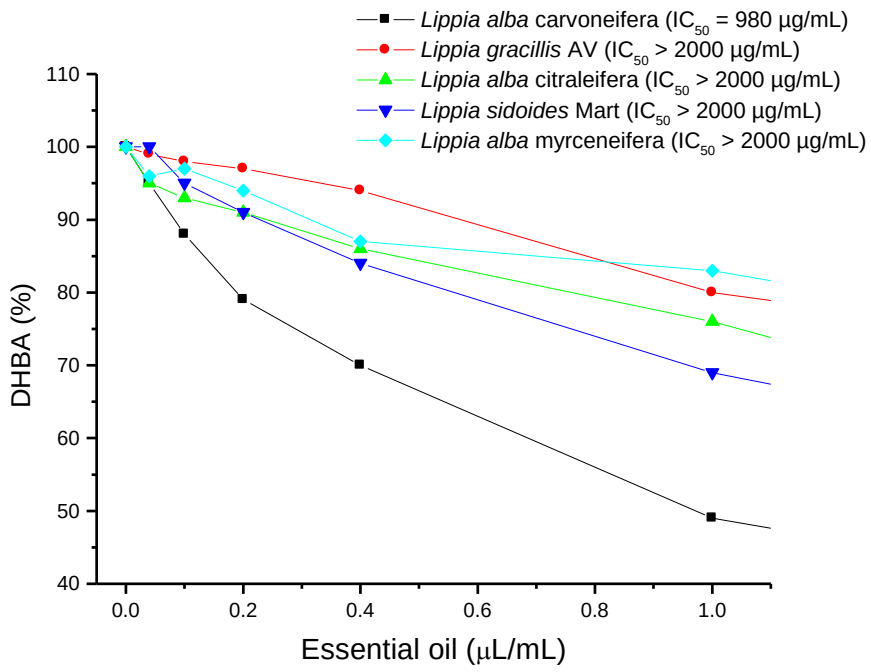


Figure 1. Inhibition of reactive oxygen species attack on salicylic acid by essential oils extracted from the leaves of *Lippia* species in the HPLC-based hypoxanthine/xanthine oxidase assay.

3.3. Antioxidant capacity of pure essential oils

The major substances detected in the hydro-distillates of the fresh green leaves, namely neral, geranial, thymol, carvacrol, carvone and limonene, purchased from a commercial source, were not only tested in the HPLC-based hypoxanthine/xanthine oxidase, but also in the DPPH, FRAP and ORAC assays. Of these, carvone (Figure 2) was the stronger (IC_{50}, 330 µM; $p < 0.05$) antioxidant in the HPLC-based hypoxanthine/xanthine oxidase assay, correlating extremely well with the data for the crude essential oils. Carvacrol (IC_{50}, 730 µM), geranial (IC_{50}, 960 µM), and neral (IC_{50}, 1040 µM) were also very active, whereas limonene (IC_{50}, > 2 mM) was relatively inactive in this system. Unfortunately due to its inherent insolubility in the assay buffer, thymol could not be reliably tested in this system. The pure oils were all very inactive in the DPPH and FRAP assays, whereas thymol and carvacrol were highly active in the ORAC assay, displaying ORAC units relative to Trolox (1 µM) of 1.88 and 1.74 respectively.

Table 2. Effect of the major pure essential oils identified in *Lippia* species in a range of antioxidant assays.

Oil	HX/XO[a] IC_{50} (µM)	DPPH[b] IC_{50} (mM)	FRAP[c] EC_{50} (mM)	ORAC[d] (units)
Carvone	330*	inactive	inactive	0.29
Carvacrol	730	1.18	8.3	1.74*
Geraniol	960	inactive	inactive	0.65
Nerol	1040	inactive	inactive	0.67
Thymol	Insoluble	1.96	6.8	1.88*
Limonene	>2000	inactive	inactive	0.35

[a]HPLC-based hypoxanthine/xanthine oxidase assay
[b]2,2-diphenyl-1-picrylhydrazyl assay
[c]Ferric reducing ability of plasma assay
[d]Oxygen radical absorbance capacity assay relative to Trolox (1 µM)
*Significantly more effective (ANOVA; $p < 0.05$)

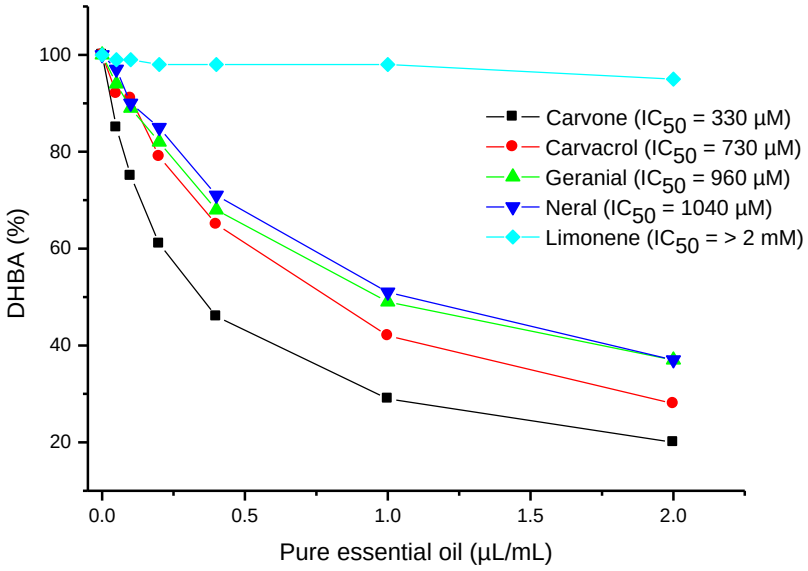


Figure 2. Inhibition of reactive oxygen species attack on salicylic acid by the major pure authentic essential oils identified in *Lippia* species in the HPLC-based hypoxanthine/xanthine oxidase assay.

3.4. Characteristics of the ethanol extracts

The yield of raw ethanol extracts from the fresh leaves of *Lippia* species was also variable (5.3-11.6 %; Table 3). The greater yield was from *Lippia sidoides* Mart. (11.6 %) with the least from *Lippia gracilis* AV (5.3 %). The extracts, displayed variable concentrations of secondary phenolic plant substances (a HPLC chromatogram of the ethanol extract of *Lippia alba* carvoneifera, dissolved in methanol is depicted in Figure 3) of which several of the components such as **1**: calceolarioside E, **2**: acteoside, **3**: isoacteoside (Figure 4a) were clearly identifiable in comparison to the data of Owen et al, [25] and Hennebelle et al, [27]. However the HPLC chromatograms, also displayed a variety of flavonoid substances, and using a range of chromatographic strategies, the flavonoids were separated and identified by HPLC-ESI-MS and reference data (Table 4) as **4**: luteolin, **5**: 5,7,3´,4´-tetrahydroxy-3,6,-dimethoxy-flavone, **6**: naringenin, **7**: apigenin, **8**: 6-methoxy apigenin (hispidulin), **9**: 5,7,3´-trihydroxy-3,6,4´-trimethoxy flavone, **10**: 5,7,3´-trihydroxy-3,6,-dimethoxy-flavone, **11**: naringenin-4´-methyl ether, **12**: 5,7-dihydroxy-3,6,4´-trimethoxy flavone (santin), **13**: 5,7-dihydroxy-6,4´-dimethoxy flavone (pectolinaringenin) and **14**: 5-hydroxy-3,7,4´-trimethoxy flavone (Figure 4b). The concentration of phenolic compounds detected in the ethanol extracts of *Lippia* species is shown in Table 5.

Table 3. Yield of ethanol extracts from the dried leaves (5.0 g) of *Lippia* species.

Lippia species	Yield (%)	Voucher Nº
Lippia alba carvoneifera	8.8	24149
Lippia gracilis AV	5.3	13308
Lippia alba citraleifera	7.3	24151
Lippia sidoides Mart	11.6	84749
Lippia alba myrceneifera	6.6	24151

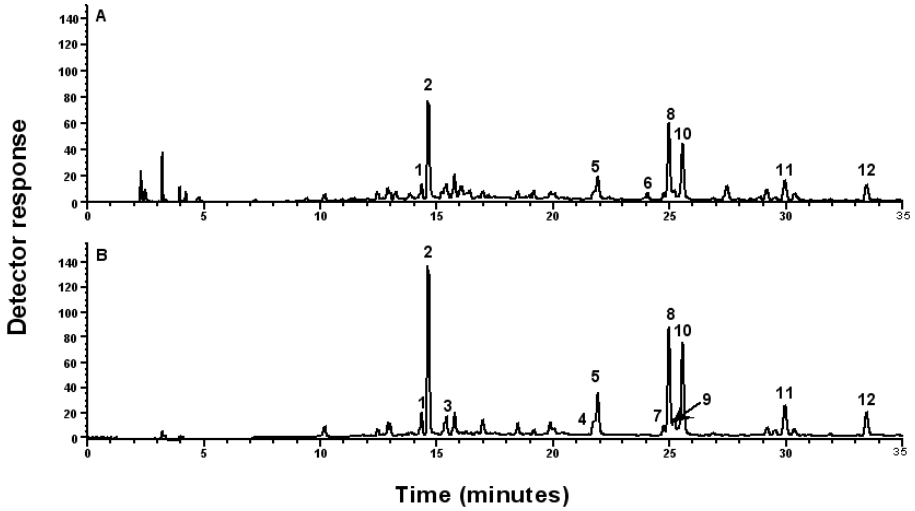
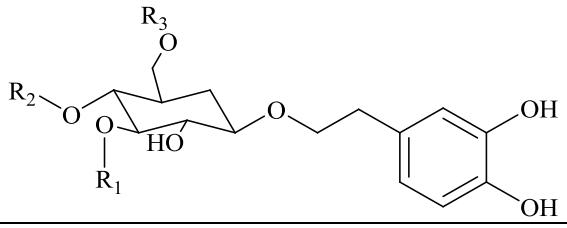


Figure 3. Analytical reverse-phase HPLC chromatogram of an ethanol extract of the leaves of *Lippia alba* carvoneifera; absorption at 278 nm (Frame A) and 340 nm (Frame B) vs. retention time (minutes) is shown. 1: calceolarioside E, 2: acteoside (verbascoside), 3: isoacteoside (isoverbascoside), 4: luteolin, 5: 5,7,3´,4´-tetrahydroxy-3,6-dimethoxy flavone, 6: naringenin, 7: apigenin, 8: hispidulin, 9: 5,7,3´-trihydroxy-3,6,4´-trimethoxy flavone, 10: 5,7,3´-trihydroxy-3,6-dimethoxy flavone, 11: santin, 12: pectolinaringenin.



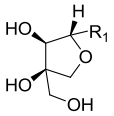
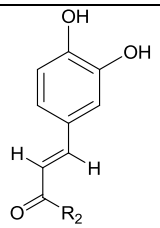
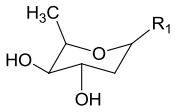
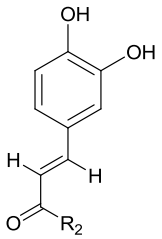
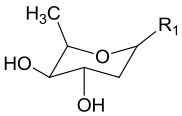
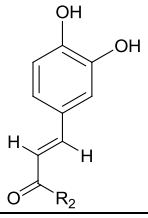
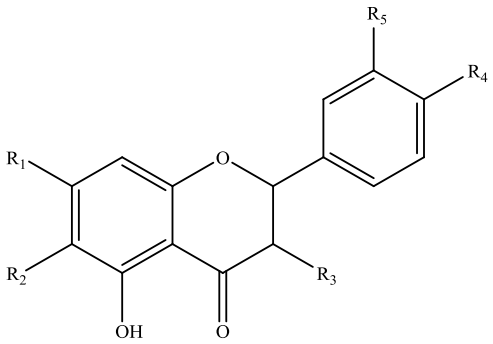
Lippia alba carvoneifera	**R1**	**R2**	**R3**
1. Calceolarioside E			H
2. Acteoside (Verbascoside)			H
3. Isoacteoside Isoverbascoside		H	

Figure 4a. Structures of the iridoids in ethanol extracts of *Lippia alba* carvoneifera.



Lippia species	**R1**	**R2**	**R3**	**R4**	**R5**
4. Luteolin	OH	H	H	OH	OH
5. Spinacetin	OH	OCH_3	OCH_3	OH	OH
6. Naringenin	OH	H	2H	OH	H
7. Apigenin	OH	H	H	OH	H
8. Hispudulin	OH	OCH_3	H	OH	H
9. 5,7,3'-trihydroy-3,6,4'-trimethoxy-flavone	OH	OCH_3	OCH_3	OCH_3	OH
10. 5,7,4'-trihydroxy-3,6-dimethoxy-flavone	OH	OCH_3	OCH_3	OH	H
11. naringenin-4'-methyl ether	OH	H	2H	OCH_3	H
12. 5,7-dihydroxy-3,6,4'-trimethoxy flavone (santin)	OH	OCH_3	OCH_3	OCH_3	H
13. 5,7-dihyroxy-6,4'-Dimethoxy flavone (pectolinaringenin)	OH	OCH_3	H	OCH_3	H
14. 5-Hydroxy-3,7,4'-trimethoxy-flavone	OCH_3	H	OCH_3	OCH_3	H

Figure 4b. Structures of the flavonoids identified in ethanol extracts of *Lippia* species.

Table 4. HPLC-ESI-MS data for the polyphenolic compounds isolated from ethanol extracts of *Lippia* species.

No	Phenolic compound	Formula	Exact mass (calc.)	HPLC-ESI-MS (*m*/*z*)			Lit. Ref
				Rt (min)	$[M-H]^-$	$[2M-H]^-$	
1	Calceolarioside E	$C_{28}H_{34}O_{15}$	610.190	14.360	609.2	1219.3	[27]
2	Acteoside	$C_{29}H_{36}O_{15}$	624.205	14.642	623.2	1247.3	[25]
3	Isoacteoside	$C_{29}H_{36}O_{15}$	624.205	15.396	623.2	1247.7	[25]
4	Luteolin	$C_{15}H_{10}O_6$	286.048	21.750	285.1	571.1	[25]
5	5,7,3´,4´-Tetrahydroxy-3,6-dimethoxy flavone (Spinacetin)	$C_{17}H_{14}O_8$	346.069	21.909	345.1	nd	[30]
6	Naringenin	$C_{15}H_{12}O_5$	272.068	24.032	271.1	543.1	[31]
7	Apigenin	$C_{15}H_{10}O_5$	270.053	24.729	269.1	539.1	[25]
8	6-Methoxy apigenin (Hispidulin)	$C_{16}H_{12}O_6$	300.063	24.972	299.1	nd.	[28]
9	5,7,3´-Trihydroxy-3,6,4´-trimethoxy flavone	$C_{18}H_{16}O_8$	360.085	25.211	359.1	nd.	[32]
10	5,7,4´-Trihydroxy-3,6-dimethoxy flavone	$C_{17}H_{14}O_7$	330.074	25.551	329.2	nd	[28]
11	Naringenin-4´-methyl ether	$C_{16}H_{14}O_5$	286.084	27.750	285.1	571.1	[29]
12	5,7-Dihydroxy-3,6,4´-trimethoxy flavone (Santin)	$C_{18}H_{16}O_7$	344.090	29.960	343.1	nd.	[28]
13	5,7-Dihydroxy-6,4´-dimethoxy flavone (Pectolinaringenin)	$C_{17}H_{14}O_6$	314.079	33.457	313.1	nd	[28]
14	5-Hydroxy-3,7,4´-trimethoxy flavone	$C_{18}H_{16}O_6$	328.095	36.770	327.2	nd	[33]

nd = not detected

Table 5. Concentration of polyphenolic compounds (mg/kg dry weight) detected in ethanol extracts of *Lippia* species.

No	Phenolic compound	*Lippia* species				
		1	2	3	4	5
1	Calceolarioside E	84.6	nd	nd	nd	nd
2	Acteoside	444.0	nd	nd	nd	nd
3	Isoacteoside	72.6	nd	nd	nd	nd
4	Luteolin	12.0	nd	nd	nd	nd
5	5,7,3´,4´-Tetrahydroxy-3,6-dimethoxy flavone (Spinacetin)	66.6	nd	nd	nd	nd
6	Naringenin	14.8	6224.4	555.4	395.2	nd
7	Apigenin	10.4	nd	nd	nd	nd
8	6-Methoxy apigenin (Hispidulin)	118.6	nd	413.2	224.2	34.4
9	5,7,3´-Trihydroxy-3,6,4´-trimethoxy flavone	28.8	nd	nd	nd	nd
10	5,7,4´-Trihydroxy-3,6-dimethoxy flavone	146.8	nd	nd	nd	34.4
11	Naringenin-4´-methyl ether	nd	1564	nd	nd	nd
12	5,7-Dihydroxy-3,6,4´-trimethoxy flavone (Santin)	56.8	nd	nd	nd	nd
13	5,7-Dihydroxy-6,4´-dimethoxy flavone (Pectolinaringenin)	36.0	542.2	225.4	182.2	46.6
14	5-Hydroxy-3,7,4´-trimethoxy flavone	nd	nd	nd	nd	51.4
Total (mg/kg)		**1092**	**8331**	**1193**	**802**	**167**

1. *Lippia alba* carvoneifera, 2. *Lippia sidoides* Mart, 3. *Lippia alba* myrceneifera, 4. *Lippia alba* citralifera, 5. *Lippia gracilis* AV, nd = not detected

3.5. Antioxidant capacity of ethanol extracts

The greater antioxidant capacity assessed by the HPLC-based hypoxanthine/xanthine oxidase assay was determined in the ethanol extract obtained from *Lippia alba* carvoneifera (IC_{50} = 1.23 mg/mL) while the remainder were less effective (IC_{50} > 2000 µg/mL). The concentration dependent scavenging of reactive oxygen species by the extracts is depicted in Figure 5.

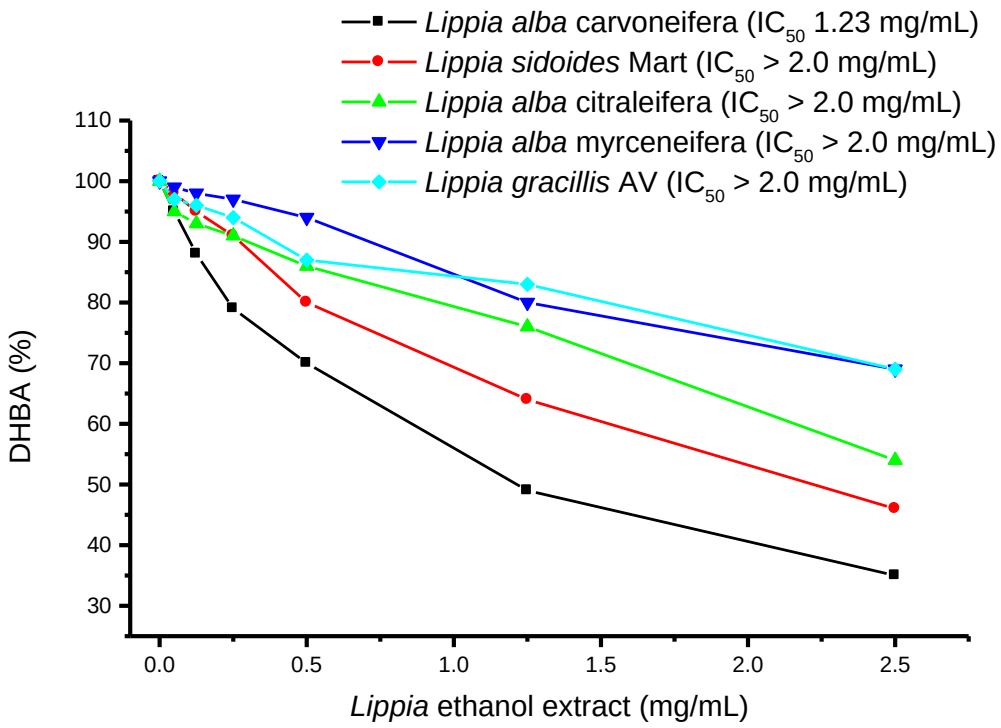


Figure 5. Inhibition of reactive oxygen species attack on salicylic acid by ethanol extracts from the leaves of *Lippia* species in the HPLC-based hypoxanthine/xanthine oxidase assay.

The results of this study show that the yield of essential oils from *Lippia* species is variable, with *Lippia alba* carvoneifera and *Lippia sidoides* Mart providing over four times more than *Lippia alba* myrceneifera. The antioxidant capacity, as measured by the HPLC-based hypoxanthine/xanthine oxidase assay, for the essential oils of each *Lippia* species was generally lower in comparison to *Ocimum* species, except that of *Lippia alba* carvoneifea, which lies in the mid-range described for *Ocimum* essential oils [22]. In this assay, the significantly higher (980 µg/mL; $p < 0.05$) antioxidant capacity of the oil from *Lippia alba* carvoneifera is clearly related to the proportion of the major monoterpenoid, carvone (48.6 %), not present in the other species, with an IC_{50} of 330 µM. However, it should be noted that, of the pure essential oils tested, while all were relatively inactive in the DPPH and FRAP assays, carvacrol and thymol were highly active in the ORAC assay.

The significantly higher ($p< 0.05$) antioxidant capacity of *Lippia alba* carvoneifera ethanol extracts can be attributed to the presence of iridoids (301 mg/kg) not detected in the other *Lippia* extracts [27].

Based on the antioxidant assays, the essential oils and ethanol extracts of *Lippia species*, have potential, in terms of future applications in traditional medicine, incorporation into functional foods, and possibly an influence on healthy life-style factors, especially those of *Lippia alba* carvoneifera.

Acknowledgements

Grateful thanks are extended to DAAD/CAPES for generously funding Ricardo A. Marques for a six month visit to the Division of Preventive Oncology, National Center for Tumor Diseases/German Cancer Research Center (DKFZ), Heidelberg, Germany. Maria T.S. Trevisan extends thanks to CNPq-INSTITUTO NANO(BIO)SIMES.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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