

Full Length Research Paper

## Chemical composition and antioxidant activity of *Lippia* species

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**Chemical composition of the hexanic fraction, antioxidant activity (DPPH assay) and total phenolic content (Folin-Ciocalteu assay) of aerial parts of sixteen *Lippia* species were determined. Hexanic fraction was analyzed by gas chromatography-mass spectrometry (GC-MS). The compounds  $\beta$ -myrcene, limonene,  $\gamma$ -terpinene, linalool,  $\beta$ -caryophyllene, germacrene D,  $\alpha$ -copaene,  $\beta$ -bourbunene,  $\beta$ -elemene, aloaromadendrene, bicyclogermacrene, and  $\delta$ -cadinene appeared in the chemical analysis in most of the *Lippia* hexanic fraction studied. This fraction showed low radical scavenging activity; however, the ethanolic fraction of *Lippia* species showed higher radical-scavenging activity than the commercial antioxidant butylated hydroxy toluene (BHT). Total phenolic content of the polar fraction, as gallic acid equivalents, ranged from 105.5 mg/g in *Lippia pseudo-thea* to 255.4 mg/g in *Lippia sericea*. The presence of phenolic compounds in the ethanolic fraction of *Lippia* spp. may also be the major cause of its high radical-scavenging activities.**

**Key words:** Verbenaceae, gas chromatography-mass spectrometry (GC-MS), phenolic content, antioxidant assay.

### INTRODUCTION

The genus *Lippia* (Verbenaceae) is widely distributed in tropical and subtropical America and Africa, and it consists of approximately 250 species of herbs, shrubs, and small trees which often contain a chemical aromatic structure (Moldenke, 1980). One of the main diversity centers of the genus is located at Espinhaço range, Minas Gerais State, Brazil (Viccini et al., 2006).

There are many economically important *Lippia* species and some of them have been used in traditional medicine mainly for gastrointestinal and respiratory diseases

(Pascual et al., 2001). Brazilian species, such as *Lippia alba* (Mill.) N.E. Br and *Lippia sidoides* Cham., have shown antiulcerogenic, antimicrobial, anti-inflammatory, antihelmintic, antioxidant, gastroprotective, and cytostatic properties (Aguiar et al., 2008; Camurça-Vasconcelos et al., 2007; Carvalho et al., 2003; David et al., 2007; Fontenelle et al., 2007; Monteiro et al., 2007; Pascual et al., 2001). These effects are partially attributed to the essential oils and phenolic compounds produced by both species. However, the chemical composition and pharmacological aspects of many *Lippia* spp. still have not been studied.

The present study was done in order to determine: (1) the chemical composition of hexanic fraction of sixteen

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*Lippia* spp. using gas chromatography-mass spectrometry (GC-MS) analysis, (2) the total phenolic content of ethanolic fraction using the Folin-Ciocalteu reagent, and (3) the antioxidant capacity, through the capture of radical 2,2-diphenylpicrylhydrazyl (DPPH) of hexanic and ethanolic fractions of *Lippia* spp.

## MATERIALS AND METHODS

### Plants

Leaves of *Lippia* aff. *microphylla* Cham., *Lippia aristata* Schauer., *Lippia corymbosa* Cham., *Lippia diamantinensis* Glaz., *Lippia filifolia* Mart. & Schauer ex Schauer, *Lippia filipei* Moldenke, *Lippia hermannioides* Cham., *Lippia lupulina* Cham., *Lippia martiana* Schauer, *Lippia microphylla* Cham., *Lippia pseudo-thea* (St. Hil.) Schauer, *Lippia pohliana* Schauer, *Lippia rosella* Moldenke, *Lippia rubella* Moldenke, *Lippia salviifolia* Cham., and *Lippia sericea* Cham. were collected at the Espinhaço Range, Minas Gerais State, Brazil, in March and April of 2007. The plants were identified by Dr. Fátima Regina Gonçalves Salimena from Botany Department (Federal University of Juiz de Fora). The voucher of each species was deposited at the CESJ Herbarium of the Federal University of Juiz de Fora.

### Preparation of hexanic and ethanolic fractions (HF and EF)

Fresh leaves of each species (3 g) were collected and transferred to a conical tube with ethanol P. A. (30 ml). Each preparation was macerated during one week at room temperature. After filtration, the extracts were taken and mixed to the same quantity of ultrapure water and were partitioned twice with hexane. Ethanolic and hexanic fractions were conserved at -20°C until use.

### GC-MS analysis

The hexanic fractions were analyzed using a Shimadzu GC-MS model QP5050A equipped with a flame ionization detector (FID) and a DB-5 fused silica capillary column (35 × 0.2 mm, film thickness 0.10 µm), using helium as the carrier gas (1.0 ml/min). The injection temperature was 200°C and the column oven program was 50 to 250°C at 4°C min<sup>-1</sup>. Mass spectra were obtained by electronic impact 70 eV and the range from 50 to 500 *m/z* was scanned. Data acquisition and handling were done via CLASS 5000 Shimadzu software. Retention index (RI) in the range of 900 to 3000 was generated from analysis of a standard mixture containing C<sub>9</sub> to C<sub>30</sub> hydrocarbons. The identification of the compounds was based on the comparison of their mass spectra with those in a Shimadzu spectral database and retention indices (Adams, 1995).

### Assay for total phenolic content in the ethanolic fraction

Total phenolic constituents were determined using the Folin-Ciocalteu reagent and gallic acid as the standard (Slinkard and Singleton, 1997). The reaction mixture contained sample (50 µl), Folin-Ciocalteu reagent (250 µl), 20% sodium carbonate (500 µl), and distilled water (4.2 ml). Calibration curve was prepared with gallic acid solutions ranging from 0 to 700 µg/L. Total phenols of the extract, as gallic acid equivalent, were determined using the absorbance of the extract measured at 765 nm. Results were expressed as µg/L. Tests were carried out in triplicate.

### Antioxidant assay

The radical scavenging activity of plant extracts was determined by DPPH method (Sreejayan and Rao, 1997). An aliquot of ethanolic solution of the HF or EF (50 µl or 0.97 to 250 µg/ml) was added to a 0.05 mM ethanol DPPH solution (150 µl) in a 96 well microplate and was incubated at room temperature for 30 min. A blank (consisting of the extract and ethanol) was used to remove the influence of the color of the sample. An ethanolic solution of DPPH was used as negative control. Ascorbic acid and butylated hydroxy toluene (BHT) were used as antioxidant reference compounds, at the same concentrations as those used for the sample. Results were expressed as mean of inhibiting concentration (IC<sub>50</sub>) which was calculated using Equation 1 as follow:

$$IC_{50}(\%) = 100 \times (A_0 - A_s)/A_0 \quad (1)$$

where A<sub>0</sub> and A<sub>s</sub> are the values for the absorbance of the negative control and the absorbance of the sample, respectively. Tests were carried out in triplicate. The antioxidant activity of the *L. lupulina* and *L. microphylla* were not analyzed, because there was not sufficient amount of extract.

## RESULTS

The hexanic fractions of *Lippia* spp. analyzed by GC-MS showed mainly terpenoid compounds such as hydrocarbons, alcohols, aldehydes, ketones, and esters (Table 1). Sixty-one compounds have been identified, representing 74.88 to 98.49% of the total compounds detected.

The monoterpenes hydrocarbons β-myrcene, limonene, γ-terpinene and linalool were identified in at least 50% of the hexanic fraction. In the present study, β-myrcene concentrations ranging from 0.43 to 12.93% as percentage of peak area, being one of the main compounds of *L. filipei*, *L. lupulina*, *L. pseudo-thea*, and *L. rosella*. Limonene concentration ranging from 0.44 to 10.19%, and was one of the main components of *L. filifolia* and *L. rubella*. However, γ-terpinene appeared in lower concentrations (between 0.57 and 5.46%). Linalool concentration varied from 0.26 to 6.96% of HF from *L. filipei* and *L. pohliana*, and was one of the major components in these species.

In addition, the HF of *Lippia aristata*, *L. filifolia*, and *L. rosella* exhibited high content of δ-3-carene (15.98%), camphene (11.98%) and α-phellandrene (14.46%), respectively. α-pinene was the most abundant in *L. aristata* (5.94%) and *L. martiana* (10.64%) extracts. On the other hand, sabinene was mainly found in *L. aristata* (16.01%) and *L. pseudo-thea* (8.69%).

Oxygenated monoterpenes were the most abundant components observed in some species. The neral and geranial appeared in *L. diamantinensis* with 9.38 and 14.53%, respectively. Camphor (25.27%) was the main component of *L. filifolia* and *cis*-limonene epoxide was the major component in *L. rubella* while 1.8-cineole appeared as a major compound in *L. pseudo-thea* and in *L. rosella* (25.47 and 34.64%, respectively).

**Table 1.** Chemical composition of HF (%) of several *Lippia* spp.

Relative percentage of compound	RI <sup>a</sup>	LAF <sup>b</sup>	LAR <sup>c</sup>	LCO <sup>d</sup>	LDI <sup>e</sup>	LFF <sup>f</sup>	LFP <sup>g</sup>	LHE <sup>h</sup>	LLU <sup>i</sup>	LMA <sup>j</sup>	LMI <sup>k</sup>	LPS <sup>l</sup>	LPO <sup>m</sup>	LRO <sup>n</sup>	LRU <sup>o</sup>	LSA <sup>p</sup>	LSE <sup>q</sup>
<b>Monoterpene hydrocarbon</b>																	
α-pinene	-	6.81	5.94	-	-	-	-	-	-	10.64	-	-	-	-	-	2.91	-
Canfene	-	0.42	-	-	-	11.98	-	-	-	-	-	-	-	-	-	-	-
Sabinene	-	0.92	16.01	-	-	-	-	2.00	-	-	-	8.69	-	7.81	-	1.02	-
β-pinene	978	1.54	0.51	0.46	1.37	-	-	2.82	-	1.16	-	-	-	-	-	1.32	-
β-myrcene	993	0.47	0.99	0.98	-	2.15	8.60	-	12.93	2.86	-	8.24	-	10.27	0.43	-	-
α-phellandrene	1007	-	-	-	-	-	-	-	-	-	-	-	-	14.46	-	-	-
δ-3-carene	1014	0.66	15.98	-	-	-	-	-	-	4.83	-	-	-	-	-	-	-
p-cymene	1028	-	-	0.61	1.54	1.54	-	-	-	-	-	1.44	-	0.52	-	-	-
Limonene	1032	0.48	-	0.81	2.02	6.52	3.69	0.44	-	2.98	-	0.68	0.62	-	10.19	0.76	0.93
β-phellandrene	1037	-	-	-	-	-	-	-	-	-	-	-	-	2.85	-	-	-
cis-β-ocimene	1041	0.43	-	-	-	2.15	0.95	-	-	7.66	-	-	-	-	-	-	-
trans-β-ocimene	1052	1.63	-	-	4.06	2.61	1.12	-	-	3.80	1.12	0.56	-	1.65	-	0.75	-
γ-terpinene	1062	-	-	2.11	-	5.46	-	0.57	-	0.34	-	2.48	-	0.64	3.70	-	-
Terpinolene	1093	0.47	-	-	-	1.30	-	-	-	4.66	-	0.23	-	0.43	-	-	-
<b>Oxygenated monoterpene</b>																	
1.8-Cineole	1035	-	-	-	-	3.06	-	2.84	-	-	-	25.47	-	34.64	-	-	-
Linalool	1107	0.89	-	0.89	-	-	6.71	1.54	-	-	-	2.57	6.96	0.26	-	0.70	-
cis-limonene epoxide	1148	-	-	-	-	-	-	-	-	-	-	-	-	-	34,38	-	-
Camphor	1154	-	-	-	-	25.27	-	-	-	-	-	2.03	-	-	-	-	-
Myrcenone	1158	-	-	-	-	-	-	-	-	-	-	18.70	-	-	-	-	-
trans-limonene epoxide	1176	-	-	-	-	-	-	-	-	-	-	-	-	-	1.23	-	-
α-terpineol	1200	-	-	-	-	-	-	0.28	-	-	-	2.88	-	-	-	-	-
Neral	1257	-	-	-	9.38	-	-	-	-	-	-	-	-	-	-	-	-
Geranial	1288	-	-	-	14.53	-	-	-	-	-	-	-	-	-	-	-	-
<b>Sesquiterpen hydrocarbon</b>																	
δ-elemene	1346	-	-	3.19	-	-	-	0.48	3.14	-	-	-	-	-	0.23	-	-
α-cubebene	1360	-	-	0.46	-	-	-	-	-	-	-	-	1.07	-	0.51	-	-
α-copaene	1384	4.79	0.63	1.58	1.61	1.56	2.37	0.94	1.46	10.63	6.02	-	1.85	1.93	0.71	1.82	8.74
β-bourbonene	1394	0.89	-	1.84	-	-	1.23	2.44	1.31	-	-	-	-	-	0.21	0.71	-
β-cubebene	1399	1.04	0.50	-	-	-	-	0.94	-	-	1.13	-	-	-	1.58	-	-
β-elemene	1400	-	-	18.37	2.02	2.60	4.63	-	2,50	-	-	-	1.79	0.64	-	-	-

Table 1. Contd.

β-caryophyllene	1431	5.57	21.11	9.79	6.68	14.37	16.34	23.40	10.87	20.40	17.82	4.68	33.55	5.43	8.55	7.25	10.19
β-gurjunene	1440	0.69	-	0.65	-	-	0.49	0.58	-	-	-	-	2.13	0.29	-	-	2.40
γ-elemene	1442	-	-	1.43	-	-	-	0.15	-	-	-	-	-	-	0.39	-	-
trans- α-bergamotene	1444	0.45	-	-	-	-	-	-	-	1.81	-	-	-	0.39	-	-	-
α-guaiene	1449	2.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.01
α-caryophyllene	1465	1.88	1.30	3.61	1.59	3.79	1.21	8.67	3.83	1.52	9.21	0.29	4.07	0.34	5.14	5.10	22.21
Aloaromadendrene	1473	2.54	0.97	3.36	-	-	2.23	2.35	2.37	0.62	2.26	-	3.06	0.97	0.57	2.37	7.07
γ-muurolene	1489	-	-	2.17	-	-	-	-	-	0.88	1.68	-	2.76	0.59	-	0.98	4.49
Germacrene D	1494	39.13	27.42	22.15	8.04	1.75	13.73	27.84	41.97	-	20.37	0.65	4.11	11.47	12.91	25.18	3.87
β-selinene	1500	0.72	-	1.69	-	-	-	-	-	-	-	-	1.38	-	-	0.93	1.79
Bicyclogermacrene	1510	11.45	3.57	6.78	-	3.57	9.33	5.10	11.27	-	11.67	-	3.99	1.54	0.75	3.08	-
α-muurolene	1512	-	-	-	-	-	-	-	-	1.46	-	-	-	-	-	-	5.75
Germacrene A	1519	-	-	2.13	-	-	0.39	-	-	-	-	-	-	-	-	2.30	-
δ-guaiene	1519	1.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.36
β-curcumene	1527	-	-	-	-	-	-	-	-	-	-	-	-	-	1.34	-	-
γ-cadinene	1529	0.48	-	1.34	-	-	-	-	-	-	-	-	1.44	0.39	-	0.68	1.92
δ-cadinene	1536	3.10	-	2.07	1.05	0.96	-	0.68	1.03	6.95	3.60	-	3.70	0.76	-	1.09	10.44
Cadina-1,4-diene	1547	-	-	-	-	-	-	-	-	-	-	-	0.66	-	1.42	-	-
α-cadinene	1552	-	-	1.08	-	-	-	-	-	-	-	-	0.74	0.22	-	-	-
Germacrene B	1574	-	-	2.79	-	-	-	1.06	4.66	-	-	-	1.56	-	1.32	-	-
<b>Oxygenated sesquiterpene</b>																	
Nerolidol	1575	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29.65	-
Spathulenol	1597	0.51	-	-	-	-	1.78	0.21	-	-	-	-	-	-	-	0.75	-
Cariophyllene oxide	1600	-	-	-	-	-	1.06	-	-	0.39	-	0.69	1.42	-	-	-	-
Ledol	1605	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.49
Globulol	1609	-	-	-	-	-	1.36	-	-	-	-	-	0.66	-	-	-	1.49
Guaiol	1611	-	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-epi-muurolol	1661	1.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.64
α-muurolol	1665	-	-	-	-	-	-	-	-	-	-	-	1.79	-	-	-	1.31
α-cadinol	1674	1.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.22
<b>Hydrocarbon</b>																	
Undecane	1107	-	-	-	4.18	-	-	-	-	-	-	-	-	-	-	-	-
Tridecane	1319	-	-	-	4.57	-	-	-	-	-	-	-	-	-	-	-	-
Pentadecane	1508	-	-	-	24.88	-	-	-	-	-	-	-	-	-	-	-	-

a, Retention index; b, *L. aff. mycrophylla*; c, *L. aristata*; d, *L. corymbosa*; e, *L. diamantinensis*; f, *L. filifolia*; g, *L. filippeii*; h, *L. hermannioides*; i, *L. lupulina*; j, *L. martiana*; k, *L. mycrophylla*; l, *L. pseudo-thea*; m, *L. pohliana*; n, *L. rosella*; o, *L. rubella*; p, *L. salviifolia*; q, *L. sericea*; r, not identified or absent.

**Table 2.** Antioxidant activity and total phenolic content of *Lippia* spp.

Species	Hexanic fraction (HF) IC <sub>50</sub> (µg/ml)	Ethanollic fraction (EF) IC <sub>50</sub> (µg/ml)	Total phenolic content (mg/g)
<i>L. aff. microphylla</i>	61.90	10.07	* <sup>a</sup>
<i>L. aristata</i>	81.70	4.90	*
<i>L. corymbosa</i>	218.82	19.22	234.1
<i>L. diamantinensis</i>	334.68	16.43	163.5
<i>L. filifolia</i>	171.56	13.11	190.6
<i>L. filipei</i>	162.05	28.27	157.9
<i>L. hermannioides</i>	437.78	31.30	109.8
<i>L. martiana</i>	57.00	5.00	*
<i>L. pohliana</i>	149.14	18.81	209.5
<i>L. pseudo-thea</i>	195.69	67.89	105.5
<i>L. rosella</i>	166.43	39.61	185.3
<i>L. rubella</i>	62.38	26.62	212.6
<i>L. salviifolia</i>	68.20	4.80	190.6
<i>L. sericea</i>	203.63	25.99	255.4

<sup>a</sup>Not analyzed.

In our investigation,  $\beta$ -caryophyllene was present within the main component of all analyzed species, and also appeared at high concentrations (4.68 to 33.55%). The isomer  $\alpha$ -caryophyllene was also found in all *Lippia* spp. at low concentrations (0.29 to 9.21%), except in *L. sericea* where it appeared as the most abundant component (22.21%).

Germacrene D was also identified as a common component in the studied species, except in *L. martiana*. The compound was found as the major constituent in *L. aff. microphylla* (39.13%), *L. aristata* (27.42%), *L. corymbosa* (22.15%), *L. hermannioides* (27.84%), *L. lupulina* (41.97%) and *L. microphylla* (20.37%), while in *L. filifolia* and *L. pseudo-thea* the concentrations were lower than 2%. Moreover, the sesquiterpenes  $\alpha$ -copaene (ranging from 0.63 to 10.63%),  $\beta$ -bourbunene (0.21 to 2.44%),  $\beta$ -elemene (0.64 to 18.37%), alooromadendrene (0.57 to 7.07%), bicyclogermacrene (0.75 to 11.67%), and  $\delta$ -cadinene (0.68 to 10.44%) have been identified for most of the *Lippia* studied. Oxygenated sesquiterpenes were detected in minor amounts. However, nerolidol (29.65%) was the major constituent of *L. salviifolia*.

Besides terpenoids, hydrocarbons common on the surface of plants such as undecane, tridecane and pentadecane were identified in a high percentage in *L. diamantinensis* (4.18, 4.57, and 24.88%, respectively).

The antioxidant activity of HF, expressed as the concentration that inhibited 50% DPPH free radical (IC<sub>50</sub>), ranged from 57.00 to 437.78 µg/mL (Table 2), showing a low radical scavenging activity as compared to ascorbic acid (2.50 µg/mL) and BHT (11.82 µg/mL). However, the ethanolic fraction of the majority of species examined showed high antioxidant activity as compared to BHT (11.82 µg/mL). The concentrations that led to 50%

inhibition (IC<sub>50</sub>) are given in Table 2. When we analyzed the total of phenol content these fractions estimated as tannic acid equivalent, it ranged from 105.5 mg/g in *L. pseudo-thea* to 255.4 mg/g in *L. sericea* (Table 2).

## DISCUSSION

Hexanic fractions obtained from fresh leaves of sixteen *Lippia* spp. showed a great chemical diversity. Among the monoterpenes hydrocarbons, it was possible to detect limonene and linalool, two components that can be found in higher quantities in essential oils of other *Lippia* spp. (Pascual et al., 2001). Myrcene and myrcenone were identified as the major components of the essential oils from the leaves and flowers of *L. lacunosa*, while limonene and myrtenal was observed in *Lippia rotundifolia* (Leitão et al., 2008).

The oxygenated monoterpenes, such as the neral and geranial were the most abundant components observed in some species (e.g. *L. diamantinensis*). These compounds have been described as important constituents of others species of *Lippia* such as *L. citriodora* (Argyropoulou et al., 2007) and *L. rugosa* (Tatsadjieu et al., 2009). The citral (mixture of neral and geranial) showed potent antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi (Onawunmi, 1989), while anti-inflammatory, analgesic and antifungal properties are assigned to the 1.8-cineole, the main compound in *L. pseudo-thea* and in *L. rosella* (Santos and Rao, 2000; Vilela et al., 2009).

Sesquiterpenes were mainly represented by  $\beta$ -caryophyllene, one component commonly found in essential oils of the genus *Lippia* (Pascual et al., 2001).

Previously,  $\beta$ -caryophyllene was identified as the major component of volatile oil from leaves and flowers of *Lippia chevalieri* (Mevy et al., 2007). In our investigation, this component was observed as one of the main components of all analyzed species, and it also appeared at high concentrations. The isomer,  $\alpha$ -caryophyllene, was also found in *Lippia* spp. These findings constitute evidences that both  $\beta$ -caryophyllene and  $\alpha$ -caryophyllene have anti-inflammatory and anti-allergic effects (Passos et al., 2007).

In general, the present data are in accordance with those previously reported (Pascual et al., 2001; Terblanché and Kornelius, 1996), which showed high variability of the chemical composition of the essential oils in *Lippia* spp. Thus, the HF represents a qualitative alternative to analyze the volatile secondary metabolites. The possibility of using HF instead of essential oil extraction increases the capability of *Lippia* chemical studies since many species possess few and very small leaves.

The literature emphasizes that, within species, a variety of geographical and ecological factors, the isolation method and the analytical conditions can influence the volatile oil composition (Santos-Gomes et al., 2005). In spite of these findings, our data were in accordance with previous studies for *L. microphylla* collected in the northeast of Brazil (Costa et al., 2005). Only thymol and 1, 8-cineole were not identified in *L. aff. microphylla* and *L. microphylla* analyzed in the present study.

The antioxidant activity of hexanic fraction of *Lippia* spp. showed low radical scavenging activity. Similar results were found for other species of the same genus. Both the essential oil from *Lippia berlandieri* (Rocha-Guzmán et al., 2007) and *L. chevalieri* (Mevy et al., 2007) also showed a lower antioxidant property. However, the limonene-carvone chemotype of *L. alba* (Stashenko et al., 2004) and *L. sidoides* (Monteiro et al., 2007), which were rich in thymol, showed good antioxidant potential. In our investigation, carvone and thymol were not identified.

Thus, it is noted that the DPPH method shows a better response to polar samples when compared with the nonpolar samples (Sultana et al., 2007). Literature reports that the low response to the test for free radical scavenging can be attributed to the absence of conjugation to form stable resonance structures during the formation of radicals in the low polarity molecules (Di Majo et al., 2005).

On the other hand, the ethanolic fraction of the majority of species showed high antioxidant activity. Based on previous data, it is possible that the powerful antioxidant activity of polar extracts can be attributed to phenolic compounds (Mensor et al., 2001), although, in the present study, the results did not show a conclusive relationship between the total phenolic content and antioxidant activity. In addition, the presence of these compounds in the ethanolic fraction of *Lippia* spp. may also be the major cause of their high radical-scavenging

activity. Several studies have also reported the absence of this kind of relationship. The molecular antioxidant response to free radicals varies markedly, depending on the chemical structure and the oxidation conditions. Phenol constitutes one of the major groups of compounds that act as primary antioxidants (Muchuweti et al., 2006). Antioxidant compounds neutralize chemically active products of the metabolism, such as free radicals which can damage cells and tissues. Phenolic compounds, with their potential to act as antioxidants, may play a key role on the prevention of various pathological conditions such as cancer, cardiovascular and neurodegenerative diseases that some authors believe to be associated with oxidative stress (Losso et al., 2007).

Conclusively, this is the first report of chemical composition and antioxidant activity of sixteen *Lippia* spp. Chemical analysis revealed the presence of various terpenoid compounds which are common in this genus. Furthermore, the compounds of the EF from leaves of *Lippia* spp. showed high antioxidant activity suggesting the potential of these plants as a natural source of strongly antioxidant substances that can be used as a natural additive in food and pharmaceutical industries.

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