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Comparative analysis of chemical profiles and antioxidant activities of essential oils obtained from species of *Lippia* L. by chemometrics

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ABSTRACT

Due to the importance of diseases associated with oxidative stress, the search for natural antioxidants proves to be essential. This work aimed to compare the chemical composition and antioxidant potential of essential oils from the genus *Lippia* L. through chemometric analysis. The essential oils were characterized by gas chromatography coupled with mass spectrometry. Antioxidant potentials were determined by DPPH, ABTS, Deoxyribose and β-carotene protection, Iron chelation and reduction methods. All data were related by multivariate analyzes. Essential oils showed low similar chemical compositions and no statistically significant relationship. These showed relevant antioxidant activity, especially for *L. sidoides* that obtained IC_{50} of 5.22 ± 0.08 μg/mL in ABTS capture. Multivariate analyzes showed the effectiveness of *L. alba* compounds to DPPH scavenging, Fe^{3+} reduction and β-carotene protection, and *L. gracilis* components to deoxyribose protect. Thus, studies proving the antioxidant potential of *Lippia* compounds against oxidative stress and their use in food conservation are fundamental.

1. Introduction

Reactive oxygen species (ROS) are oxidizing agents capable of causing direct damage to biomolecules such as lipids, proteins and DNA, where their excessive production directly contributes to an oxidative imbalance that often results in oxidative stress, a set of harmful biochemical events that act as a trigger for the development of numerous diseases, leading to irreversible damage and cell death (Chatterjee, 2016).

Given the importance of oxidative stress-associated diseases in the context of global health, the search for natural antioxidants capable of neutralizing the action of free radicals has attracted the interest of researchers all over the world (Dziąbowska-Grabias et al., 2021). Natural antioxidants can be found in a wide variety of plant species, as they are produced by their secondary metabolism and stored in different parts of vegetables, including fruits, which encourages the consumption of these products to take advantage of their biological activities (Safaeian et al., 2020).

In this context, studies show that essential oils are endowed with pharmacological properties that make them useful in the management of diseases associated with oxidative stress (Goudjil et al., 2020). Essential oils are complex substances formed by volatile compounds such as monoterpenes and sesquiterpenes hydrocarbons and their oxygenated derivatives. Importantly, the therapeutic potential of many of these compounds has been previously reported (Giacometti et al., 2018).

The genus *Lippia*, the second largest genus in the family Verbenaceae, includes more than 200 species of herbs, shrubs, and small trees characterized by the presence of essential oils (Silva et al., 2018). In Brazil, this genus is the principal representant of the family, with 88 species, 68 of which are endemic and distributed in biomes such as the cerrado and

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rupestrian fields (Salimena & Múlgura, 2015).

Species of *Lippia* have been widely used in the treatment of parasitic infections, asthma, stomach pain, and menstrual disorders. Additionally, Due to the significant production of essential oils, many of these species are used in the cosmetic industry, especially in the production of perfumes (Soto-Armenta et al., 2019). Accordingly, previous studies have reported the analgesic, anti-inflammatory, antipyretic, antihypertensive, and antimicrobial properties of species belonging to the genus (Siqueira-Lima et al., 2019).

Studies have demonstrated the antioxidant potential of essential oils obtained from species of *Lippia*. Importantly, evidence indicates that these substances can be successfully used in the food industry, as they are capable of increasing the concentration of phytochemicals, as well as improving the quality and conservation of food for consumption (Ishkeh et al., 2019). Despite the chemical complexity and diversity of essential oils in this genus, it has been suggested that the food-improving properties of this substances are directly related to the action of phenolic monoterpenes present in the composition of these species (Cantú-Valdéz et al., 2020).

The mechanisms underlying the antioxidant interactions of mixtures of compounds have not been sufficiently elucidated, and for essential oils only general statements have been made, indicating, for the most part, that the antioxidant activity of the majority composition can be modulated by other components present in these mixtures (Ciesla et al., 2016). In this context, chemometric techniques can represent important alternatives in the characterization of the properties of individual constituents, as they enable the evaluation of multiple variables and their interactions, as well as allow the obtaining of a single and global response for multiple dependent variables (Diedrich et al., 2021).

Considering the above-mentioned evidence, this work aimed to compare the chemical composition and antioxidant potential of essential oils from three species of the genus *Lippia* L. using chemometric analysis.

2. Materials and methods

2.1. Botanical material and essential oil extraction

Fresh leaves of *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson (878 g) and *Lippia sidoides* Cham. (3000 g) were collected in the Garden of Medicinal Plants of the Regional University of Cariri (7°14′20.1″ S 39°24′53.1″ W) in April 2019, and exsiccate of each species was identified and deposited at the Herbarium Caririense Dárdano de Andrade Lima (HDCAL/URCA) under the registry numbers 13,907 and 3038, respectively. The leaves of *Lippia gracilis* Schauer (418 g) were collected in the Gisélia Pinheiro district, Crato, Ceará, Brazil (7°13′05.2″ S 39°25′44.9″ W) in April 2019, and the species exsiccate were identified and registered at the Herbarium Prisco Bezerra of the Federal University of Ceará (registry number 44456). For both collection areas, according to the Köppen classification, the climate is AW, corresponding to a humid tropical climate, with an average annual rainfall of 850 mm, an average air temperature of 27 °C and a relative humidity of around 75% (Vásquez et al., 2019).

Essential oil extraction was performed by hydrodistillation in a Clevenger-type apparatus. Fresh leaf samples were crushed and subjected to distillation for 2 h (ANVISA 2019). After extraction, the essential oils of *L. alba* (LaEO), *L. sidoides* (LsEO) and *L. gracilis* (LgEO) were dried with anhydrous sodium sulfate (Na_2SO_4), presenting yields (w/w) of 0.18%, 0.52%, and 0.89% respectively.

2.2. Chemicals and solvents

All chemicals were analytical grade. Dichloromethane, chloroform, and methanol (MeOH) were purchased from Merck KGaA (Darmstadt, Hesse, Alemanha). Ascorbic acid, Na_2SO_4, hydrocarbons (C8-C40), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS), H_2O_2, $FeSO_4$, Deoxyribose, KH_2PO_4, trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeCl_3$, Tris-HCl, *o*-phenanthroline (*o*-phe), linoleic acid, Tween 40, and *β*-carotene were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.3. Essential oil analysis

The volatile constituents were analyzed by Gas Chromatography coupled with Mass Spectrometry (GC/MS) using a Shimadzu model GC–MS QP2010 apparatus equipped with an Rtx-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm), both provided by Shimadzu Scientific Instruments Inc. (Columbia, Maryland, USA). The temperature was programmed as follows: 60–240 °C at 3 °C/min; 240–280 °C at 10 °C/min, ending at 280 °C for with 10 min. Helium was used as the carrier gas with a flow rate of 1.5 mL/min and split mode with a 1:50 ratio. The injection port was set at 220 °C. Operating parameters of MS quadrupole: interface temperature = 240 °C; Electron impact ionization set at 70 eV with scan mass ranging from 40 to 350 *m/z* and sampling rate of 1.0 scan/s. Injected volume: 1 μL of a 5 μg/mL solution in dichloromethane. The constituents were identified by computational search using digital libraries of mass spectral data (NIST 08) and by comparing their authentic mass spectra. The Kovats retention index was obtained by injecting a mixture of C8-C40 linear hydrocarbons under the same conditions as the samples, as described by Van Den Dool and Kratz (1963). The identity of the compounds was confirmed by comparing their retention indices and mass spectra with those taken from the literature (Adams, 2007).

2.4. Antioxidant activity analysis

2.4.1. DPPH free radical scavenging

The DPPH free radical scavenging was determined following the method proposed by Rufino et al. (2007a). To this end, each essential oil was diluted to concentrations ranging from 5 to 1000 μg/mL. Then, wells on a 96-well plate were filled with 20 μL of the essential oil and 280 μL of a 0.06 mM DPPH solution. After 30 min of reaction in a dark environment, the readings were taken at 515 nm in a spectrophotometer Kasuaki DR-200BS (Araucária, Paraná, Brazil). MeOH and ascorbic acid were used as the blank and positive control, respectively, while the solvent + DPPH was used as the negative control. The results were calculated according to equation 1, where Abs means absorbance:

$$AA\% = 100 - -\{[(AbsSample - -AbsBlank)/AbsNegative\,Control]x100\}$$

2.4.2. $ABTS^{\bullet+}$ free radical capture

These analyses were carried out according to the method described by Rufino et al. (2007b). Briefly, 30 μL of each essential oil solution (5–1000 μg/mL) was transferred to test tubes containing 3.0 mL of $ABTS^{\bullet+}$ radical and kept reacting in the absence of light for 6 min. Then, the readings were performed at 734 nm. The same controls described in the previous section were included in this assay, and results were calculated using equation 1.

2.4.3. Deoxyribose oxidative degradation assay

The ability of the essential oils to inhibit deoxyribose degradation was assessed through the methodology of Puntel et al. (2005). The reactive mixture consisted of 240 μL of H_2O_2 (0.8 mM), 240 μL of $FeSO_4$ (0.08 mM), 450 μL of potassium phosphate buffer (7.5 mM, pH 7.4), 150 μL of deoxyribose (1.5 mM) and 320 μL of H_2O. For each extract concentration (5 to 1000 μg/mL), 100 μL of the sample was added to the reaction, followed by incubation at 37 °C for 60 min. After this period, the reactive mixture was added with 750 μL of 2.8% TCA and 750 μL of 0.8% TBA, followed by an additional incubation at 100 °C for 20 min. The negative control and blank consisted of the reactive mixture components in the absence of extracts and deoxyribose, respectively. After cooling, the readings were performed at 532 nm and the results were

expressed as a percentage of protection according to equation 2:

$$\text{Protection}(\%) = \{[\text{AbsControl} - (\text{AbsSample} - -\text{AbsBlank})]/\text{AbsControl}\} \times 100$$

2.4.4. Fe^{2+}-chelating activity and Fe^{3+}-reducing power test

To analyze the Fe^{2+}-chelating activity and Fe^{3+}-reducing power of the essential oils we used the *o*-phe test, as reported by Minotti and Aust (1987), with some modifications. Eppendorf tubes were filled with 500 μL of extract solution (at concentrations ranging from 5 to 500 μg/mL), 500 μL of $FeSO_4$ (1000 μM) or 500 μL of $FeCl_3$ (1000 μM), separately. After 2 min of reaction, 50 μL of this mixture were added to each well on a 96-well plate containing a mix of Milli Q water, Tris-HCl (0.1 M, pH 7.4), and *o*-phe (300 μM). Fe^{2+} and Fe^{3+} controls were obtained by replacing the extract solution with Milli Q water, while the blank was obtained by adding Milli Q water with the corresponding ferrous solution. The readings were performed in a spectrophotometer at 510 nm and the results were expressed in percentage, according to equation 2. Fe^{3+} reducing power was determined by comparing the results with those of Fe^{2+} controls.

2.4.5. Co-oxidation of β-carotene/Linoleic acid method

The analysis followed the methodology proposed by Rufino et al. (2006). The reaction mixture consisted of 40 μL of linoleic acid, 530 μL of Tween 40, 50 μL of β-carotene (20 mg/mL), and 1 mL of chloroform. After homogenizing the mixture, an oxygenator was used to vaporize the chloroform and Milli Q water was added until reaching an absorbance ranging between 0.6 nm and 0.7 nm at 470 nm. For testing, 250 μL of this reactive mixture and 10 μL extract solution (5 to 1000 μg/mL) were kept reacting on wells on a microplate. The blank and controls were obtained by replacing respectively, the mixture components and extract solutions with Milli Q water. The results were expressed as a percentage of oxidation inhibition according to equation 2.

2.5. Statistical analysis

The values obtained in the antioxidant assays were expressed as mean ± standard deviation of three sample replicates (n = 3). For antioxidant activity analysis, after data normalization, a non-linear regression was applied to obtain the IC_{50} values. Then, ANOVA and Tukey's test was applied for multiple comparisons between pairs. The GraphPad Prism software version 8.0 for Windows (GraphPad Software, San Diego, California, USA) was used in all analyzes and results with a $P < 0.05$ were considered statistically significant. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to compare the composition of essential oils among the three species, as well as to correlate them with their antioxidant activities. Multivariate analyzes were performed using Jamovi software version 1.6.16 (Jamovi Project, Sydney, Australia).

3. Results and discussion

3.1. Essential oil analysis

The compounds identified in the essential oils, as well as their percentages and retention indices, are listed in Table 1. Our analysis identified 96.51%, 98.80%, and 98.34% of the total constituents of LaEO, LsEO, and LgEO, respectively. Qualitatively, all of them presented monoterpenes as the most abundant compounds (LaEO: 83.88%, LsEO: 91.16%, and LgEO: 84.29%), which is a common feature among species of this genus (Pascual et al., 2001).

The main constituents found in *L. alba* oil were geranial (43.20%), neral (30.17%), and (*E*)-caryophyllene (5.76%), thus being classified as belonging to the Citral chemotype, which is recognized for having numerous biological activities such as analgesic, anti-inflammatory,

Table 1
Chemical compounds identified in the essential oils of *L. alba*, *L. sidoides* and *L. gracilis*.

N°	Compound	RI[1]	RI[2]	LaEO	LsEO	LgEO
1.	Aromadendrene	1665	1662	–	0.37 ± 0.01[a]	–
2.	11-Bicyclo [7,2,0] undecan-3-ol	1643	1644	–	0.22 ± 0.01[a]	–
3.	δ-Cadinene	1528	1530	0.53 ± 0.01[a]	–	0.39 ± 0.00[a]
4.	Camphene	953	953	–	–	0.28 ± 0.00[a]
5.	δ-3-Carene	1012	1011	–	–	0.30 ± 0.01[a]
6.	Carvacrol	1307	1307	–	0.31 ± 0.01[a]	10.06 ± 0.02[b]
7.	Carvacryl acetate	1357	1354	–	–	0.62 ± 0.01[a]
8.	(*E*)-Caryophyllene	1423	1423	5.76 ± 0.01[a]	3.55 ± 0.02[a]	6.76 ± 0.04[a]
9.	9-*epi*-(*E*)-Caryophyllene	1443	1442	–	0.78 ± 0.01[a]	–
10.	Caryophyllene oxide	1589	1589	1.02 ± 0.01[a]	1.65 ± 0.01[a]	0.23 ± 0.02[a]
11.	(*E*)-methyl-Cinnamate	1387	1386	–	–	6.03 ± 0.00[a]
12.	α-Copaene	1386	1387	–	–	0.38 ± 0.01[a]
13.	*o*-Cymene	1025	1027	0.90 ± 0.01[a]	–	–
14.	*p*-Cymene	1026	1026	–	44.83 ± 0.00[a]	12.25 ± 0.01[b]
15.	β-Elemene	1395	1394	3.95 ± 0.01[a]	–	–
16.	Eucalyptol	1032	1032	–	8.52 ± 0.00[a]	0.23 ± 0.02[b]
17.	Geranial	1274	1270	43.25 ± 0.03[a]	–	–
18.	Geraniol	1257	1256	1.20 ± 0.01[a]	–	–
19.	Geranyl acetate	1379	1378	0.39 ± 0.00[a]	–	–
20.	Germacrene D	1485	1485	1.37 ± 0.01[a]	0.27 ± 0.01[a]	–
21.	6-methyl-Hept-5-en-2-one	986	986	3.33 ± 0.04[a]	–	–
22.	α-Humulene	1458	1458	–	–	0.32 ± 0.01[a]
23.	Limonene	1029	1029	–	1.33 ± 0.01[a]	0.72 ± 0.01[a]
24.	Linalool	1101	1101	0.99 ± 0.00[a]	0.28 ± 0.01[a]	0.38 ± 0.01[a]
25.	Myrcene	991	991	0.63 ± 0.01[a]	0.24 ± 0.01[a]	1.77 ± 0.00[a]
26.	Neral	1244	1242	30.17 ± 0.00[a]	–	–
27.	Nerol	1230	1228	1.24 ± 0.00[a]	–	–
28.	α-Pinene	933	933	–	1.45 ± 0.00[a]	6.11 ± 0.01[a]
29.	β-Pinene	980	980	–	–	0.35 ± 0.01[a]
30.	Spathulenol	1584	1584	–	0.29 ± 0.01[a]	–
31.	Terpinen-4-ol	1180	1180	–	1.22 ± 0.01[a]	0.39 ± 0.00[a]
32.	α-Terpinene	1018	1018	–	–	0.68 ± 0.01[a]
33.	γ-Terpinene	1059	1059	–	–	2.68 ± 0.01[a]
34.	α-Terpineol	1193	1195	–	0.70 ± 0.01[a]	–
35.	Thuja-2,4(10)-diene	1007	1009	0.43 ± 0.01[a]	–	–
36.	α-Thujene	923	923	–	–	0.32 ± 0.01[a]
37.	Thymol	1298	1298	–		

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	RI[1]	RI[2]	LaEO	LsEO	LgEO
					29.92 ± 0.01[a]	34.75 ± 0.01[a]
38.	Thymol methyl ether	1237	1237	–	1.56 ± 0.01[a]	12.38 ± 0.01[b]
39.	Verbenone	1176	1176	–	0.38 ± 0.01[a]	–
40.	Vinyl amyl carbinol	942	941	1.45 ± 0.00[a]	0.45 ± 0.01[a]	–
41.	Viridiflorene	1500	1501	–	0.25 ± 0.01[a]	–
42.	Zonarene	1527	1526	–	0.26 ± 0.01[a]	–
	Total			**96.66 ± 0.10**	**98.92 ± 0.09**	**98.47 ± 0.08**

RI[1]: Experimental retention index; RI[2]: Literature retention index; LaEO: *L. alba* essential oil; LsEO: *L. sidoides* essential oil; LgEO: *L. gracilis* essential oil. Compound elution order: 36, 28, 40, 4, 29, 21, 25, 35, 5, 32, 13, 14, 23, 16, 33, 24, 39, 31, 34, 27, 38, 26, 18, 17, 37, 6, 7, 19, 12, 11, 15, 8, 9, 22, 20, 41, 42, 3, 30, 10, 2, 1. Means followed by different letters differ by Tukey test with a $P < 0.05$.

antipyretic, sedative, anti-asthmatic, anti- hypertensive, antispasmodic, emmenagogue, diaphoretic, among others (Silva et al., 2018).

P-cymene (44.83%), thymol (29.92%), and eucalyptol (8.52%) were identified as the major compounds in the essential oil of *L. sidoides*, partially differing from previous reports identifying thymol as a major compound among specimens collected in the Northeast of Brazil (Santos et al., 2015). Studies have demonstrated that the chemical composition and yield of essential oils obtained from *Lippia* species can be influenced by abiotic factors, such as seasonality, water availability, light exposure, temperature, plant development stage, and plant nutritional status, as well as by genetic factors and procedures during and after the harvest (Soares & Tavares-Dias, 2013). These factors may be linked to the lower content of thymol content in the oil analyzed by this study, while its precursor (*p*-cymene) was found in greater concentrations.

On the other hand, the essential oil of *L. gracilis* proved to be rich in thymol (34.75%), thymol methyl ether (12.38%), and *p*-cymene (12.23%), which is in line with previous studies analyzing species collected in the state of Maranhão, reporting the same compounds among the major components (Franco et al., 2014). Curiously, carvacrol, an isomer of thymol, represented only 10.06% of the total composition of LgEO, corroborating the evidence showing a linear relationship between the contents of these isomers among species, i.e., if the concentration of thymol is high, the concentration of carvacrol is low, and vice-versa (Nezhadali et al., 2014).

In addition to these major compounds, myrcene, linalool, caryophyllene oxide, and (*E*)-caryophyllene were identified in the essential oils of the species, the latter representing 5.76%, 3.56%, and 6.70% of the essential oils of LaEO, LsEO, and LgEO, respectively.

3.2. Antioxidant activity

At a concentration of 1000 μg/mL, the essential oils of *L. gracilis*, *L. sidoides* and *L. alba* showed DPPH-scavenging activities, with inhibitions of 60.05%, 58.53%, and 29.89%, respectively (Fig. 1A). In addition to presenting very similar scavenging capacity, LgEO and LsEO present statistically comparable IC_{50} values, as shown in Table 2. It has been demonstrated that the essential oils obtained from fresh and dried leaves of *Lippia thymoides* Mart. & Schauer (which are rich in thymol) showed DPPH-scavenging activities that were correlated with the concentration of thymol, with inhibition percentages ranging from 65.28 to 89.97% (Nascimento et al., 2021).

The structural characteristics of thymol, especially the presence of phenolic hydroxyl and alkyl groups in the *ortho* and *meta* positions favor its antioxidant properties. The latter increases the electronic density of phenoxyl radicals formed by hydrogen donation, stabilizing them by inductive effects and thus enhancing the anti-radical activity (Aprotosoaie et al., 2019).

All essential oils evaluated in this study showed significant ability to capture the ABTS radical, with an emphasis on the activity of LsEO and LgEO, which presented inhibition percentages corresponding to 99% at the concentrations of 500 and 1000 μg/mL, respectively. These percentages were very close to that obtained by ascorbic acid, which had 100% inhibition efficiency at 500 μg/mL, as shown in Fig. 1B. Importantly, LaEO, LsEO and LgEO presented significantly lower IC_{50} values than that of the positive control (ascorbic acid), confirming their relevant antioxidant potential (Table 2).

The ABTS radical is comparatively more reactive than the DPPH radical, as the oxidant mechanism of the former involves electron transfer, while the latter involves the transfer of H atoms (Kaviarasan et al., 2007). In addition, the ABTS test can be used for both water-soluble and fat-soluble samples, whereas the DPPH reagent should be preferentially used for organic solvents (Sucupira et al., 2014). These factors may explain the differences in the results obtained from these analyses.

The deoxyribose protection test showed that the essential oils were effective in neutralizing the $OH^{\bullet}$ radical, where the percentages of inhibition followed the order LaEO > LsEO > LgEO, as shown in Fig. 1C. The IC_{50} of the samples did not differ statistically from ascorbic acid, except for LgEO, which was less effective when compared to the others (Table 2).

She et al. (2019), following the composition of the essential oil of *Litsea cubeba* (Lour.) Pers., for three months, identified citral as the major constituent of the essential oils of the samples studied in this period, demonstrating that this compound has significant $OH^{\bullet}$-neutralizing activity, with IC_{50} of 0.14%, 0.04% and 0.31% (v/v). Furthermore, *in vivo* studies showed that citral is effective in reducing both intracellular and extracellular concentrations of ROS (Safaeian et al., 2020).

On the other hand, when compared to quercetin ($IC_{50} = 4.61$ μg/mL) and carvacrol ($IC_{50} = 8.00$ μg/mL), thymol ($IC_{50} = 0.23$ μg/mL) had more potent $OH^{\bullet}$-eliminating activity (Stoilova et al., 2008). Evidence indicates that the radical elimination mechanism by thymol occurs through the production of the phenoxyl radical as a transient species, which is formed mainly from adducts, with the addition of the $OH^{\bullet}$ radical both in the *ortho* position (which is more energetically favorable) and in the phenolic group, which undergoes dehydration, generating the phenoxyl radical (Venu et al., 2013).

Thymol is also recognized to be able to increase levels of antioxidant compounds and oxygen uptake in plant tissues, including enzymatic and non-enzymatic systems, which leads to increased oxygen radical uptake and hydroxyl radical scavenging capacity of tissues, improving disease resistance and minimizing physiological degradation (Perumal et al., 2021). In addition, both thymol and citral hold GRAS status, generally recognized as safe, and are approved by the United States Food and Drug Administration (FDA) and can be used as food flavoring and preservatives (Masyita et al., 2022).

The Fe^{3+} reduction test is used to evaluate the ability of compounds to reduce Fe^{3+} to Fe^{2+} through the donation of hydrogen atoms (Bouzenna et al., 2017). Our analysis showed that both LaEO and LgEO exhibited higher Fe^{3+} reducing power than the positive control ascorbic acid (Fig. 1D). All the essential oils, as well as ascorbic acid, exhibited a concentration-dependent effect, whereas LsEO presented the lowest IC_{50} among the essential oils (Table 2). The isolated compound citral is recognized for its ability to donate electrons, presenting a concentration-dependent Fe^{3+}-reducing activity, with an IC_{50} of 125 μg/mL (Bouzenna et al., 2017), which corroborates the results obtained with the LaEO.

Also, all the essential oils exhibited Fe^{2+} chelating activity, among which LgEO, at the concentrations of 250 and 500 μg/mL, stands out for presenting higher chelation percentages than that of ascorbic acid (Fig. 1E). As for the Fe^{3+}-reducing activity, LsEO showed the lowest IC_{50} among the essential oils, followed by LaEO and LgEO. However, the positive control presented a significantly lower IC_{50} (Table 2). These

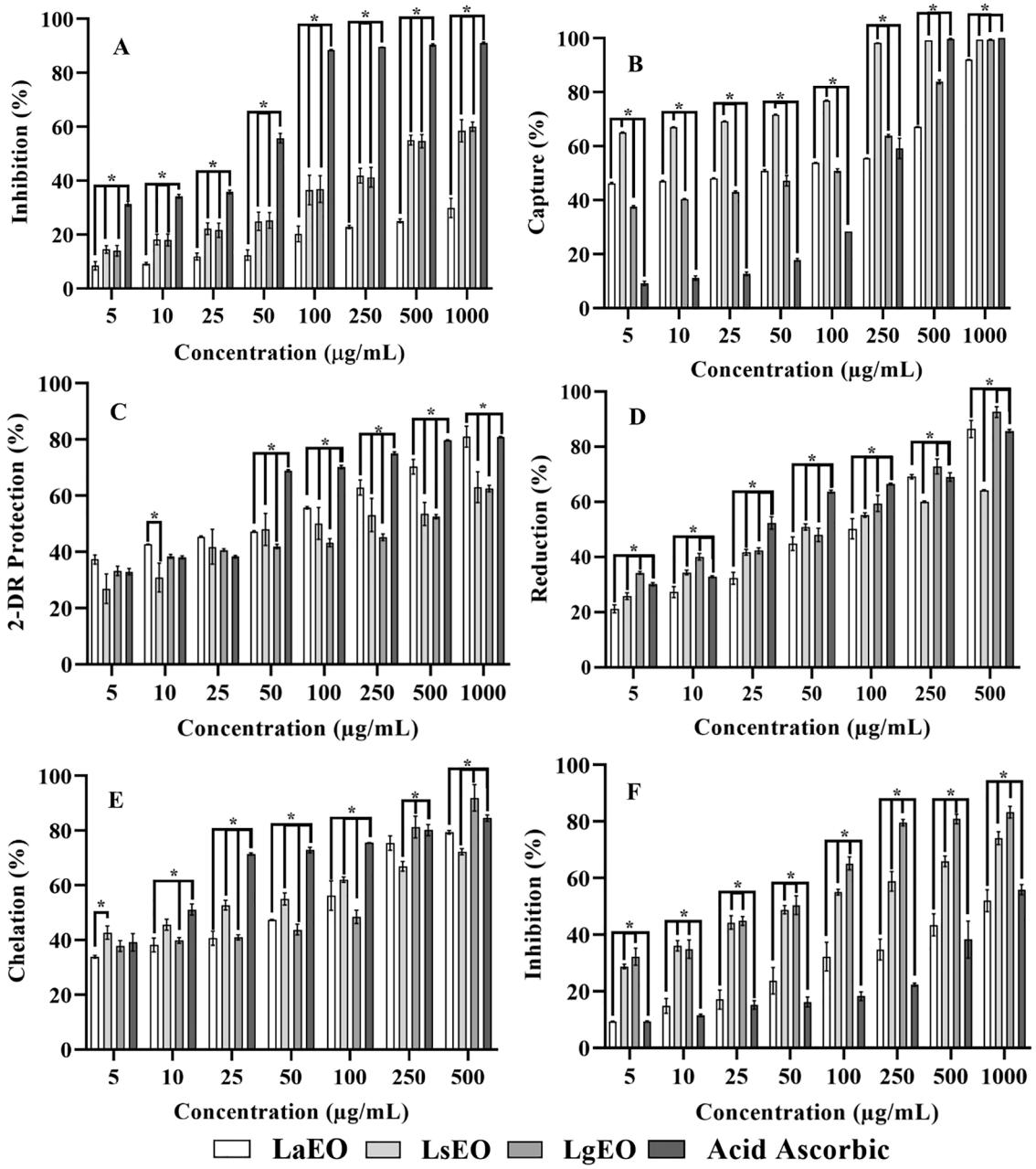

Fig. 1. Antioxidant activity of the essential oils of *L. alba*, *L. sidoides* and *L. gracilis* by different mechanisms. A: DPPH free radical scavenging; B: ABTS radical sequestration; C: Deoxyribose protection; D: Fe^{3+} reduction; E: Fe^{2+} chelation; F: Inhibition of linoleic acid degradation. Ascorbic acid was used as a standard antioxidant control. *: $P < 0.05$. Data were analyzed by ANOVA and Tukey's post hoc test.

Table 2
IC_{50} values of the essential oils of *L. alba*, *L. sidoides* e *L. gracilis* obtained through different antioxidant mechanisms.

Sample	IC_{50} (µg/mL)					
	DPPH	ABTS	Deoxyribose protection	Fe^{3+} Reduction	Fe^{2+}Chelation	Co-oxidation
LaEO	5,849 ± 0.00[a]	45.79 ± 1.81[a]	59.06 ± 2.15[a]	67.42 ± 6.94[a]	56.10 ± 4.15[a]	737 ± 57.97[a]
LsEO	377 ± 34.04[b]	5.22 ± 0.08[b]	42.53 ± 5.06[a]	50.05 ± 1.90[b]	22.74 ± 5.49[b]	69.08 ± 0.28[b]
LgEO	397 ± 53.06[b]	88.91 ± 1.27[c]	456 ± 34.04[b]	62.49 ± 1.08[a]	102 ± 0.60[c]	40.25 ± 2.03[c]
Ascorbic Acid	34.02 ± 0.60[c]	195 ± 3.39[d]	33.51 ± 0.58[a]	27.52 ± 0.64[c]	9.79 ± 0.95[d]	905 ± 4.85[d]

These results are expressed as mean ± SD (n = 3). Means followed by different letters differ by Tukey test with a $P < 0.05$.

results demonstrate the effectiveness of the essential oils as antioxidants that participate in the catalysis in the Fenton and Haber-Weiss reactions, originating reactive oxygen species such as hydroxyl radicals ($HO^{\bullet}$) and superoxide ($O_2^{\bullet -}$) (Barreiros et al., 2006).

The co-oxidation test analyzed the ability of the essential oils to prevent lipid peroxidation by inhibiting the attack to β-carotene double bonds by the radicals generated during the oxidation of linoleic acid (Sucupira et al., 2014). The essential oils were found to neutralize the

peroxide radicals, where LsEO and LgEO showed stronger activities than ascorbic acid at all tested concentrations, as shown in Fig. 1F. Additionally, these essential oils presented the best IC_{50} values (Table 2), followed by the LaEO and ascorbic acid, respectively.

The ability to inhibit lipid peroxidation in the co-oxidation test was demonstrated by several especies whose essential oils presented thymol as a major constituent. In this context, *Origanum vulgare* subsp. *vulgare* presented inhibitory activities of 99.89%, (Sarikurkcu et al., 2015). Furthermore, Kazemi and Rostami (2015), studying the biological activities of the essential oil of *Achillea wilhelmsii* L., found that even though thymol is not its major compound of the species, it represented 65% of the antioxidant activity presented by its oil.

3.3. Multivariate analysis

Principal Component Analysis (Fig. 2) was conducted in order to explore the correlations between the chemical compositions and antioxidant potential of the essential oils. The number of principal components in the analysis was determined using the minimum eigenvalue criterion (greater than1) considering the variability of the dimensions of the antioxidant responses and correlation coefficients, explaining 100% of the total variance of data.

The analysis of graphically represented PCA scores shows that the chemical compositions of essential oils had little significant correlation with each other. Both PC1 (weight – 25.10) and PC2 (weight – 22.90) played important roles in this differentiation, with PC1 showing positive charges and PC2 showing both negative and positive charges. On the other hand, all antioxidant activities showed positive values for PC1 demonstrating that the chemical composition of *L. gracilis* and *L. alba* oils significantly influence their activity.

Accordingly, the presence of components such as camphene, δ-3-carene, carvacrol, carvacrol acetate, α-copaene, *o*-cymene, α-humulene, α- and β-pinene, α-terpinene and α-thujene was correlated with the antioxidant activity of the essential oils on deoxyribose degradation protection. On the other hand, compounds δ-cadinene and (*E*)-caryophyllene showed correlations with the antioxidant activity on the ABTS and iron-chelating assays.

The present PCA analysis also indicates that most antioxidant activities are directly correlated with *o*-cymene, β-elemene, geranial, geraniol, geranyl acetate, germacrene D, 6-methyl-Hept-5-en-2-one, linalool, neral, nerol, thuja- 2,4 (10)-diene, and vinyl amyl carbinol. These results showed that most of the antioxidant activity and essential oil content had a high load on PC1, showing *L. gracilis* and *L. alba* as species whose essential oils present the highest antioxidant activity and best essential oil content. On the other hand, *L. sidoides* had the most negative scores on PC1 and PC2, corroborating the weakest antioxidant activity and poorest essential oil content.

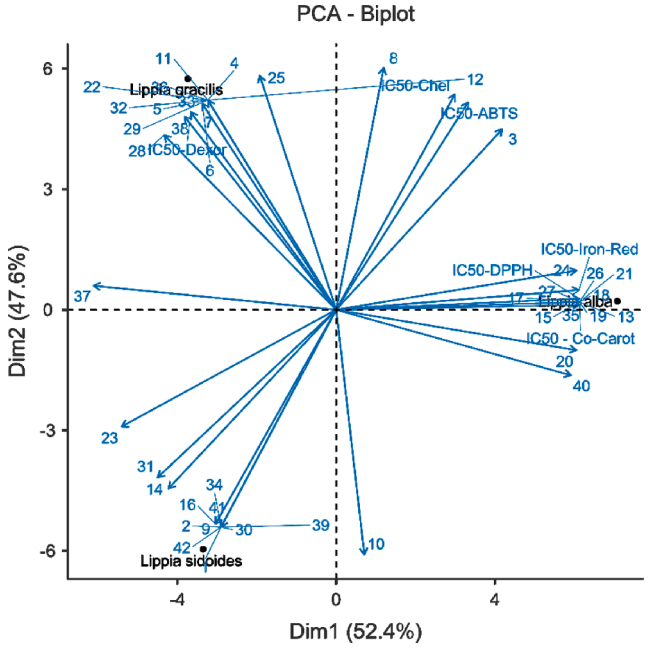

Fig. 2. Principal Component Analysis Biplot (*Score* e *loading*) showing correlations between the chemical compositions and antioxidant activities of *Lippia* L. essential oils.

The chemical composition similarity among the species was analyzed by Euclidean distance matrix using heatmap cluster analysis. This visual representation of numerical data shows individual values as colors where higher and lower numerical values are represented by darker-colored and lighter-colored squares, respectively and dendrograms demonstrate the relationships between groups in rows and columns.

A heatmap showing the two-dimensional grouping of species and variables (chemical composition and antioxidant activity) is shown in Fig. 3, where the grouping relationships at the top represent the species, while those on the left represent the variables. The variability dispersion analysis clearly confirms that the species under investigation are chemically differentiated. Cluster 1 is characterized by the variables *o*-cymene, β-elemene, geranial, geraniol, geranyl acetate, germacrene D, 6-methyl-Hept-5-en-2-one, linalool, neral, nerol, thuja-2, 4 (10)-diene, and vinyl amyl carbinol, which have relatively high weights for *L. alba*. On the other hand, cluster 2, composed of the variables 9-*epi*-(*E*)-caryophyllene and δ-cadinene, contribute with low weight for *L. sidoides* and intermediate weight for *L. alba* and *L. gracilis*, while in cluster 3, 11-bicyclo[7, 2,0]undecan-3-ol, 9-*epi*-(*E*)-caryophyllene, *p*-cymene, eucalyptol, limonene, spathulenol, terpinen-4-ol, α-terpineol, verbenone, viridiflorene, and zonarene have relatively high weight for *L. sidoides* and intermediate weight for *L. alba* and *L. gracilis*. Finally, cluster 4 demonstrates the chemical composition of greater weight for *L. gracilis* and less weight for *L. sidoides* and *L. alba*.

The variables at the top of the heatmap demonstrate that *L. sidoides* and *L. gracilis* (cluster 2) give a smaller contribution to the antioxidant activity, while cluster 1, represented by *L. alba*, demonstrates a greater contribution to the antioxidant capacity. Thus, we can conclude that the chemical composition of the essential oil of *L. alba* has greater general antioxidant power compared to the other species evaluated in this study.

4. Conclusions

The present study demonstrated the relationship between the chemical composition and the *in vitro* antioxidant activity of species of genus *Lippia* found in Cariri, Ceará. The essential oils of *L. alba*, *L. sidoides* and *L. gracilis* showed little similar chemical compositions and no statistically significant relationship. However, these oils presented relevant antioxidant activity in view of the different mechanisms studied, hich is related to the action of different and non-majority compounds, as demonstrated by the multivariate analyses, especially those that have double bonds and hydroxyls in their structure.

Multivariate analyzes demonstrated the effectiveness of compounds present in *L. alba* on DPPH free radical scavenging, reduction of Fe^{3+} ion, and inhibition of β-carotene oxidation, while deoxyribose degradation protection was linked mainly to the compounds identified in *L. gracilis*. On the other hand, ABTS radical capture and Fe^{2+} ion chelation have intermediate relationships with the composition of *L. alba* and *L. gracilis*. The antioxidant potential of the essential oils of these species for food systems still needs to be deepened with studies that seek to verify their direct action as natural preservatives as well as their applications in enriched packaging films, being able to determine their performance and their effective use in food products.

Finally, both cluster analysis and PCA analysis classified the variables by their similarity, reducing data redundancy, demonstrating that chemometric tools are valuable to correlate the antioxidant activity and chemical composition of a given species and therefore, can be used in

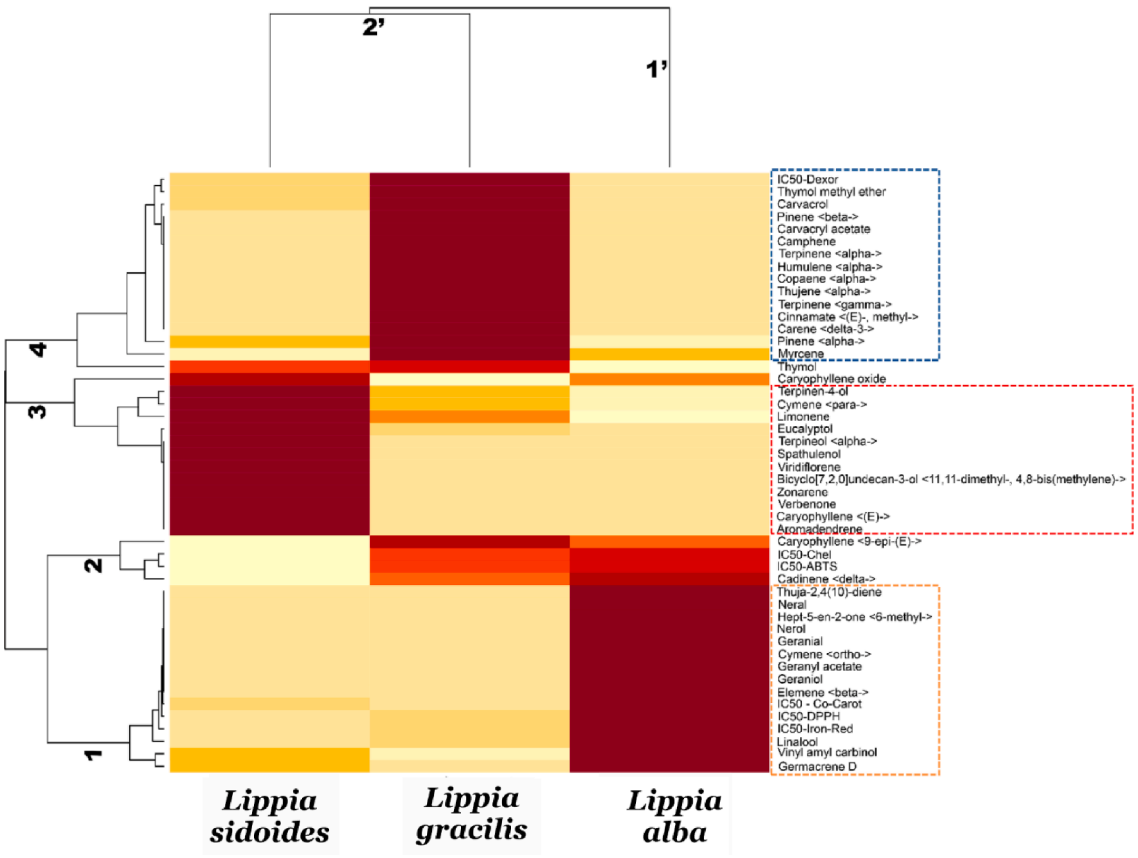

Fig. 3. Heatmap cluster analysis of *Lippia* species and variables (chemical composition and antioxidant activity).

the future to select the components that have a greater contribution to the antioxidant potential of natural products.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Carla de Fatima Alves Nonato: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Cicera Janaine Camilo:** Investigation. **Débora Odília Duarte Leite:** Investigation. **Mário Gustavo Lúcio Albuquerque da Nobrega:** Writing – review & editing. **Jaime Ribeiro-Filho:** Writing – review & editing. **Irwin Rose Alencar de Menezes:** Formal analysis, Writing – review & editing. **Josean Fechine Tavares:** Investigation, Writing – review & editing. **José Galberto Martins da Costa:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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