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Essential oil composition, antifungal activity and leaf anatomy of *Lippia alba* (Verbenaceae) from Brazilian Chaco

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This study aims to determine the essential oil chemical composition of *Lippia alba* (Mill.) N.E.Br. *ex* Britton & P. Wilson collected in the Brazilian Chaco where plants grow in conditions of high temperatures in the summer, periodic flood, low temperatures and air humidity in the winter. We also evaluate the oil antifungal activity against the animal and plant pathogenic fungi *Aspergillus flavus, A. fumigatus*, *A. niger*, *A. terreus*, *Fusarium* sp., *Penicillium funiculosum* and *Sclerotinia sclerotiorum*. Leaf essential oils were extracted by Clevenger hydrodistillation and characterized by GC-MS. The major essential oil components were linalool (38.26%), *trans*-ocimenone (6.57%) and caryophyllene oxide (6.48%). At first time *L. alba* from Brazilian Chaco was identified as a chemotype producing linalool. The essential oils showed antifungal activity, mainly against *S. slerotiorum*, a fungi species related with diseases in soybean plants, with 100% of growth inhibition. These results suggest the potential alternative of this species to synthetic fungicides and confirm its popular uses as an important medicinal plant in South America.

Key words: Brazilian Chaco, erva cidreira, essential oil, pathogenic fungi, glandular trichome, terpenes.

INTRODUCTION

Lippia alba (Mill.) N.E.Br. *ex* Britton & P. Wilson (Verbenaceae), popularly known as bushy matgrass, bushy lippia, hierba negra, pitiona and erva cidreira, is an aromatic subshrub, with chamaephyte life form, widely

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distributed throughout the Americas and found in different environments, such as forests, fields, and roadsides (Salimena and Múlgura, 2015). It is commonly used in South American folk medicine as an analgesic, anti-inflammatory, cold remedy, as well as a treatment for hepatic afflictions (Oliveira et al., 2006).

Essential oils from the leaves of *L. alba* have been categorized into different chemotypes, depending on their major constituents, such as linalool, citral, and carvone (Pandeló et al., 2012). Several biological properties of this plant, such as cytotoxicity, antioxidant, antibiofilm, anesthetic, antitumor, antibacterial, antifungal, anti-inflammatory, antispasmodic activities and anxiolytic-like effects differ according to essential oil chemotype (Glamočlija et al., 2011; Trevisan et al., 2016; Tofiño-Rivera et al., 2016; Pandey et al., 2016, García et al., 2017).

Aspergillus species are associated with a wide range of diseases, including allergic bronchopulmonary aspergillosis (ABPA) and various forms of invasive Aspergillosis (Uniyal et al. 2012). Over many decades, the percentage of fungal infection by *Aspergillus* species has risen dramatically (Kocié-Tanackov and Dimié, 2013). Furthermore, *Aspergillus* species produce aflatoxins, a mycotoxin contaminant in several foods. Aflatoxins are genotoxic, hepatocarcinogenic and immunotoxic, harming both human and animal health (Passone et al., 2013).

Several essential oils have been described as inhibitors of aflatoxin and mycelial growth in *Aspergillus* species; thus, they are an attractive alternative method to avoid food contamination (Pandey et al., 2016; Passone et al., 2013). *Sclerotinia* is one of the most devastating and cosmopolitan of plant pathogens (Dalili et al., 2015). This pathogen infects more than 500 species of plants worldwide, including important field crops and numerous weeds (Saharan and Mehta, 2008). Chemical fungicides have been used against plant pathogenic fungi, and even though several of them are available, they are usually expensive, ineffective, and hazardous (Lu, 2003).

Plant essential oils are also good candidates for the control of fungus, as they consist of many bioactive chemicals with antifungal activity (Kocié-Tanackov and Dimié, 2013; Mahilrajan et al., 2014).

Therefore, this study aimed to determine the chemical composition of *L. alba* essential oils in specimens collected in the humid Brazilian Chaco and then evaluate their antifungal activity against *Penicillium funiculosum*, *Sclerotinia sclerotiorum* and some *Aspergillus* and *Fusarium* species.

In addition, histochemical, morphological and micromorphological analyses of glandular trichomes can provide support for studies useful to taxonomy, phylogeny and exploitation of substances produced by *Lippia* species. In this context, a histological analysis of glandular trichomes associated with oil accumulation in leaves of *L. alba* is presented.

MATERIALS AND METHODS

Plant materials

Plants were collected in the Brazilian Chaco region (21°41'56"W, 57°52'57"S) (Figure 1A). All experiments were conducted with leaves obtained from 20-30 individuals of *L. alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae) (Figure 1B and C), and specimens were collected in June, 2015 and April, 2016. For anatomical, histochemical and micromorphological analyses, about 20 leaves were processed from five individuals. *Lippia alba* was identified by comparison with descriptions found in literature and with samples growing in the botanical garden at INBIO/UFMS. Representative dried specimens of the studied plants are preserved in the herbarium CGMS/UFMS under number 66619.

Extraction of leaf essential oils

Fresh leaves (30-40 g) of *L. alba* were collected in June 2015 and April 2016. They were cut and submitted to hydrodistillation using a Clevenger-type apparatus for 3 h. Essential oils were pooled and refrigerated until GC-MS analyses.

Gas chromatography-mass spectrometry (GC-MS) analysis

Samples of leaf essential oils were analyzed in a Shimadzu GCMS-QP2010 using a DB-5MS column (30 m x 0.25 mm, 0.25 mm in thickness). The initial oven temperature was 60°C, raised to 240°C at 3°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min and at a pressure of 79.7 kPa. The injector temperature was 250ºC, applying the split ratio of 1:5. Mass spectra were obtained using electron ionization source at 70 eV. The essential oil was solubilized in dichloromethane at concentration 2 mg/mL and 1 µL was injected on the chromatographic system. To identify constituents, the mass spectra were compared to data from NIST 08, FFNSC 1.3 and WILEY 7 libraries. A comparison of retention indices reported in the literature was also performed (Adams and Sparkman, 2007). Values were calculated using alkanes from C9 to C22.

Characterization of isolated fungal strains

The fungal species used in the experiments were *Aspergillus* CPV34.2A, *Aspergillus flavus* SM3, *A. fumigatus*, *A. niger*, *A. terreus* MP 31, *Fusarium* sp., *P. funiculosum*, *Aspergillus* sp., *Aspergillus* sp. SM8 and *S. sclerotiorum*. Filamentous fungi were identified by Clarice Rossato Marchetti, in accordance with their morphological characteristics and deposited in the fungi collection of the Laboratory of Biochemistry and Microorganisms, UFMS, Campo Grande, Brazil. Stock cultures were propagated at 30°C on slats of solid potato dextrose agar (PDA) media and stored at 4°C.

Antifungal activity assays

For mycelial growth inhibition, 20 µL of essential oil were incorporated into PDA, giving a final concentration of 1 µg/mL. The medium with essential oil was poured into a Petri dish 9 cm in diameter. Culture medium without the essential oil served as control. After the incorporation of embedded discs (5 mm in diameter) with spores (6.10^6/mL) on PDA medium, the plates were incubated for six days at 28°C. The diameters of the inhibition zones were measured in millimeters, and their means were calculated. Each treatment was tested on four plates as replications. The percentage of fungal growth inhibition was

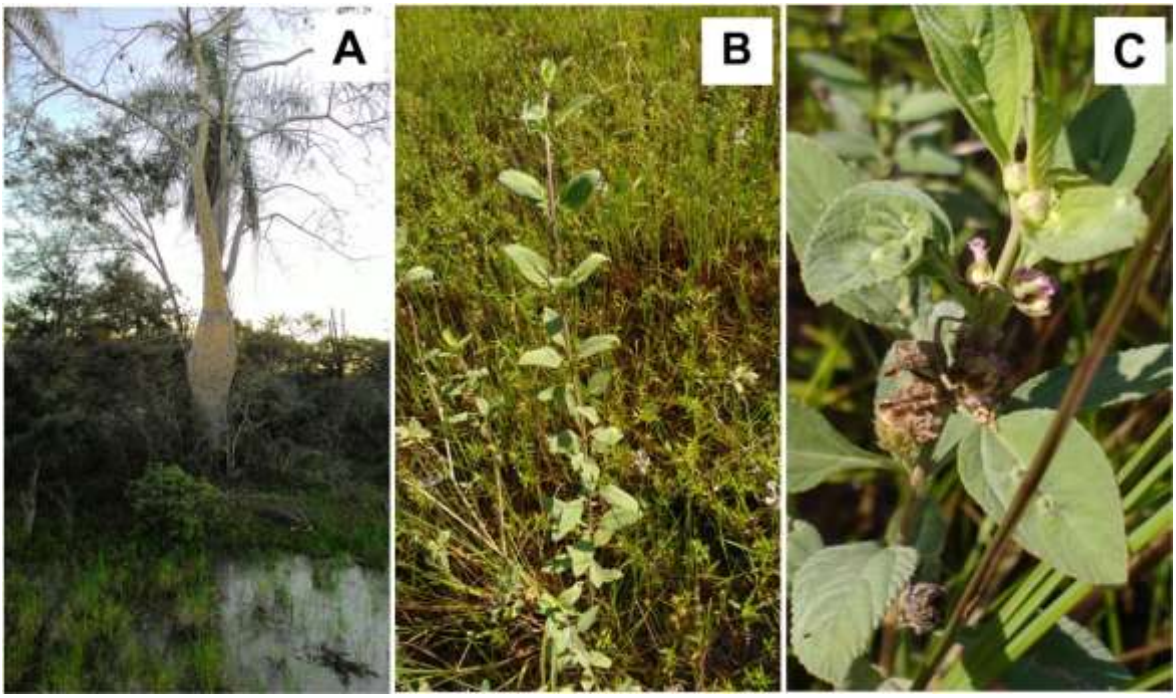

Figure 1. *Lippia alba* (Verbenaceae), plant collection site. A. Chaquean vegetation during periodic flooding in Mato Grosso do Sul, Brazil. B. *L. alba*, habit (arrow). C. Detail of grayish leaves due the numerous non-glandular trichomes.

calculated according to McCalley and Torres-Grifol (1992), as Growth Inhibition % (MIC) = [(growth in the control – growth in the sample) / growth in the control)] x 100.

Leaf anatomical and micromorphological (SEM) analysis

Leaves were collected and fixed in buffered neutral formalin 10% solution (Lillie 1947) for 48h, dehydrated in ethanolic series, and conserved in ethanol 70%. Leaf segments were embedded in plastic resin (Leica®); sections 0.5-0.8 µm thickness was then stained with Toluidine Blue 1% (O'Brien et al., 1965). To analyze epidermis, epidermal peels were prepared using leaf segments dissociated in hydrogen peroxide and glacial acetic acid solution (1:1) and heated in an oven at 60º C for 12 h (Franklin 1945). These samples were stained with 0.25% ethanolic solution of basic fuchsin (20 seconds), washed in distilled water, and mounted in 50% glycerin. Analyses with scanning electron microscopy (SEM) were performed on herborized leaves about 0.4cm² coated with a thin layer of gold (Denton Vacuum Desk IV Standard Sputter Coater). The specimens were examined in a JEOL JSM-6380LV scanning electron microscope (JEOL, Japan). For histochemical analysis, free-hand and plastic resin-embedded sections (Leica®, Heidelberg, Germany) obtained with a rotary microtome (0.5-0.8 µm thickness) were prepared and exposed to the following reagents: Sudan IV for total lipids (Pearse, 1972); Nile blue for acidic lipids (Cain, 1947); Nadi for essential oils and terpenoids (David and Carde, 1964); ruthenium red for pectin (Johansen, 1940) and phloroglucinol and hydrochloric acid for lignified cell walls (Foster, 1950). All samples were rinsed and mounted in distilled water on slides with cover slips. Control sections were performed simultaneously. Photomicrographs (with scale bars) were obtained with the Leica DM5500 B light microscope and Leica Application Suite (LAS) V3 Program.

RESULTS

The chemical analysis of *L. alba* leaf essential oils collected in Chaco, Mato Grosso do Sul, showed the monoterpene linalool (38.26%) (Figure 2) as the main component, followed by caryophyllene oxide (6.48%), *trans*-ocimenone (6.57%), p-mentha-1,8-dien-3-one (4.61%), and humulene epoxide II (4.03 %) (Table 1). Based on these major chemical constituents, Brazilian Chaco *L. alba* could be classified as a linalool chemotype.

Accordingly, the effects *L. alba* essential oils against pathogenic fungi were evaluated in the present study. Specifically, the linalool chemotype from Chaquean *L. alba* showed inhibition of mycelial growth at 1 µg/ mL (Table 2). The extension diameter (mm) of hyphae from the center to the sides of each dish, including replicates, was measured every 24 h for 6 days.

The fungal species tested did not grow after 3 days of incubation, except for *A. niger* (8 mm) and *Aspergillus* sp SM8 (10 mm). When compared with the control (55-90 mm of mycelial growth), the essential oil showed moderate antifungal activity against the growth of all fungal species tested (0-35 mm of mycelial growth) after 6 days of incubation.

Results showed variable inhibition between 77 to 100% and 50 to 100%, for 3 and 6 days, respectively (Table 3). *L. alba* essential oil was effective against *S. sclerotiorum*, *Aspergillus* sp5, and *Aspergillus* sp1 CPV34.2A with a

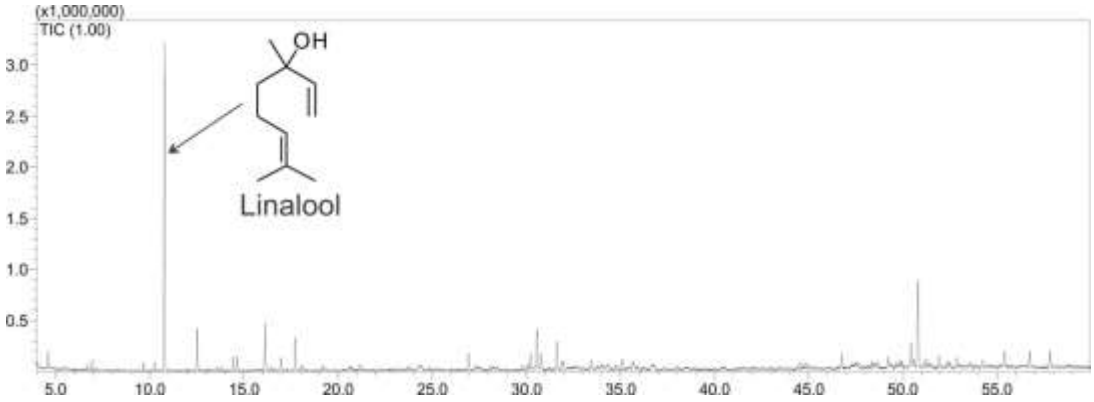

Figure 2. GC-MS chromatogram of the leaf essential oil of *Lippia alba.*

Table 1. Constituents identified of the leaf essential oil from Chaquean *Lippia alba* by GC-MS, June (2015).

Peak	RT (min)	Compounds	Area (%)	RI
1	4.60	Tiglicacid	1.67	901
2	6.93	β-Myrcene	0.94	991
3	9.67	*cis*-Linalool oxide	0.78	1073
4	10.25	*trans*-Linalool oxide	0.71	1087
5	10.78	Linalool	38.26	1099
6	12.52	n.i.	5.43	1146
7	14.42	*cis*-3-Hexenyl butyrate	1.77	1190
8	14.64	α-Terpineol	1.64	1194
9	16.13	*trans*-Ocimenone	6.57	1231
10	16.96	n.i.	1.73	1251
11	17.72	p-Mentha-1,8-dien-3-one	4.61	1269
12	26.94	β-Selinene	2.25	1488
13	30.24	Ledol	1.67	1572
14	30.58	Caryophyllene oxide	6.48	1581
15	30.77	Spathulenol	2.11	1585
16	31.63	Humuleneepoxide II	4.03	1607
17	31.97	n.i.	1.63	1617
18	35.10	n.i.	1.32	1701
19	46.77	n.i.	1.85	2069
20	49.21	n.i.	0.80	2151
21	50.81	n.i.	13.75	2205
-	-	Total identified (%)	73.49	-
-	-	Monoterpenes	55.28	-
-	-	Sesquiterpenes	16.54	-

n.i., not identified; RT, Retention time; RI, retention indices on DB-5 capillary column.

growth inhibition average of 100, 78.3 and 72.2%, respectively.

Samples of *L. alba* herein investigated presented grayish color owing to a large number of trichomes covering the leaf surface (Figure 3A, B and C). The epidermal cells present right anticlinal wall and a very thick periclinal wall covered by a smooth wax layer and thin cuticle (Figure 3G, 3J). The *L. alba* leaf is amphystomatic with scarce stomata randomly distributed on the adaxial surface, whereas on the opposite side,

Table 2. Effect of essential oils on mycelial growth after 6 days (144 h) of incubation.

Days	*Aspergillus* sp1 CPV34.2A		*S. sclerotiorum*		*Penicillium funiculosum*		*A. flavus* SM3		*Aspergillus* sp5	
	C	**ZI**	**C**	**ZI**	**C**	**ZI**	**C**	**ZI**	**C**	**ZI**
1	15	-	5	-	10	-	12	-	6	
2	28	-	10	-	20	-	28	-	10	-
3	45	-	30	-	30	-	35	-	22	-
4	60	5	55	-	40	7	48	17	31	5
5	75	18	70	-	50	15	65	25	40	11
6	90	25	90	-	62	25	80	32	60	13

Days	*A. terreus*31		*A. niger*		*Aspergillus* SM8		*A. fumigatus*		*Fusarium* sp.	
	C	**ZI**	**C**	**ZI**	**C**	**ZI**	**C**	**ZI**	**C**	**ZI**
1	6	-	10	-	10	-	8	-	10	-
2	13	--	30	-	28	-	20	-	20	-
3	18	-	45	8	45	10	38	-	30	-
4	24	5	55	20	55	25	45	10	45	10
5	36	14	65	30	60	30	50	20	50	17
6	60	24	85	40	70	35	55	25	55	25

C, PDA medium without the essential oil served as control; **ZI**, the diameters of the inhibition zones were measured in millimeters and their means were calculated. Mean growth measurements were calculated from four replicates for each of the fungal species.

Table 3. Percentage of growth inhibition* of fungal species in agar diffusion plate assay by *Lippia alba* essential oils.

Days	*Aspergillus* sp1 CPV34.2A	*S. sclerotiorum*	*Penicillium funiculosum*	*A. flavus* SM3	*Aspergillus* sp5	*A. terreus*31	*A. niger*	*Aspergillus* sp SM8	*A. fumigatus*	*Fusarium* sp.
1	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	82.0	77.0	100	100
4	91.6	100	82.3	64.5	83.8	79.1	63.3	54.5	77.00	77.0
5	76.0	100	70.0	61.5	72.5	61.0	53.4	50.0	6.0	66.0
6	72.2	100	59.6	60.0	78.3	60.0	53.0	50.0	5.5	54.5

*Growth inhibition (MIC) of treatment against control was calculated by percentage (%), using the formula [(C – T/ C) x 100], where C is an average of four replicates of hyphal extension (mm) of control and T is an average of four replicates of hyphal extension (mm) of plates treated with essential oil.

substitute for synthetic fungicides based on their antibacterial, insecticidal and antifungal properties (Feng and Zheng, 2007). These natural compounds interact with microbial membranes and disrupt the permeability barrier leading to the leakage of cell content and impairing energy production (Tian et al., 2012).

The essential oil of *L. alba* showed 100% growth inhibition against *S. sclerotiorum*. Similar stomata are numerous and organized in patches protected by numerous non-glandular trichomes (Figure 3D and E).

Growth inhibition (MIC) of treatment against control was calculated by percentage (%), using the formula [(C – T/ C) x 100], where C is an average of four replicates of hyphal extension (mm) of control and T is an average of four replicates of hyphal extension (mm) of plates treated with essential oil.

Two types of trichomes are observed on both leaf surfaces: non-glandular and glandular (Figure 3A and B). Non-glandular trichomes are long, unicellular and present a thin cuticle layer, producing a dense layer, especially on abaxial surface (Figure 3B, C and H). Cylindrical ornamentations were observed on the trichome surface.

Glandular trichomes are multicellular and can show one or two head cells with different dimensions, termed as type I, type II and type III. In *L. alba*, type I has a unicellular, spherical and voluminous head, nearly 45 µm in diameter. Type I is sustained by two flattened cells that exhibit a thick cutinized cell wall forming a collar (Figure 3G and J). In *L. alba*, this type of glandular trichome is the most abundant, is located in higher level than other epidermal cells, and presents positive reaction to histochemical tests for terpenoids (Figure 3G). Interestingly, although alkaloids were detected in type I trichomes (Figure 3H), with histochemical tests, our extractions did not give evidenced of this type of metabolite. Types II and III, which present uni or bicellular head, respectively, are sparsely distributed. The secretory cells (one or both cells) are about 20 µm in diameter and are supported by a short pedicle with two cells. Type II and III glandular trichomes presented positive reaction to both Sudan and Nile Blue, indicating the presence of general and acidic lipids (Figure 3F and I).

Leaves of *L. alba* found in Brazilian Chaco present isolateral mesophyll, i.e., palisade parenchyma on both leaf sides in a compact organization. Lipophilic drops were detected in chlorenchyma cells. The vascular system in *L. alba* leaves is composed of collateral vascular bundles forming projections to the abaxial leaf surface. The vascular bundles are surrounded by a parenchyma sheath (endodermis) connecting vascular tissues to epidermis. In the main vein cortical region, parenchyma cells are voluminous, producing a translucent tissue, possibly related to water storage.

DISCUSSION

Chaco region is located in the western region of Brazil where Chaquean vegetation occupies an area of approximately 70,000 km². Chaco climate is marked by strong seasonality with hot summers and maximum temperatures reaching 49°C, but dry, cold winters with occasional frost. During and after the rainy season, flooding may occur owing to the poor drainage of compact soil (Pennington et al., 2000). In the collection area, the rainy season occurs from November to February (rainfall ≥ 100 mm) with soil flooding in subsequent months. The dry season starts in April; in September, water deficit occurs (Freitas et al., 2013). The soil is whitish in color and saline.

Conditions in particular environments can be determinants of both quantity and quality of essential oils. Studies done by Tavares et al. (2005) showed variation of leaf essential oils of *L. alba* which indicated three chemotypes from different regions in Brazil. These were characterized by the production of citral (Rio de Janeiro), carvone (Ceará) and linalool (São Paulo), as major constituents. Leaf essential oils of *L. alba* from Paraná (Brazil) presented geranial (50.94%) and neral (33.32%) as the main components, representing 97.69% of total essential oil identified (Glamočlija et al., 2011). Production of essential oils in plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply (Gobbo-Neto and Lopes, 2007). Linalool is a common monoterpene in leaf essential oils of Lamiaceae and Verbenaceae plants, and it presents several biological activities against bacteria and fungi (Lang and Buchbauer, 2012).

A revision of 52 *Lippia* species, as reported by Pascual et al. (2001), showed *p*-cimene, camphor, linalool, α-pinene, β-caryophyllene and thymol as the compounds most frequently detected in leaf essential oils. Ketone with unsaturated carbonyl and *trans*-ocimenone, a natural organic compound classified as a monoterpene and used in the perfume industry, were also revealed. In fumigant and contact assays with plant essential oils, ketone-rich plants were verified as having insecticidal activity (Germinara et al., 2012; Herrera et al., 2014).

The presence of carbonyl groups has also been reported to increase toxicity. Herrera et al. (2014) verified the high toxicity of ocimenone against *Sitophilus zeamais,* a beetle that attacks maize. Assays against fungus using essential oils from *Tagetes mendocina* (Asteraceae), which is rich in ocimenone, showed activity *in vitro* against several yeasts, filamentous fungi, dermatophytes, Gram-positive and Gram-negative bacteria, and protozoa (Lima et al., 2009). *L. alba* cultivated in the Chaco ecosystem is subjected to dry and rainy seasons with temperatures ranging from warm to cold (Freitas et al., 2013), as well as periodic flooding. This chemical characterization of leaf essential oil is important in order to identify the components that are effective against fungi.

The antifungal activity of different chemotypes of *L. alba* essential oil against pathogenic fungi was also

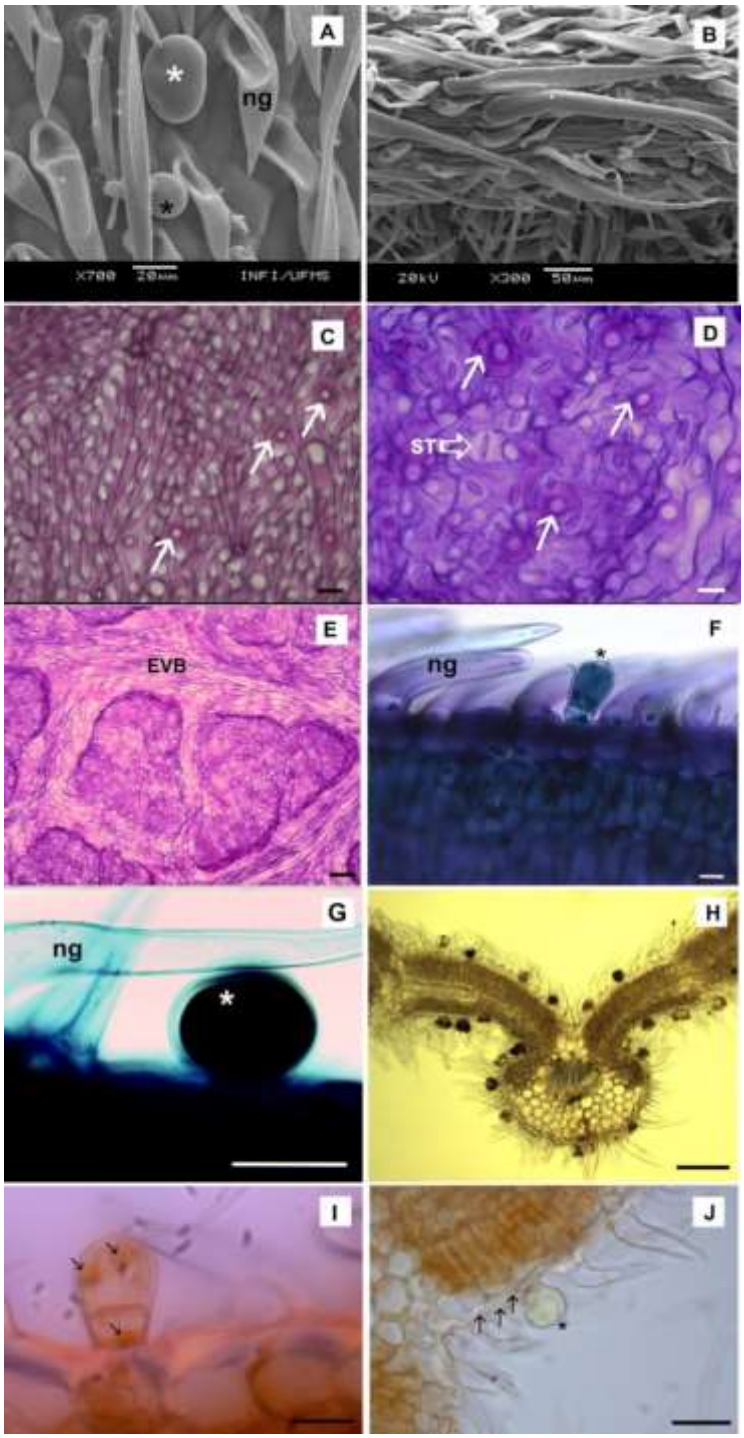

Figure 3. *Lippia alba* (Verbenaceae): leaf micromorphological and anatomical details. **A and B:** Scanning electron microscopy of leaf epidermis. A. Glandular trichomes (white asteristic in type I and black asteristic in type II) and non-glandular trichomes (arrow). **B.** Non-glandular trichomes. **C-J:** Light microscopy. **C, D and E:** Adaxial and abaxial surface in front view showing trichomes (fine arrows) and stomata (horizontal arrow,ST). **E.** stomata are organized in patches. **F-J:** In situ histochemical reactions. **F:** Nile Blue for acidic lipids; **G,** Nadi for essential oil and terpeneoids; **H:** Ellran test for alkaloids: black content in type I glandular trichomes; **I and J:** Sudan VI evidencing lipophilic drops in type II glandular trichomes (I, arrows) and mesophyll cells (J, arrows) and in collar cells of type I glandular trichome.

recently investigated (Rao et al., 2000; Mesa-Arango et al., 2009; Glamočlija et al., 2011; Geromini et al., 2015; Pandey et al., 2016). Results similar to those of the present study were obtained from leaf essential oils at a concentration of 1 µg/mL against *Aspergillus* sp CPV34.2A, *A. flavus*SM3, *A. fumigatus*, *A. niger*, *A. terreus*MP 31, *Fusarium*sp. *P. funiculosum*, *Aspergillus* sp, *Aspergillus* sp SM8 and *S. sclerotiorum*.

Therefore, essential oils, as borne out by the results of the present study, show promise as an effective results were obtained using vinclozolin fungicide at 1 µg/ mL against *S. sclerotiorum* (Mueller et al., 2002). Benjilali et al. (1984) examined the antifungal effects of essential oils obtained from three chemotypes of wild wormwood, thyme, eucalyptus and rosemary against *Aspergillus* and *Penicillium* species and other fungi.

The antifungal activity of essential oil from *L. alba* was investigated against *Aspergillus ochraceus, A. versicolor, A. niger, A. fumigatus, Penicillium ochrochloron, P. funiculosum* and *Trichoderma viride.* With a MIC of 0.300-1.250 mg/mL, the present study showed this essential oil to be a potential alternative to synthetic fungicides for green molds (Glamočlija et al., 2011).

The essential oil from *L. alba* also showed activity against *S. cerevisiae, A. flavus, A. niger* and *C. albicans* at a concentration of 500 µg.disc^{-1} (Ara et al., 2009). In another work, the ranges of reduction in the growth of 91 isolates of *S. sclerotiorum* on potato dextrose agar (PDA) amended with thiophanate methyl and vinclozolin were 18 to 93% and 93 to 99%, respectively (Mueller et al., 2002). The essential oil of *L. gracillis* showed MIC of 5.0 µg/mL against *Cladosporium sphaerospermum* and *C. cladosporioides* (Franco et al., 2014).

Kumar et al. (2010) suggest that eugenol at a concentration of 0.2 µL/mL acts as a fungicide against *Alternaria alternate, Aspergillus candidus, A. fumigates, A. niger, A. paradoxus, A. terreus, A. versicolor, Cladosporium cladosporioides, Culvularia lunata, Fusarium nivale, F. oxyporum and Penicillium* species, while essential oil could achieve fungicidal effect on the tested fungi at a concentration of 0.3 µL/mL. The essential oils of *S. aromaticum, C. limon, C. aurantium* and *M. piperita* showed antifungal activity against *A. niger* and *C. candidum* (Verma et al., 2011).

According to Metcalfe and Chalk (1979), species of Verbenaceae show a diverse type of glandular trichomes, describing up to 16 types. In glandular trichomes, the head is a storage compartment located on the tip of the hair, and it is part of the glandular cell, or cells, which are metabolically active (Glas et al., 2012).

Argyropoulou et al. (2010) pointed out the presence of alkaloids in a variation of type I trichome in *L. citriodora*, termed by them as type D. Glandular trichomes are considered as an important front in the chemical defense of plants, therefore, they can accumulate and synthesize chemicals that can be released by the touch of herbivorous insects, for example. In *Lippia alba*, our chemical analyzes did not indicate the presence of alkaloids, although histochemical tests revealed the presence of these substances.

Further investigation on the content of isolated trichomes will confirm these results as it is notably the glandular trichomes that are on the surface of the leaf, more exposed, and must have an important defensive function for the plant. Sunflower leaves (*Helianthus annuus* L., Asteraceae) present different trichomes: non-glandular, capitate glandular and linear glandular trichomes, each one with distinctive chemical composition of and distribution among epidermal cells being this pattern useful to infer the ecophysiological roles of metabolites (Brentan Silva et al., 2017).

Similarly, other species of *Lippia* can present different types of glandular trichomes, e.g., *L. origanoides* and *L. stachyoids*, with five and four types of glandular trichomes, respectively (Tozin et al., 2015). In *L. citriodora*, five types of glandular trichomes were also described, four of them having unicellular head (Argyropoulou et al., 2010). Most studies on glandular trichomes use histochemical methods in order to identify chemical class and sites of accumulated synthesized substances (Nikolakaki and Christodoulakis, 2004, 2007). Although chemical analysis did not evidence alkaloids in leaves, our histochemical results did reveal that head cell vacuoles of type I glandular trichomes store substances that have the same reaction to this compound.

Lipophilic drops were detected in chlorenchyma cells. Leaves of *L. alba* found in Brazilian Chaco present isolateral mesophyll, that is, palisade parenchyma on both leaf sides in a compact organization. Lipophilic drops were detected in chlorenchyma cells. In contrast, morphotypes of *L. alba* growing under laboratory conditions presented dorsiventral leaves with loose parenchyma cells (Jezler et al., 2013).

L. alba growing in the Chaquean region exhibits leaf features typically observed in plants living in dry environments with saline soils, that is, thick external cell walls, numerous non-glandular trichomes, forming a barrier protecting stomata from evapotranspiration, the development of translucent parenchyma cells, most likely to improve water storage, and abundance of palisade parenchyma (Dickison 2000).

In sum, terpenoids play important roles in primary plant metabolism by their relationship to the production of chlorophylls, quinones, phytohormones and other signaling molecules. However, most terpenoids are functionally related to plant defense or attraction of pollinators (Gleason and Chollet, 2012). In *L. alba* analyzed here, we detected a high production of linalool as the major oil component in plants from Brazilian Chaco, possibly playing a role in direct defense against pests, as they have a deterrent or repellent, often toxic, effect (Glas et al., 2012).

This study demonstrated the antifungal activity of *L. alba* essential oils against *Aspergillus* species, such as *A.*

flavus, A. fumigatus, *A. niger*, *A. terreus*, and against *Fusarium* sp, *P. funiculosum* and *S. sclerotiorum*, but mainly *S. slerotiorum*. Importantly, these results strongly indicate the potential of such essential oils as an alternative to synthetic fungicides, with the concomitant advantages of avoiding the development of resistance by pathogenic microorganisms and reduced environmental impact, especially if plants are growing in proper conditions. In addition, our study also points to the medicinal potential of *L. alba* plants already highlighted by the population in several locations in Brazil and South America.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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