Fronteiras: Journal of Social, Technological and Environmental Science
http://periodicos.unievangelica.edu.br/fronteiras/
ISSN 2238-8869

Article

Morphoanatomical Characterization and Chemical Composition of Essential oils of *Lippia lupulina* Cham. and *Lippia pohliana* Schauer (Verbenaceae)

Sandra Ribeiro de Morais [1], Thiago Levi Silva Oliveira [2], Dayana Figueiredo Abdalla [3], Douglas Vieira Thomaz[4], Leonice Manrique Faustino Tresvenzo [5], Edemilson Cardoso da Conceição [6], Maria Helena Rezende [7], Tatiana de Sousa Fiuza [8], Pedro Henrique Ferri [9], José Realino de Paula[10]

[1] Doutorado. Universidade Paulista. ORCID: 0000-0001-7128-4838. E-mail: sandrarmorais@hotmail.com

[2] Doutorado. Universidade Paulista. ORCID: 0000-0003-3848-5261. E-mail: thiagolevi.farmacia@hotmail.com

[3] Doutorado. Instituto Federal de Educação, Ciência e Tecnologia de Goiás, Campus Itumbiara. ORCID: 0000-0001-6639-950X. E-mail: dayanaabdallabio@gmail.com

[4] Mestrado. Universidade Federal de Goiás. ORCID: 0000-0003-0000-3466. E-mail: douglasvthomaz@gmail.com

[5] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0003-0014-5069. E-mail: manrique@farmacia.ufg.br

[6] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0003-4113-2686. E-mail: edemilson_conceicao@ufg.br

[7] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0001-7873-3595. E-mail: mhrezende@uol.com.br

[8] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0003-0135-177X. E-mail: tatianaanatomia@gmail.com

[9] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0002-8744-3829. E-mail: pedro_ferri@ufg.br

[10] Doutorado. Universidade Federal de Goiás. ORCID: 0000-0002-4424-7692. E-mail: pjrpaula@gmail.com

ABSTRACT

Lippia lupulina Cham. and *Lippia pohliana* Schauer are species of Verbenaceae used in folk medicine in the Brazilian Midwest. In order to identify the characteristics with taxonomic value that differentiate these species, the morphoanatomical characterization of the leaves and description of the chemical composition of the essential oils was carried out. The investigation of the anatomical characteristics of each species allowed the identification of different structural characters, related to the types of glandular and non-glandular trichomes, types of stomata, structural organization of the petiole's vascular system and the presence of sclereids in the petiole. Regarding the chemical composition of essential oil, in *L. lupulina* the main constituents were E-caryophyllene, caryophyllene oxide and dauca- 5,8-diene, while in *L. pohliana*, E-caryophyllene was the main constituent, followed by α-humulene and amorpha-4,7(11)-diene. Therefore, the results found have taxonomic value, as they allow the identification of species and help in the knowledge of the genus. And the characters studied contribute to future morphoanatomical, taxonomic and pharmacological studies.

Keywords: Cerrado; foliar anatomy; essential oil; medicinal plants; Verbenaceae.

RESUMO

Lippia lupulina Cham. e *Lippia pohliana* Schauer são espécies da família Verbenaceae utilizadas na medicina popular no Centro-oeste brasileiro. Realizou-se a caracterização morfoanatômica das folhas e descrição da composição química dos óleos essenciais, a fim de identificar as características com valor taxonômico que diferenciam essas espécies. A investigação das características anatômicas de cada espécie permitiu a identificação de diferentes caracteres estruturais, como tipos de tricomas glandulares e não glandulares, tipos de estômatos, organização estrutural do sistema vascular do pecíolo e presença de esclereídeos no pecíolo. Em relação à composição química do óleo essencial, os principais constituintes identificados em *L. lupulina* foram E-cariofileno, óxido de cariofileno e dauca-5,8-dieno, enquanto em L. pohliana, o E-cariofileno foi o principal constituinte, seguido por α-humuleno e amorfa-4,7(11)-dieno.

Submissão: 26/12/2020 Aceite: 09/01/2023 Publicação: 30/12/2022

v.11, n.4, 170-184. 2022 • p. 170-184. • DOI http://dx.doi.org/10.21664/2238-8869.2022v11i4.p 170-184.

Assim, os resultados encontrados possuem valor taxonômico, pois permitem a identificação das espécies e auxiliam no conhecimento do gênero. Os caracteres estudados podem subsidiar futuros estudos morfoanatômicos, taxonômicos e farmacológicos.
Palavras-chaves: Cerrado; anatomia foliar; óleo essencial; plantas medicinais; Verbenaceae.

1. Introduction

Lippia is a remarkable genus from Verbenaceae family whose traditional uses in folk medicine suggests diverse applications in pharmaceutical industry (Siqueira-Lima et al. 2019; Cádiz-Gurrea et al. 2018; Cavallaro et al. 2018). Although most species of this genus do evidence biological activity, the differential identification of some *Lippia* species is still somewhat hindered by the existence of homonyms, synonyms and infraspecific taxon, what implies the importance of further stablishing species differentiation parameters (Silva & Salimena 2002).

Considering occurrence of *Lippia* in Brazil, *Lippia lupulina* Cham. and *Lippia pohliana* Schauer are the main representatives of this genus in central-western and northeastern regions. These plants present noteworthy uses in traditional medicine, being used against throat infection and to alleviate flu symptoms, respectively (Costa-Neto & Oliveira 2000; Rodrigues & Carvalho 2001). Moreover, *L. lupulina* and *L. pohliana* are considered endemic species in 'cerrado-rupestre', and showcase similar features, what denotes the importance of establishing morphoanatomical and chemical parameters to aid differentiation, given the pharmaceutical relevance of these species.

Literature reports regarding *L. lupulina* and *L. pohliana* differential identification were based on chromosome number determination techniques and pollen characterization (Silva & Salimena 2002; Viccini et al. 2005), however there was up to date no outreach concerning the comparative study of leaves morphoanatomy and essential oil composition for these species. In this sense, studies regarding structural features on *L. lupulina* and *L. pohliana* could shed light on possible differentiation parameters as well as foment better understanding of these plants. Moreover, the determination of essential oil chemical profile could aid both species differentiation as well as provide answers to the biological effects attributed to these plants in ethnopharmacological investigations (Gomide et al. 2013; Funari et al. 2012; Singulani et al. 2012).

Considering the relevance of the genus *Lippia* and the pharmaceutical applicability of its main representatives in the Cerrado, this work aimed at the morphoanatomical characterization of the leaves and the analysis of essential oils of *L. lupulina* and *L. pohliana,* aiming to identify characteristics that differentiate the species and that have taxonomic value.

2. Materials and Methods

2.1. Plant material

L. lupulina and *L. pohliana* leaves were collected in Serra Dourada Biological Reserve, Mossâmedes Municipality, Goiás state, Brazil (altitude 1010 m, 15° 47' 44" S, 48° 49' 57" W). The species were identified by Professor Dr. Marcos José da Silva, Federal University of Goiás, and a sample voucher of each plant was deposited in the Herbarium of the Federal University of Goiás under the codes: UFG-47321 and UFG-47322, respectively.

2.2. Morphologic characterization

The morphological analyzes of *L. lupulina* and *L. pohliana* were performed by naked eye observations at the collection site, photographic record using the Canon EOS Digital Rebel XTI / 400D camera and in the laboratory with the aid of a stereoscopic microscope.

2.3. Anatomic characterization

Samples of fully expanded leaves from the 3rd and 4th nodes below the floral branch were collected from three individuals of *L. lupulina* and from *L. pohliana*. Fragments of the middle third of leaf lamina and petiole were fixed in FAA 70 (formaldehyde, acetic acid, 70% ethanol) 1:1:18 (v/v) for 48 h (Johansen 1940) and later stored in 70% ethanol.

Transverses sections of the plant material were clarified in 5% sodium hypochlorite solution, washed in distilled water and subjected to double staining with 0.1% aqueous basic fuchsin and 0.3% astra blue solutions (Roeser 1972). Subsequently, the histological slides were assembled using 50% glycerin solution.

Fragments of the samples were dehydrated in ethanolic series, later infiltrated and included in plastic resin (Leica Historesin) to obtain histologic blocks. The blocks were sectioned using Leica® RM2245 rotary microtome equipped with disposable TC-65 tungsten blade. Cross sections of 7-10 μm thickness were stained with 1% toluidine blue in 0.2 mol L-1 sodium phosphate buffer, pH 8.2 (O'Brien et al. 1965). Permanent histological slides were assembled with Acrilex® colorless synthetic resin (Paiva et al. 2006).

For the analysis of epidermal structures in frontal view, fragments of 1 cm from the middle third of the leaf blade were subjected to a maceration process with chromic and nitric acids (Johansen, 1940) stained with 1% safranin in 50% ethanol and mounted between slides and coverslips with 50% glycerin solution.

Photomicrographic recordings were performed using Leica® microscope and ICC50 digital camera, equipped with image capture program LAD EZ version 1.8.1.

2.4. Ultra-structural analysis

For the ultrastructural analysis the samples were fixed in FAA 70, dehydrated in ethanol series and subjected to critical-point drying in Autosamdri® 815, Series A. Subsequently, the material was metallized with gold in the Denton Vacuum, Desk V. Image capture was performed in Scanning electron microscope (SEM) JSM – 6610, equipped with EDS, Thermo Scientific NSS Spectral Imaging.

2.5. Essential oils extraction

The leaves of *L. lupulina* and *L. pohliana* were dried at room temperature, grounded and subjected to hydrodistillation in Clevenger apparatus for two hours. The essential oils were separated from hydrolates, desiccated using anhydrous sodium sulfate and thereafter stored in freezer at -10 °C.

2.6. GC-MS analysis

The essential oils were analyzed on GC-MS Shimadzu QP5050A using a fused silica capillary column (CBP-5; 30 m x 0.25 mm x 0.25 μm). Experimental conditions consisted of helium flow of 1 mL min^{-1}, programmed temperature of 60 - 240 °C for 2 min (3 °C min^{-1}), then 240 – 280 °C for 10 min (10 °C min^{-1}). The ionization energy was of 70 eV, injection volume of 1 μL of samples diluted in dichloromethane (20% w/v) in Split mode (ratio of 1:50).

Retention rates were calculated by co-injection of hydrocarbon mix (C8 - C32) and application of Van Den Dool & Kratz equation (Van Den Dool & Kratz 1963). The identification of the essential oils components was performed by comparing the mass spectra and retention indexes with the values reported in literature (Adams 2007).

3. Results

3.1. Morphologic characterization

L. lupulina (Figure 1A-B) is a subshrub plant which exhales distinct aroma, it presents itself in a slightly branched shape and grows about 0.70 - 1.1 m high. *L. lupulina* leaves are simple, oval, opposite crossed phyllotaxis, moreover the leaves showcase short-lenght petiole. Its inflorescence is terminal with oval bracts colored in different shades of pink. Its flowers are pentamera, diclamideas, bisexual and super ovary.

Regarding *L. pohliana* (Figure 1C), results showcased that it is also an aromatic subshrub plant, it grows about 0.90 - 1.2 m high. *L. pohliana* stem is quadrangular and tomentose, while leaves are indumented and simple, opposite crossed phyllotaxis. Its inflorescence is terminal similarly to *L. lupulina*, with oval bracts colored in different shades of white to yellow. Its flowers are diclamideas, tubulous, bisexual and super ovary.

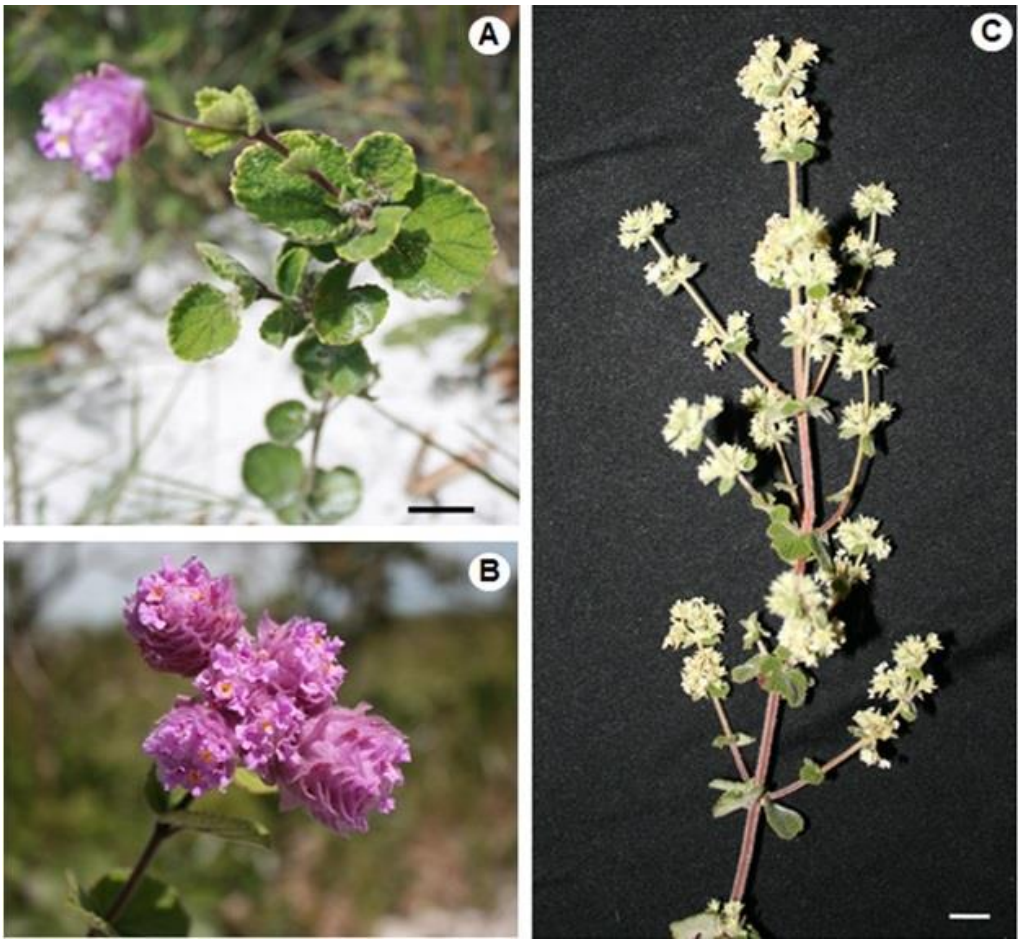

Figure 1. A: *Lippia lupulina* Cham. morphological features. B: Detail of *Lippia lupulina* Cham. inflorescence. C: *Lippia pohliana* Schauer morphological features. Bar = 1 cm

3.2. Anatomic characterization

In the frontal view of the leaf blade, the results showed that in *L. lupulina* and *L. pohliana*, on the adaxial face, the epidermal cells have straight curved anticline walls (Figures 2A, D-F) and the cells of the abaxial epidermis, with wavy to sinuous anticline walls (Figures 2B-C, G). The leaves of both species are anfistomatic. In *L. lupulina*, the analyzes showed anisocytic and anomocytic stomata on the laminae of the adaxial leaf (Figure 2A) and anisocytic, anomocytic and rare stomata twinned on the laminae of the abaxial leaf (Figures 2B-C). In *L. pohliana,* anomocytic (Figure 2D, G), anisocytic (Figure 2E) and diacitic (Figure 2F) stomata were shown on both sides of the leaf blade.

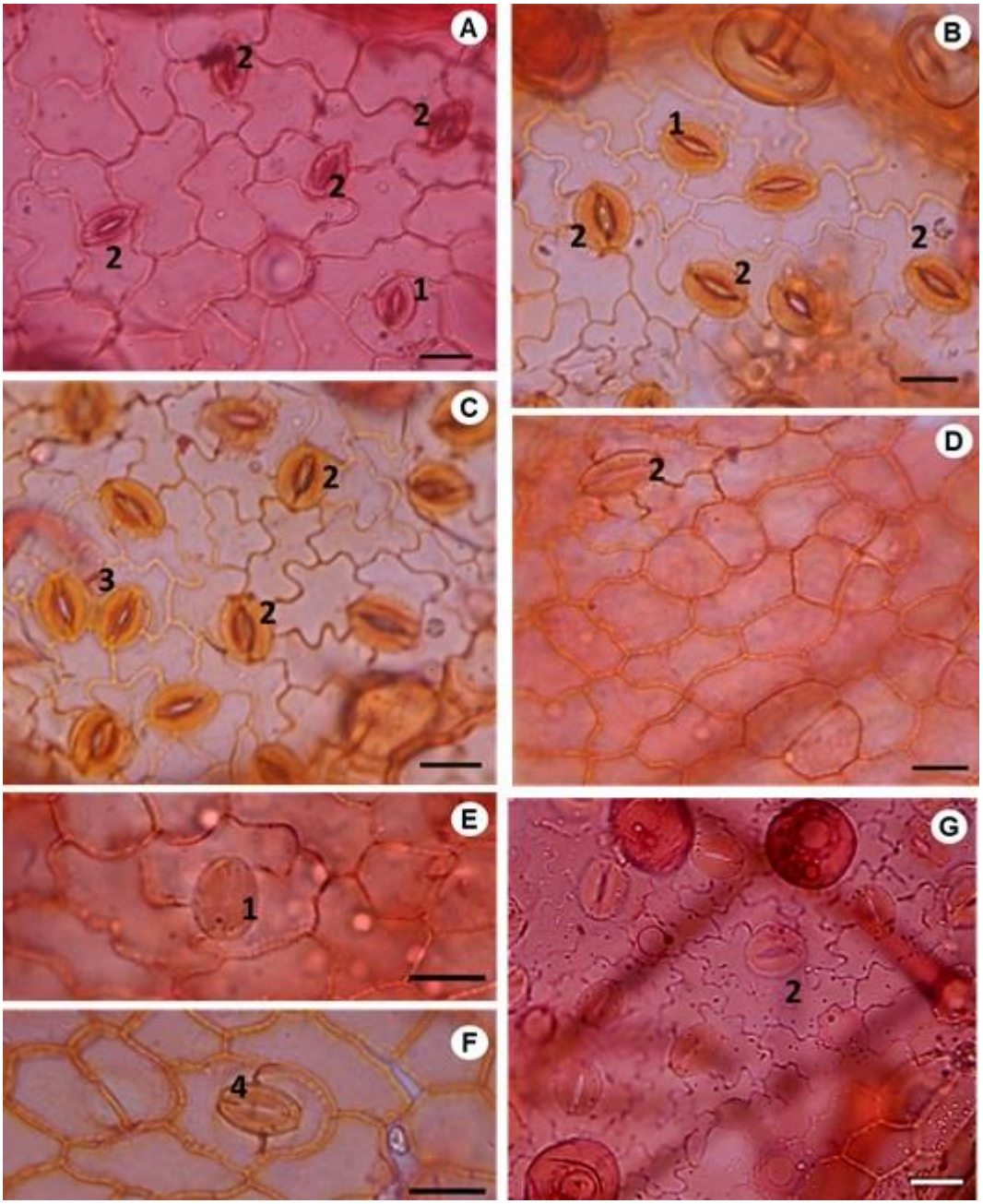

Figure 2. Leaf blade in frontal view. A-C: *Lippia lupulina* Cham. A. Adaxial epidermis. B-C. Abaxial epidermis. D-G. *Lippia pohliana* Schauer. D-F. Adaxial epidermis. G. Abaxial epidermis. Stomata:1- anisocytic, 2- anomocytic, 3- twinned 4- diacitic. Bars = 20µm (A-F); 30µm (G).

The studied species have non-glandular and glandular trichomes on both sides of the leaf blade, as evidenced by scanning electron microscopy (Figures 3A-D). In *L. lupulina*, the non-glandular trichome are multicellular and can be: short, formed from two to three cells of different shape (Figures 3E-F); long, with three to five cells (Figure 3G); glandular trichomes have different morphotypes: short, with a basal cell, neck cell and unicellular globular secretory head (Figure 3I); short, with a basal cell and a biseriate secretory head formed by several cells (Figure 3J); long pedunculate, with a basal cell, peduncle with three to four cells and unicellular secretory head (Figures 3E, K). In *L. pohliana*, the non-glandular trichomes are unicellular of different sizes (Figure 3H) and glandular trichomes of different morphotypes: short, with a basal cell, neck cell and unicellular globoid secreting head (Figure 3L); short pedunculate, with a basal cell, unicellular peduncle, neck cell and unicellular secretory head (Figure 3M); short pedunculate, with a basal cell, unicellular peduncle and tetracellular secretory head (Figure 3N); and a long pedunculate, with a basal cell, a peduncle with three cells and a unicellular globular secretory head (Figure 3O).

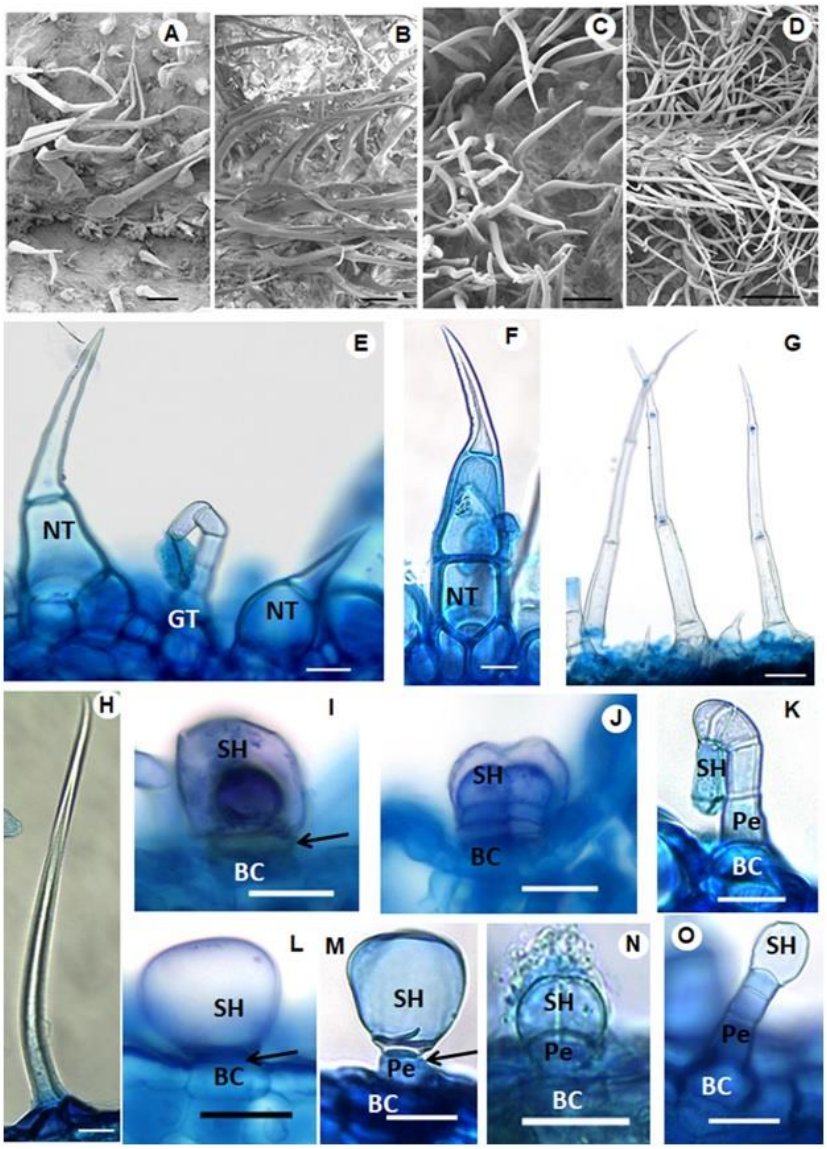

Figure 3. Leaf blade of *Lippia lupulina* Cham. (A-B; E-G, I-K) and *Lippia pohliana* Schauer (C-D; H, L-O). A-D: Trichomes evidenced in scanning electron microscopy. A-B: *L. lupulina* - adaxial and abaxial surface. CD: *L. pohliana* - adaxial and abaxial surface. E-O: Trichomes observed under light microscopy. E-H: Detail of the non-glandular trichomes. E: two types of short bicellular. F: short tricellular. G: long multicellular. H: long unicellular. I-O: Detail of the glandular trichomes. I: Short with unicellular globoid secretory head. J: short with biseriate secretory head. K: long pedunculate: peduncle with four cells

curved to the epidermis and unicellular secretory head. L: short with unicellular globular secretory head. M: short pedunculate - unicellular peduncle and globoid secretory head. N: Short pedunculate: unicellular peduncle and tetracellular secretory head. O: long pedunculate, peduncle with three cells and unicellular secretory head. BC: Basal Cell; SH: Secretory Head; Pe: Peduncle; GT: Glandular Trichome; NT: Non-glandular Trichome. Black arrow: neck cell. Bars = 100µm (A-C); 200µm (D); 50µm (E-F, H-O); 250µm (G).

In cross section, the leaf blade epidermis, in both species, is unistratified and covered by a thin cuticle, with cells of the adaxial face relatively larger than those of the abaxial (Figures 4A-C). In *L. lupulina* the stomata are at the same level as the other epidermal cells on both sides of the leaf blade (Figure 4A). And in L. *pohliana* the stomata on the adaxial face are at the same level as the epidermal cells and on the abaxial face are located above the level of the other epidermal cells and have a large sub-stomatic chamber (Figure 4B).

In both species the mesophyll is dorsiventral and palisade parenchyma is formed by cells in juxtaposition and the lacunous parenchyma by two to three layers of cells with intercellular spaces (Figures 4A-B). *L. pohliana* palisade parenchyma is compact (Figure 4B) when compared to *L. lupulina* parenchyma. The leaf edge of both species is curved to the abaxial face and has a rounded contour; it has a chlorophyll parenchyma between the last vascular bundle and the epidermis, with the epidermal cells on the adaxial face being larger than those on the abaxial face (Figure 4D). The small-caliber vascular bundles present in the mesophyll have a parenchymal sheath (Figure 4D).

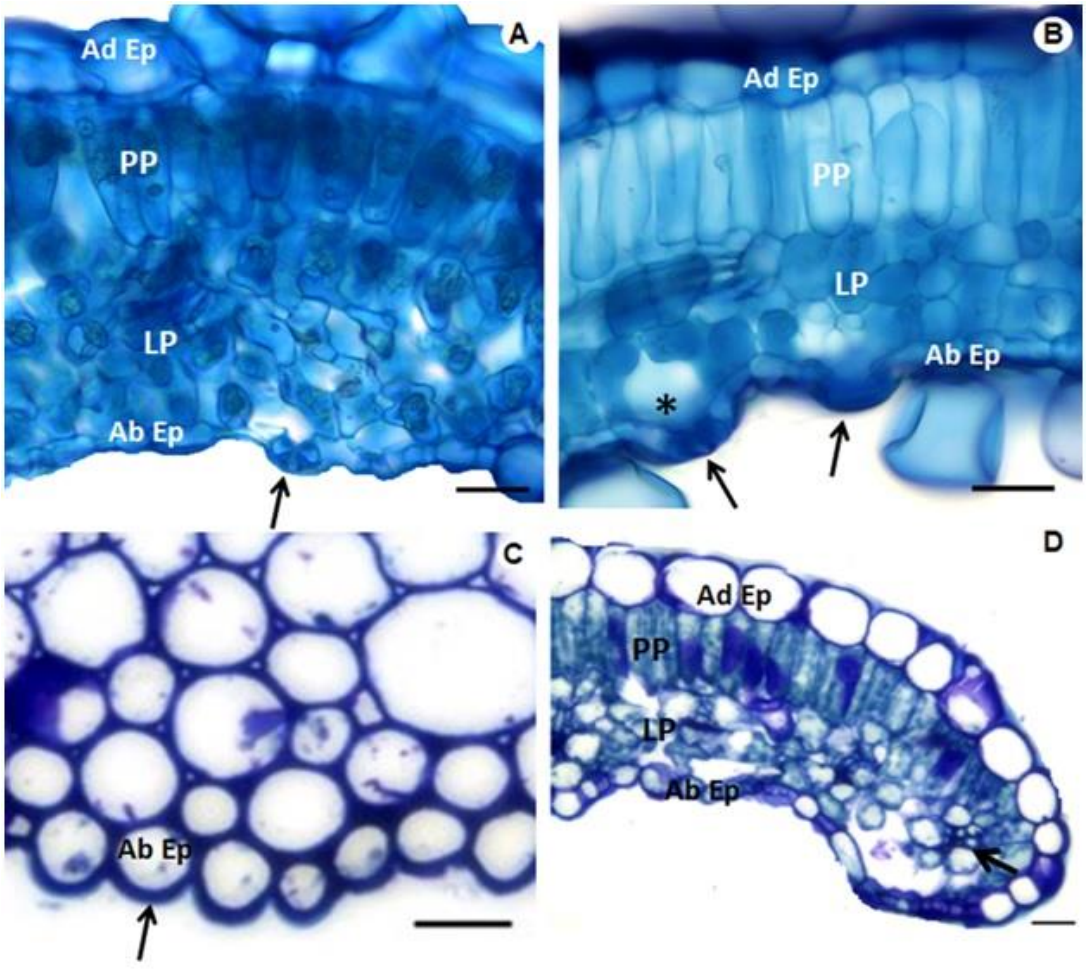

Figure 4. Leaf blade of *Lippia lupulina* Cham. (A) and *Lippia pohliana* Schauer (B-D), in cross sections. A-B: General aspect of the leaf blade showing the size of the epidermal cells on the adaxial and abaxial surfaces, stomata (black arrow) and stomatal chamber (*) on the abaxial surface and dorsiventral mesophyll. C: Detail of the abaxial epidermis, showing the thin cuticle (black arrow). D: Detail of the border, showing the epidermal cells of the adaxial face relatively larger than those of the abaxial face and a smaller caliber bundle with a sheath (black arrow). Ab Ep - Abaxial Epidermis; Ad Ep - Adaxial Epidermis; LP - Lacunous Parenchyma; PP - Palisade Parenchyma. Bars = 50µm (A-D).

The central vein of the two species has a convex-convex outline; angular collenchyma on both sides of the leaf blade, with three to five layers of cells on the adaxial surface and one to two layers on the abaxial surface; the cortical parenchyma has cells of varying sizes with small intercellular spaces (Figures 5A-D), and in *L. lupulina* the parenchymal cells have a thickened wall and some are lignified (Figure 5C) and in *L. pohliana*, the cells parenchymal cells have a thin wall (Figure 5D). The chlorophyll parenchyma in both species extends in the region of the central vein (Figures 5A-B); the vascular system consists of a collateral bundle in the shape of an open arch (Figures 5E-F), and in *L. pohliana* there are two to three accessory bundles in an adaxial position (Figure 5F). Some fibers are associated with phloem in both species (Figures 5E-F).

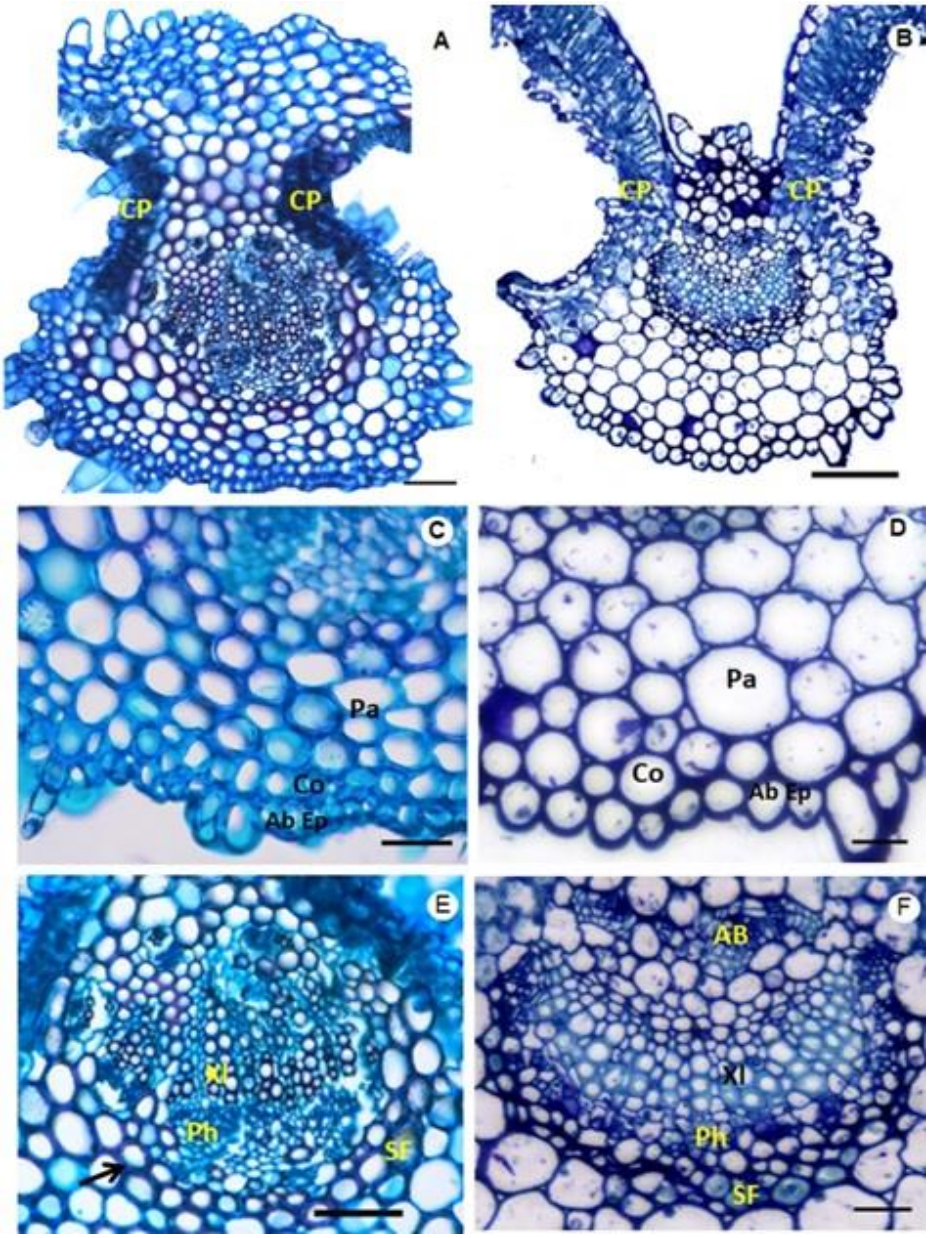

Figure 5. Leaf blade of *Lippia lupulina* Cham. (A, C, E) and *Lippia pohliana* Schauer (B, D, F), in cross sections. A-B. General aspect of the central rib showing convex-convex contour, respectively and the chlorophyll parenchyma extended in the rib region. C-D. Detail of the cortical region, showing the collenchyma and parenchyma. E-F. Detail of the vascular system showing an arc-shaped collateral bundle. E. Presence of sclerenchyma cells around the bundle (black arrow). F. Detail of accessory bundles in an adaxial position. Ab Ep: Abaxial Epidermis; AB: Accessory Bundle; Co: Collenchyma; CP: Chlorophyll Parenchyma; Pa: Parenchyma; Ph: Phloem; SF: Sclerenchymatic Fibers; Xl: Xylem. Bars = 200µm (A-B), 50µm (C-D, F), 100µm (E).

The petiole of *L. lupulina* and *L. pohliana* has unistratified epidermis covered by a thin cuticle, with the presence non-glandular and glandular trichomes similar to those of the leaf blade; the cortex consists of two to three layers of angular collenchyma cells on the adaxial side in *L. pohliana* and three to five layers of cells in *L. lupulina*; three layers of collenchymatic cells on the abaxial surface in both species (Figures 6A-B). The cortical parenchyma in *L. lupulina* has numerous sclerified cells and in *L. pohliana* it is formed by three to four layers of rounded cells with cellulosic walls, of varying sizes and small intercellular spaces, on the abaxial surface (Figures 6A-B); the vascular system in *L. lupulina* is formed by three collateral bundles surrounded by sclereids, in the shape of an open arch (Figure 6C). In *L. pohliana*, the vascular system consists of a collateral bundle in the form of an open arch with three accessory bundles in an adaxial position (Figure 6D).

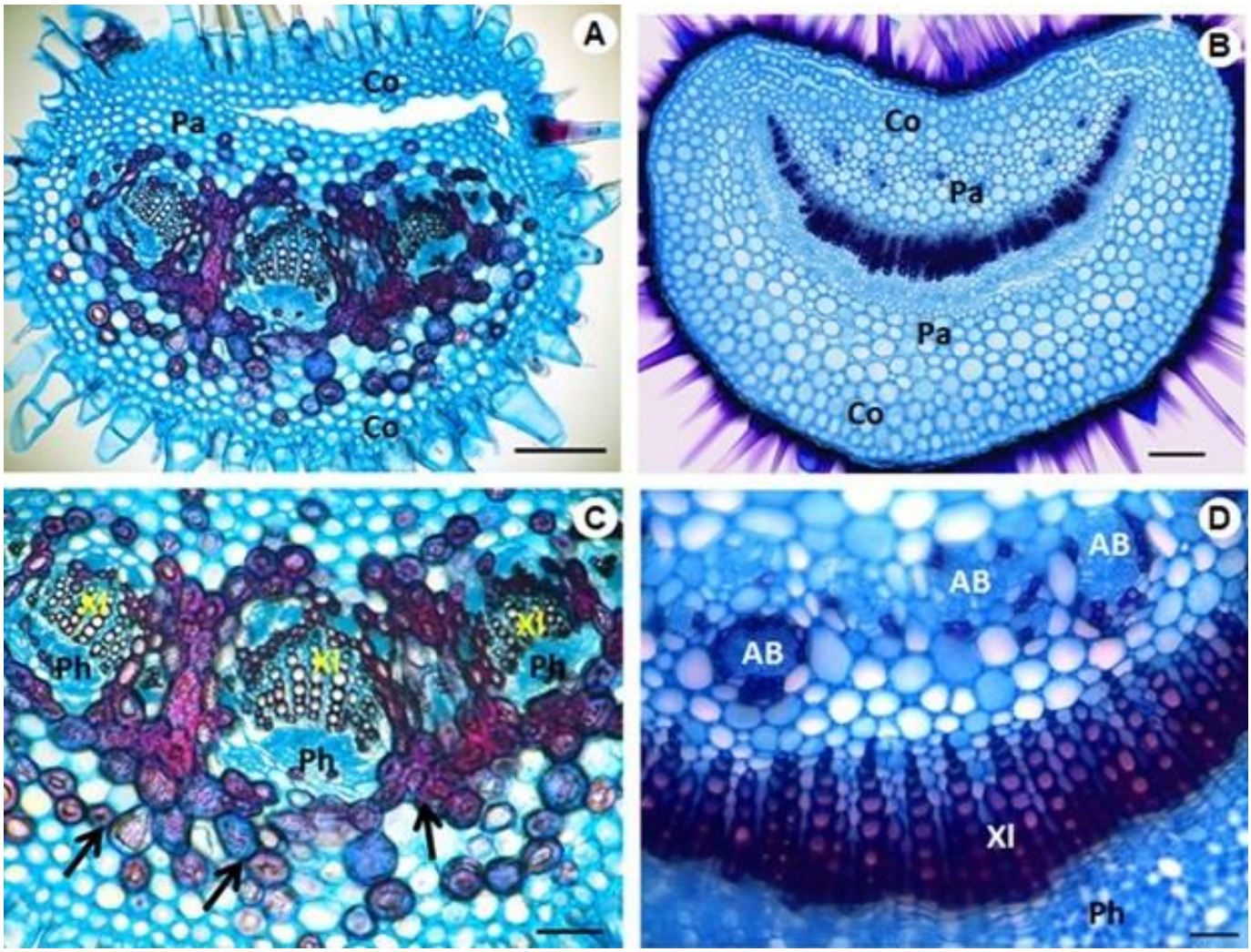

Figure 6. Petiole of *Lippia lupulina* Cham. (A, C) and *Lippia pohliana* Schauer (B, D), in cross sections. A-B: General view of the petiole. C: Vascular system showing the three collateral bundles and sclereid (black arrow) around the bundles. D: Vascular system showing the largest bundle and accessory bundles. AB: Accessory Bundle; Co: Collenchyma; Pa: Parenchyma; Ph: phloem; Xl: xylem. Bars = 200µm (A-B), 100µm (C), 50µm (D).

3.3 Essential oil constitution

The chromatograms of the essential oils of *L. lupulina* and *L. pohliana* are showcased in Figure 7. Major constituents are therein highlighted. Moreover, Table 1 displays essential oil composition. Constituents are therein showcased according to elution order.

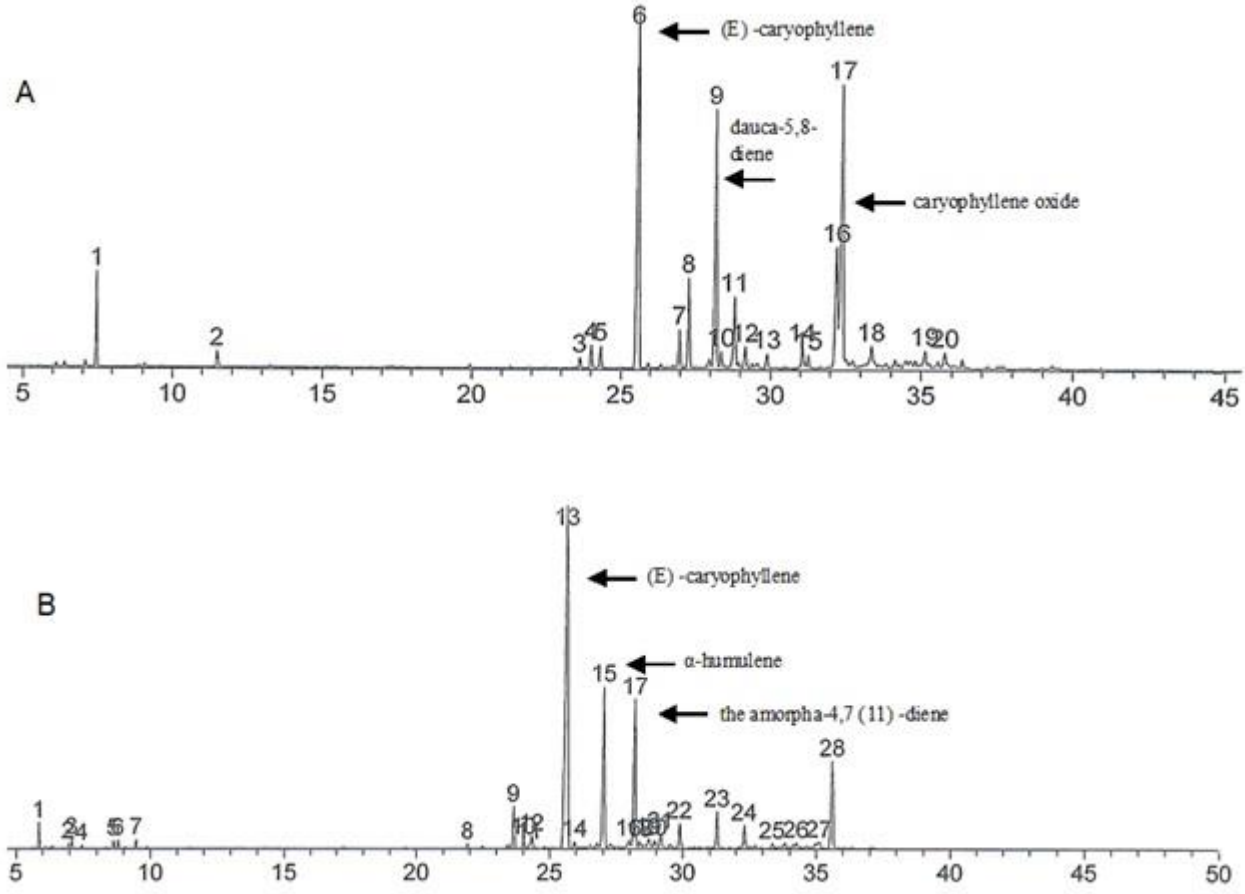

Figure 7. Total ion chromatogram of **(A)** *Lippia lupulina* Cham. and **(B)** *Lippia pohliana* Schauer essential oils. Major constituents therein highlighted.

Considering that the yield of L. lupulina essential oil was of 0.4% and 17 compounds were identified, making up of 97.2% of the chemical constituents, may suggest that sesquiterpene hydrocarbons were the most abundant (60.76%), followed by oxygenated sesquiterpenes (32.14%), monoterpene hydrocarbons (3.47%) and oxygenated monoterpenes (0.87%). The major constituents were (E) -caryophyllene (25.49%), caryophyllene oxide (21.66%) and dauca-5,8-diene (16.83%) (Figure 7.A and Table 1).

Regarding L. pohliana, considering that the yield of the essential oil was of 0.8% and 19 compounds were identified, which represented 85.28% of the essential constituents, may suggest that sesquiterpene hydrocarbons were the most abundant (82.27%), followed by monoterpene hydrocarbons (3.01%). (E) -caryophyllene (43.20%) was the major constituent, followed by α-humulene (14.42%) and the amorpha-4,7 (11) -diene (14.30%) (Figure 7.B and Table 1).

Table 1. Chemical compounds (%) and retention indices (RI) of the essential oils of leaves of *Lippia lupulina* Cham. and *Lippia pohliana* Schauer (Verbenaceae).

Compound	RI	*L. lupulina*	*L. pohliana*
Tricyclene	926	-	1.00
Sabinene	975	-	0.21
β-Pinene	979	-	0.42
Myrcene	990	3.47	0.13
ρ-Cymene	1024	-	0.38
Limonene	1029	-	0.44
(*E*)-β-ocimene	1044	-	0.43
Linalol	1096	0.87	-
δ-Elemene	1338	-	0.25
α-Ylangene	1375	0.59	2.89
β-Bourbonene	1388	1.36	0.35
β-Elemene	1390	1.29	0.82
(E)-Caryophyllene	1419	25.49	43.20
β-Copaene	1432	-	0.40
α-Humulene	1454	2.15	14.42
Allo-aromadendrene	1460	5.31	-
Dauca-5,8-diene	1472	16.83	-
ϒ-Muurolene	1479	-	0.44
Amorpha-4,7(11)-diene	1481	-	14.30
Bicyclogermacrene	1500	4.74	0.56
Germacrene A	1509	1.44	-
δ-Amorphene	1512	0.80	1.80
Germacrene B	1561	0.76	2.84
Espathulenol	1578	8.92	-
Caryophyllene oxyde	1583	21.66	-
Humulen epoxyde II	1608	0.82	-
14-hydroxi-9-epi-(*E*)-Caryophyllene	1669	0.74	-
Monoterpenes		3.47	3.01
Oxygenated monoterpenes		0.87	-
Sesquiterpenes		60.76	82.27
Oxygenated sesquiterpenes		32.14	-
Non identified		2.77	14.72
Identified total (%)		97.2	85.28
Yield (%, v/p)		0.4	0.8

- non detected

4. Discussion

This study evidenced that *L. lupulina* and *L. pohliana* presented some anatomical similarities, such as anfistomatic leaves, presence of non-glandular and glandular trichomes, dorsiventral mesophyll and vascular system in the central vein consisting of a collateral bundle in the form of an open arch with fibers associated to phloem. Morretes (1969) described the anatomy of L. *pohliana* leaves retrieved from Cerrado region and found similar anatomical features to those described in this study. The anfistomatic leaves and dorsiventral mesophyll described in *L. lupulina* e *L. pohliana* confirm the pattern described by Solereder (Solereder 1908) for Verbenaceae family, as well as the non-glandular and glandular trichomes described by Metcalfe and Chalk (Metcalfe & Chalk 1972) for *Lippia* species, which were also reported Combrinck et al. (2007) for *L. scaberrima*, and by Jezler et al. (2013) for *L. alba*.

The leaves of the two species under study showed anatomical differences. Anatomical characters can be of great taxonomic importance in differentiating between species, including Verbenaceae (Iroka et al. 2015). *L. lupulina* presented long and short multicellular non-glandular trichomes, while in *L. pohliana*, the trichomes were unicellular, of different sizes. In addition, both species showed glandular trichomes of different morphotypes. According to Cutler et al. (2011), trichomes have great taxonomic value in the identification of species, subspecies depending on the type and pattern of occurrence in plant organs. *Lippia*'s glandular trichomes have morphological and histochemical variations and are the main sites of essential oil synthesis (Tozin et al. 2015).

Analyzing the different types of stomata present in the studied species, it was found that only *L. lupulina* had a twinned type stomata and *L. pohliana* had a diacitic stomata. In addition, in *L. pohliana* the stomata on the abaxial surface are located above the level of the other epidermal cells and have a large substomatic chamber. The types of stomata and trichomes of the Verbenaceae family are important characters for the study of the family's phylogeny (Cantino 1990). And anatomical characters referring to the epidermis and its attachments, such as stomata, are valuable sources of information, which can assist in the characterization and identification of plants (Dickison 2000).

The petiole of both species is different. *L. lupulina* presents sclereids in the cortical parenchyma and around the three collateral bundles that form the vascular system. While *L. pohliana* does not have sclereids and the vascular system is a single bundle in the form of an open arch, with three accessory bundles in an adaxial position. Sclereids, their type and size have a taxonomic value, as they are inherited and controlled by genetic factors, thus being able to axillary in the identification of rates (Zhang et al. 2009).

Regarding the composition of essential oil, the species studied showed some similarities as to their major constituents, such as sesquiterpenes hydrocarbons and caryophylenes. However, *L. lupulina* presented caryophyllene oxide (21.66%) and dauca-5,8-diene (16.83%) among its main constituents, unlike *L. pohliana* which contained α-humulene (14.42%) and the amorpha-4.7(11)-diene (14.30%). This information helps in species differentiation and identification. Zoghbi et al. (2002) described the chemical profile of *L. lupulina* oils extracted from plants retrieved from three collection points of Mato Grosso state, Brazil. In the first point, the oil extracted from *L. lupulina* stems and leaves showcased terpinene-4-ol (41.3%), terpinolene (10.4%) and α-terpineol (9.2%), while the oil extracted from plants in the second point of collection yielded 1,8 cineol (15.5%), β-caryophyllene (12.3%) and endo-fenchol (10.4%). In the third collection point, β-caryophyllene (12.7%) and bicyclogermacrene (11.2%) were found in *L. lupulina* volatile oil. Concerning *L. pohliana*, this work is to the best of our knowledge the first report regarding the chemical profile of the essential oil extracted from this species. In this sense, comparisons were drawn from hexane fractions reported in literature, which outreached β-caryophyllene (33.55%) and linalool (6.96%) as major components (Singulani et al. 2012).

Considering that the largest proportion of constituents reported in our work is comprised of sesquiterpene hydrocarbons, while Zoghbi et al. (2002) reported monoterpenes, and that the collection of vegetal material for this work took place during rainy season while the cited report had the material collected during dry season, results suggest therefore the remarkable effect of region and climate on essential oil chemical profile. These findings are nonetheless corroborated by literature, which reports the influence of edaphological and climatic features on Verbenaceae essential oil composition (Elechosa et al. 2017; Kamanula et al. 2017; Silva-Junior et al. 2019).

5. Conclusion

This work presented the characterization of the morphoanatomy and essential oils of *L. lupulina* and *L. pohliana* and identified the characteristics that differentiated the two species. The study of leaf anatomy allowed to differentiate the species, observing the following structural characters: types of glandular and non-glandular trichomes, types of stomata, structural organization of the petiole's vascular system and the presence of sclereids in the petiole of one of the species. Regarding the chemical composition of essential oil, the main constituents were different; in *L. lupulina* the main ones were E-caryophyllene, caryophyllene oxide and dauca-5,8-diene, while in *L. pohliana*, E-caryophyllene was the main constituent, followed by α- humulene and amorphous-4,7 (11) -diene. In addition, the chemical profile of the essential oil of *L. pohliana* was described here for the first time. The anatomy characters of the leaf and petiole and data on the composition of essential oils are important for taxonomy and are useful in identifying the two species.

6. Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the financial support obtained from Coordination for the Improvement of Higher Education Personnel (CAPES), Brazilian National Council for Scientific and Technological Development (CNPq), and Foundation for Research Support of the State of Goiás (FAPEG). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Referencias

Adams RP 2012. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry. 4th ed, Allured Publ, Carol Stream, 804 pp.

Cádiz-Gurrea ML, Olivares-Vicente M, Herranz-López M, Arraez-Roman D, Fernández-Arroyo S, Micol V, Segura-Carretero A 2018. Bioassay-guided purification of Lippia citriodora polyphenols with AMPK modulatory activity. J Func Foods 46:514-520.

Cantino PD 1990. The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae. Journal of the Arnold Arboretum 71(3):323-370.

Cavallaro V, Baier CJ, Murray MG, Estévez-Braun A, Murray AP 2018. Neuroprotective effects of Flaveria bidentis and Lippia salsa extracts on SH-SY5Y cells. South Afr J Bot 119:318-324.

Costa-Neto EM, Oliveira MVM 2000. The use of medicinal plants in the county of Tanquinho, State of Bahia, Northeastern Brazil. Rev Bras Plant Med 2(2):1-8.

Combrinck S, Du Plooy GW, McCrindle RI, Botha BM 2007. Morphology and histochemistry of the glandular trichomes of Lippia scaberrima (Verbenaceae). Annals Bot 99:1111-1119.

Cutler DF, Botha T, Stevenson DW 2011. Anatomia Vegetal: uma abordagem aplicada. Artmed, Porto Alegre, 304 pp.

Dickison WC 2000. Integrative Plant Anatomy. Academic Press, San Diego, 533p.

Elechosa MA, Lira PDL, Juárez MA, Viturro CI, Heit CI, Molina AC, Martínez AJ, López S, Molina AM, Baren CM, Bandoni AL 2017. Essential oil chemotypes of Aloysia citrodora (Verbenaceae) in Northwestern Argentina. Biochem Syst Eco 74:19-29.

Funari CS, Gullo FP, Napolitano A, Carneiro RL, Mendes-Giannini MJS, Fusco-Almeida AM, Piacente S, Pizza C, Silva DHS 2012. Chemical and antifungal investigations of six Lippia species (Verbenaceae) from Brazil. Food Chem 135(3):2086-2094.

Gomide MS, Lemos FO, Lopes MTP, Alves TMA, Viccini LF, Coelho CM 2013. The effect of the essential oils from five different Lippia species on the viability of tumor cell lines. Rev Bras Farmacog 23(6):895-902.

Iroka CF, Okeke CU, Okereke CN 2015. Systematic implications of trichomes in the species of Stachytarpheta found in Awka. Nigeria Asian Journal of Plant Science and Research 5(4):30-34.

Jezler CN, Oliveira ARMF, Batista RS, Oliveira RA, Silva DC, Costa LCB 2013. Lippia alba morphotypes cidreira and melissa exhibit significant differences in leaf characteristics and essential oil profile. Braz J Pharmacog 23(2):217-223.

Johansen DA 1940. Plant Microtechnique. McGraw-Hill Book Company Inc, New York, 523 pp.

Kamanula JF, Belmain SR, Hall DR, Farman DI, Goyder DJ, Mvumi BM, Masumbu FF, Stevenson FC 2017. Chemical variation and insecticidal activity of Lippia javanica (Burm. f.) Spreng essential oil against Sitophilus zeamais Motschulsky. Industr Crops Prod 110:75-82.

Metcalfe CR, Chalk L 1972. Anatomy of Dicotyledons. Claredon Press, Oxford,1500 pp.

Morretes BL, Ferri MG 1969. Contribuição ao estudo da anatomia das folhas de plantas do cerrado. Boletim da Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo. Botânica. 24:7-32.

Paiva JGA, Carvalho SMF, Magalhaes MP, Ribeiro DG 2006. Verniz Vitral incolor®: uma alternativa de meio de montagem economicamente viável. Act Bot Bras 20:257-264.

Rodrigues VEG, Carvalho DA 2001. Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do Alto Rio Grande - Minas Gerais. Ciên Agrotéc Lav 25(1):102-123.

Roeser KR 1972. Die Nadei der Schwarzkiefer – Massenproduckt und Kunstwerk der Natur. Mikrokosmos 61:33-36.

Singulani JL, Silva PS, Raposo NRB, Siqueira EP, Zani CL, Alves TMA, Viccini LF 2012. Chemical composition and antioxidant activity of Lippia species. J Med Plant Res 6(27):4416-4422.

Silva TRS, Salimena FRG 2002. Novas combinações e novos sinônimos em Lippia e Lantana (Verbenaceae). Darwiniana 40(1-4):57-59.

Silva-Júnior AQ, Silva DS, Figueiredo PLB, Sarrazin SLF, Bouillet LEM, Oliveira RB, Maia JGS, Mourão RHV 2019. Seasonal and circadian evaluation of a citral-chemotype from Lippia alba essential oil displaying antibacterial activity. Biochem Syst Eco 85:35-42.

Siqueira-Lima PS, Passos FRS, Lucchese AM, Menezes IRA, Coutinho HDM, Lima AAN, Zengin G, Quintans JSS, Quintans-Júnior LJ 2019. Central nervous system and analgesic profiles of Lippia genus. Rev Bras Farm 29(1):125-135.

Tozin LRS, Carvalho SF, Machado SR, Rodrigues TM 2015. Glandular trichome diversity on leaves of Lippia origanoides and Lippia stachyoides (Verbenaceae): morphology, histochemistry, and ultrastructure. Botany 93(5):297-306.

Van Den Dool H, Kratz JDA 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromat A 11(C):463-471.

Viccini LF, Pierre PMO, Praça MM, Costa DCS, Romanel EC, Sousa SM, Peixoto PHP, Salimena FRG 2005. Chromosome numbers in the genus Lippia (Verbenaceae). Plan Syst Evol 256:171-178.

Zhang W, Hu Y, Li Z, Wang P, Xu M 2009. Foliar sclereids in tea and its wild allies, with reference to their taxonomy. Australian Systematic Botany 22(4):286-295.

Zoghbi MGB, Andrade HHA, Silva MHL, Maia JGS 2002. Volatile constituents of Lippia lupulina Cham. Flav Fragr J 17:29-31.