Leaf anatomy of *Cordiera sessilis* (Vell.) Kuntze (Rubiaceae)

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ABSTRACT. A study on the leaf anatomy of *Cordiera sessilis* (Rubiaceae), a native medicinal shrub from Brazilian Cerrado was carried out to identify features that may be useful in species recognition. Leaves were collected, fixed and processed by usual techniques, and studied by light and electron microscopy. Quantitative analyzes of stomata and trichomes were performed. In addition to the typical anatomical characteristics of Rubiaceae leaves, two types of vascular patterns were identified in the petiole: in distal part, the vascular system is arranged cylindrically surrounded by sclerenchyma sheath and in proximal part the vascular system is arranged in U-shape coupled to sclerified cells. The micromorphological organization of leaf surface, epicuticular wax types, the petiole pattern and histochemical characteristics as the presence of druses, crystal sand and alkaloids and absence of raphides in the mesophyll, midrib and petiole are considerate representative characteristics of *C. sessilis* and may be useful in the species recognition.

Keywords: "Marmelinho", plant anatomy, histochemical tests, *Cerrado*, medicinal plant.

Anatomia foliar de *Cordiera sessilis* (Vell.) Kuntze (Rubiaceae)

RESUMO. Um estudo da anatomia foliar de *Cordiera sessilis* (Rubiaceae), um arbusto medicinal nativo do Cerrado brasileiro foi realizado com o objetivo de identificar características úteis no reconhecimento da espécie. As folhas foram coletadas, fixadas e processadas por meio de técnicas usuais e estudadas em microscopia de luz e eletrônica. As análises quantitativas dos estômatos e tricomas foram realizadas. Além das características anatômicas típicas das folhas de Rubiaceae, dois padrões vasculares foram identificados no pecíolo: na porção distal, o sistema vascular disposto cilindricamente, contornado por uma bainha esclerenquimática e na porção proximal, o sistema vascular organizado em forma de U associado a células esclerenquimáticas. A organização micromorfológica da superfície foliar e da cera epicuticular, o padrão do pecíolo e as características histoquímicas como a presença de drusas, areia cristalina e alcaloides e a ausência de cristais do tipo ráfides no mesofilo, nervura central e pecíolo são consideradas características representativas de *C. sessilis* e podem ser úteis para o reconhecimento da espécie.

Palavras-chave: Marmelinho, anatomia vegetal, testes histoquímicos, Cerrado, planta medicinal.

Introduction

The genus *Cordiera* (Rubiaceae) comprises 21 species distributed in Central and South America (Delprete & Cortés-B, 2006). *Cordiera sessilis* (Vell.) Kuntze, or "marmelinho", "marmelo-do-cerrado" and "marmelada-de-cachorro" is a branched shrub with a low canopy, which is widely distributed in Brazil, especially in the states of Ceará, Mato Grosso, Goiás, Minas Gerais and São Paulo (Silva, Silva, Bolzani, & Lopes, 2006; Zappi, Calió, & Pirani, 2014). The species is the synonym for *Alibertia sessilis* (Vell.) K. Schum., *Alibertia melloana* Hook.f., *Alibertia subaurea* Zahlbr and *Gardenia sessilis* Vell. (Zappi et al., 2014).

Cordiera sessilis is native species from Brazilian *Cerrado* and has nutritional and medicinal properties (Silva et al., 2006). The wood is used in the production of firewood and charcoal, and the fruits are eaten fresh as pies and jellies. Leaf and branch portions are commonly employed as cataplasms, compresses or baths in the treatment of skin disorders (Rodrigues & Carvalho, 2001). Some chemical studies reported to the isolation of the constituents from leaves such as triterpenes, iridoids, flavonoids and esters of caffeic acid (Olea, Roque, & Bolzani, 1997; Silva et al., 2006). Silva, Bolzani, Young, and Lopes (2007) reported new antifungal phenolic derivatives, iridoids and lignins from leaves. The flowering occurs in August and September and the inflorescences are terminal and unisexual. The fruit has a fleshy and sweet pulp with many seeds. The leaves are simple, opposite and leathery, with curved and wavy edges, 10 - 16 cm long (Matheus, Bacelar, & Oliveira, 2008). There are

no studies about of leaf anatomy that may provide diagnostic characters for identification of *Cordiera sessilis*. According to Melo, Nascimento, Amorim, Lima, and Albuquerque (2004), this fact may lead to inaccurate or dangerous use of plant drugs.

The leaf anatomy studies are important for the Rubiaceae taxonomy (Campbell, Rabelo, & Cunha, 2016; Coelho, Leite, Nunes, & Ventrella, 2012; Moraes, Rabelo, Alexandrino, Silva Neto, & Cunha, 2011). Hypostomatic leaves, paracytic stomata, straight-walled epidermal cells, dorsiventral mesophyll and the collateral vascular system are usual characters of Rubiaceae family (Solereder, 1908; Metcalfe & Chalk, 1950). However, according Moraes, Barros, Silva Neto, Gomes, and Cunha (2009), the leaf surface, domatia types, epicuticular wax types and patterns of epidermis anticlinal cell walls are useful in the recognition of different species.

Numerous leaf anatomical characteristics are valuable for the systematic identification of the species and the description of the epidermis and its appendages (stomata and trichomes) are useful for this purpose (Dickison, 2000). The anatomical and micromorphological characteristics of the leaf blade are useful in the identification of fragmentary material and in the recognition of the taxon when reproductive structures are not available (Moraes et al., 2009; Defaveri, Arruda, & Sato, 2011) and may be used for phylogenetic studies on Rubiaceae (Andrade et al., 2015).

Dendroides colleters in vegetative and reproductive apices of *C. sessilis* were described by Machado, Barreiro, Rocha and Rodrigues (2012) and the morphological description of fruits and seeds was performed by Matheus et al. (2008). However, studies on *C. sessilis* anatomy are scarce. The current study describes the leaf anatomy of *C. sessilis* in order to contribute for the diagnosis of the species.

Material and methods

Plant material

Completely expanded leaves of *Cordiera sessilis* (Vell.) Kuntze (Rubiaceae) were collected in the Brazilian Cerrado in the municipality of Ijaci, southern region of the Minas Gerais State, Brazil (21°13'46"S; 44°58'32"W; average altitude 908 m). Collections were made under a permit issued by *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio) and the voucher specimen (PAMG 56551) was identified by Andréia Fonseca Silva, curator of PAMG Herbarium of the *Empresa de Pesquisa Agropecuária de Minas Gerais* (EPAMIG).

Light microscopy

The leaves were fixed in FAA 70 (formaldehyde: acetic acid: 70% ethanol, 1:1:18) and stored in 70% ethanol (Johansen, 1940). Samples were taken from leaf midrib, intercostal region, edge and petiole. Leaf anatomy was based on handmade paradermal and transverse sections. The cross-sections were clarified with a commercial solution of 50% sodium hypochlorite and stained with safranin and Astra-Blau (Safrablau), according to Bukatsch (1972) modified. The paradermal sections were stained with 1% safranin and the sections were mounted in 50% glycerin.

Quantitative studies of stomata and trichomes were performed according to Justo, Soares, Gavilanes, and Castro (2005). Ten leaves of five specimens were analyzed. Thirty fields of the leaf blade (including basal, middle and distal sections) were evaluated to determine the index and density of the stomata and the trichomes and stomata diameter (polar and equatorial). The stomata diameter was measured for one stomata (per field) with Axio Visio Rel. 4.8. The stomata and trichomes index and the density were determined according to Cutter (1986) and Labouriau, Oliveira, and Salgado-Labouriau (1961), respectively. The stomata index (I) and density (D) were calculated by the expressions: $I(\%) = SN/(SN + EC) \times 100$ and D (stom. mm^{-2}) $= SN/A$, respectively, where SN = stomata number; EC = epidermal cells number; A = area (mm^2). The same expressions were employed for trichomes, replacing SN by TN (trichomes number). Data were analyzed by ANOVA and the means were compared by Tukey's test at 5% significance. Results were given as mean ± standard deviation. Sections were examined with a Zeiss Primo Star light microscope and documented by AxioVision.

Scanning electron microscopy

The micromorphological study was carried out on 0.5 cm^2 fragments of the median third of the blade and on petiole fragments. Samples were SEM-observed (LEO Evo 40 XVP) by immersion in fixative (modified Karnovsky, 2.5% glutaraldehyde, 2.0% paraformaldehyde, 0.05 M cacodylate buffer, pH 7.2) for 24 hour, and prepared according to protocol by Bussola and Russell (1999). The epicuticular wax was classified according to Barthlott et al. (1998); the trichomes were classified according to Theobald, Krahulik, and Rollins (1979); the stomata were identified following classification of Wilkinson (Wilkinson, 1979). Results were recorded on electromicrographs with scales projected under the same optical and electronic conditions.

Histochemical analyses

The analyses were performed for the identification of cellular metabolites: ferric chloride for phenolic compounds (Johansen, 1940); zinc chloride iodine for lignified elements and starch (Jensen, 1962); reagent of Dragendorf for alkaloids (Costa, 1982); Sudan III (Sass, 1951) and IV (Gerlach, 1984) for lipids in general; lugol for starch (Johansen, 1940); and hydrochloric acid 10% (Chamberlain, 1932) for identification of calcium oxalate and carbonate crystals. The sections were examined with a Zeiss Primo Star light microscope and documented.

Results

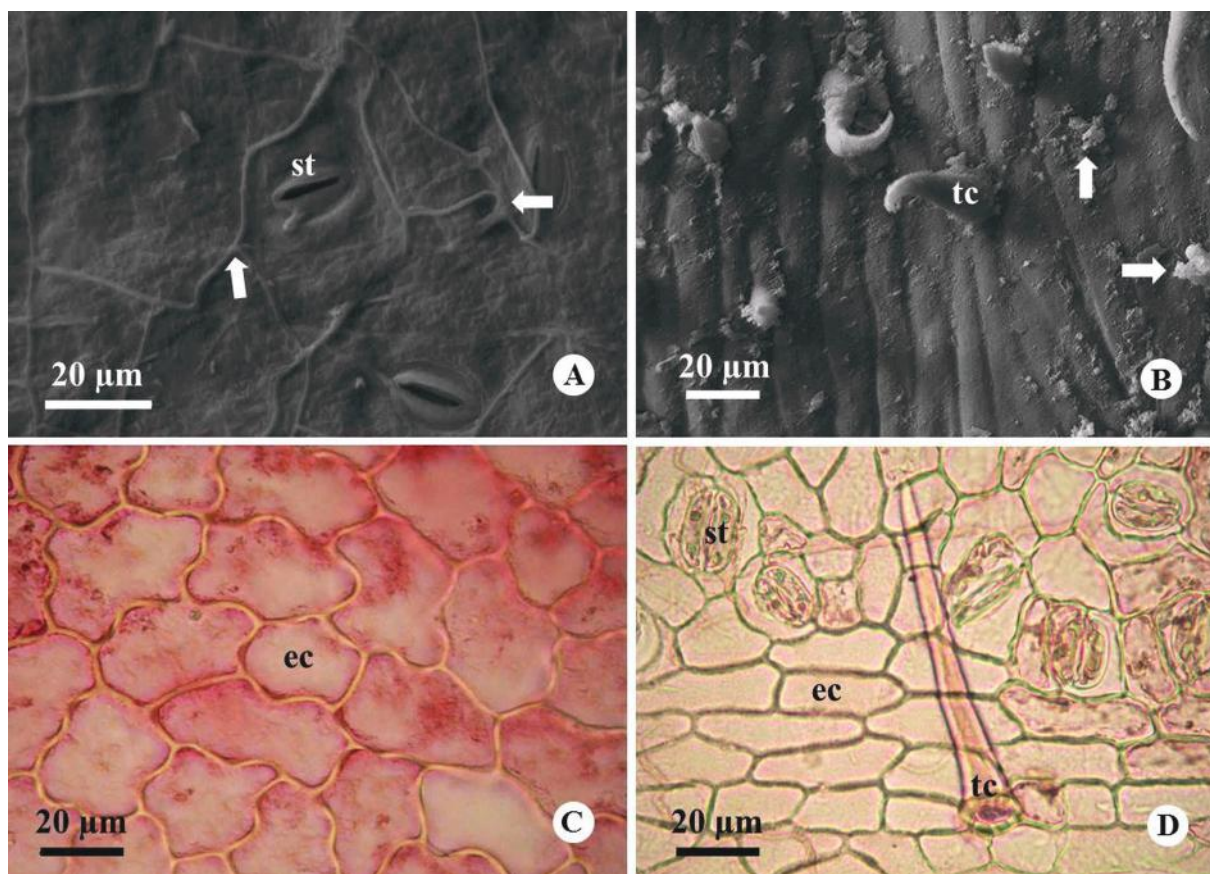### Surface

The leaf blade is completely covered with a dense wax layer making visible the stomatal pore found only on abaxial surface and crystalloids of epicuticular wax in threads were observed (Figure 1A). The crystalloids exhibit grouped granules shape on the adaxial surface of the leaf blade and petiole (Figure 1B). Simple non-glandular trichomes are found on the adaxial and abaxial surfaces of leaf blade and on the petiole (Figure 1B and 1D).

Leaf blade

In frontal view, the epidermal cells on the adaxial and abaxial surfaces have slightly sinuous and straight anticlinal walls, respectively (Figures 1C and D) and the wall is thick, especially on leaf adaxial surface. The leaves are hairy and the higher trichomes index and density were observed on the abaxial surface ($p < 0.05$) in all the regions of the leaf blade (Table 1). The leaves are hypostomatic, with paracytic stomata (Figure 1D). The stomatal index did not vary between apex, middle and leaf base ($p > 0.05$), averaging approximately $20.73 \pm 2.10\%$. However, the leaf base had lower stomatal density when compared to the apex and the middle (Table 2). Stomata exhibited an average polar and equatorial diameter of 24.00 ± 0.87 μm and 15.44 ± 0.72 μm, respectively. There was no variation in stomatal size for the different regions of the leaf ($p > 0.05$).

Figure 1. Eletromicrographs showing epicuticular waxes types in abaxial surface of blade leaf (A) and in petiole (B) (arrows: A- crystalloids in threads; B- crystalloids in grouped granules). Photomicrographs of the adaxial surface (C) and abaxial surface (D) of leaf blade. st= paracytic stomata; tc= simple non-glandular trichome; ec= epidermal cells.

Table 1. Analysis of trichomes in *Cordiera sessilis* leaves.

	Trichome Index★ (%)			Trichome Density★ (trich. mm⁻²)		
	Apex	Middle	Base	Apex	Middle	Base
Adaxial	0.19 ± 0.09aB	0.24 ± 0.52aB	0.51 ± 0.11aB	2.89 ± 0.51aB	4.89 ± 0.75aB	9.99 ± 1.07aB
Abaxial	1.66 ± 0.67aA	1.73 ± 0.48aA	1.78 ± 0.21aA	27.20 ± 8.16aA	28.74 ± 5.25aA	28.95 ± 5.54aA

★Lowercase letters compare means in the lines and capital letters compare means in the column. Means followed by the same letter do not differ at 5 % probability by Tukey´s test.

Table 2. Analysis of stomata in *Cordiera sessilis* leaves.

	Stomatal index★	Stomatal density★	Diameters	
	(%)	(stom. mm⁻²)	Polar★ (μm)	Equatorial★ (μm)
Apex	21.79 ± 2.94a	468.44 ± 41.25a	23.77 ± 1.06a	15.57 ± 0.96a
Middle	22.09 ± 1.92a	480.74 ± 37.30a	23.83 ± 0.95a	15.52 ± 0.53a
Base	18.30 ± 2.40a	358.92 ± 35.21b	24.40 ± 0.59a	15.24 ± 0.66a

★Means followed by the same letter in the column do not differ at 5 % probability by Tukey´s test.

In the cross-section, epidermis is one-layered and cells with a thin and flat outer periclinal wall occur on both surfaces. Cuticular flanges were observed and the cuticles are thickened, especially on the adaxial surface. The mesophyll is dorsiventral with a palisade parenchyma with periclinally elongated cells facing the adaxial surface, filling about half of the mesophyll (Figure 2A). The first layer of palisade parenchyma is compactly arranged (Figures 2A and D). The cells of the spongy parenchyma are loosely arranged with many intercellular spaces, with four layers facing the abaxial surface. The stomata are slightly proeminent when compared to the epidermal cells and subsidiary cells are larger than the guard cells (Figure 2B). Idioblasts with crystals and oil are frequent in the mesophyll (Figure 2C). Sclerified cells are associated to the vascular bundles (Figure 2A and D).

The blade edge is slightly revolute. The cuticle is usually thick and the epidermal cells have thin outer periclinal walls. The subepidermal region is parenchymatous (Figure 2D).

In the midrib region, the vascular system forms a prominent abaxial vein. The vascular tissues are distributed cylindrically with internal xylem and external phloem surrounded by sclerenchyma sheath and a small portion of phloem also occurs in the center of the midrib (Figure 2E). Sclerified cells in red color also appear in the medullar region of the midrib. Besides the main vascular bundles, there are also two small amphicribal bundles adjacent to the adaxial surface (Figures 2E and F). In the adaxial surface, a cortical parenchyma is located below of epidermis, but in the abaxial surface, the cortical parenchyma is separated from the epidermis by a few layers of annular collenchyma (Figure 2G). Sclerified cells also appear in the collenchyma region (Figure 2H). Calcium oxalate crystals like druses and crystal sand were observed on cells of the cortical and medullary parenchyma and collenchyma.

Petiole

In cross-section, the petiole is plan convex in its distal part (Figure 3A) and circular in its proximal part (Figure 3B). The surface is usually covered by cuticle and non-glandular trichomes are observed. The epidermis is one-layered, the cortex is parenchymatous and sclerified cells are not common in the cortical parenchyma (Figures 3C and D). In distal part of petiole (Figure 3C), the vascular system is formed by collateral bundles arranged cylindrically, surrounded by sclerenchyma sheath and a small portion of phloem occurs in the medullar parenchyma. However, in proximal part of petiole, the vascular system is arranged in U-shape coupled to sclerified cells (Figure 3D). In the two portions, besides the main vascular system, there are two accessory vascular bundles adjacent to the abaxial surface (Figures 3C and D). The isolated bundles are amphicribal. Druses and crystal sand were observed in the cortical and medullary parenchyma.

Histochemical tests

The histochemical tests showed starch in the cortical and medullar parenchyma of midrib and petiole and starch sheath in the petiole (Figure 4A), as well as phenolic compounds in epidermal cells, cortical and medullar parenchyma, and in the phloem of the midrib and petiole (Figure 4B). Alkaloids were detected in the mesophyll, in the midrib and in the petiole, especially in cortical and medullar parenchyma (Figure 4C). Lipid substances, such as cutin, were observed in the epidermal cells and trichomes surface in the leaf blade and petiole (Figure 4D). Oil droplets were reported throughout the leaf's mesophyll (Figure 2C). Lignin was observed in the sclerenchyma, phloem and xylem (Figure 4E). Idioblasts bearing calcium oxalate crystals (druses and crystal sand) were found in the leaf mesophyll (especially in the spongy parenchyma), midrib and petiole (cortical and medullary parenchyma) (Figure 4F). Calcium

oxalate crystals known as raphides were not detected in *C. sessilis* leaves.

Discussion

Hypostomatic leaves, paracytic stomata, dorsiventral mesophyll and collateral bundles in *C. sessilis* leaves agree with the general anatomical characters described for Rubiaceae family (Coelho et al., 2012; Moraes et al., 2011; Erbano & Duarte, 2010; Bremer, 2009). However, other characteristics may have diagnostic value being useful for recognition of *C. sessilis* leaves like the micromorphological organization of blade leaves, the pattern of the petiole, presence of druses and clusters of single calcium oxalate crystals and alkaloids in the mesophyll and absence of raphids.
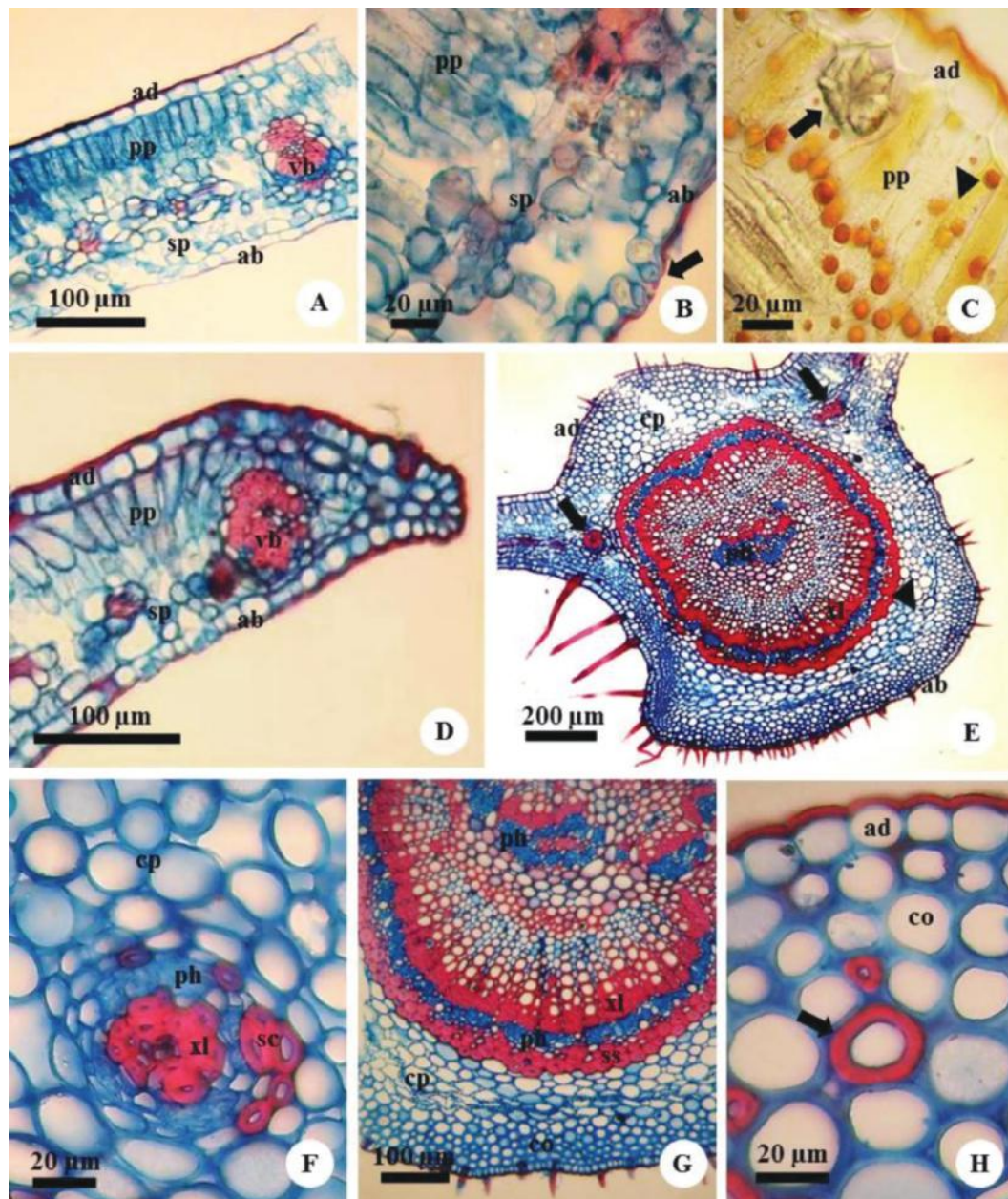
Figure 2. Photomicrographs of cross-sections of the leaf blade. (A) Dorsiventral mesophyll; (B) Detail of the cells of stomata (arrow); (C) Idioblasts in the mesophyll with crystals (arrow) and oil (arrowhead); (D) Blade edge; (E) Midrib showing cylindrical main vascular bundle, sclerenchyma sheath (arrowhead) and small amphicribal bundle (arrow); (F) Detail of small amphicribal bundle; (G) Detail of midrib; (H) Sclerified cells (arrow). ad = adaxial surface; ab = abaxial surface; pp = palisade parenchyma; sp = spongy parenchyma; vb = vascular bundles; cp = cortical parenchyma; co = collenchyma; sc = sclerified cells; ss = sclerenchyma sheath; ph = phloem; xl = xylem.
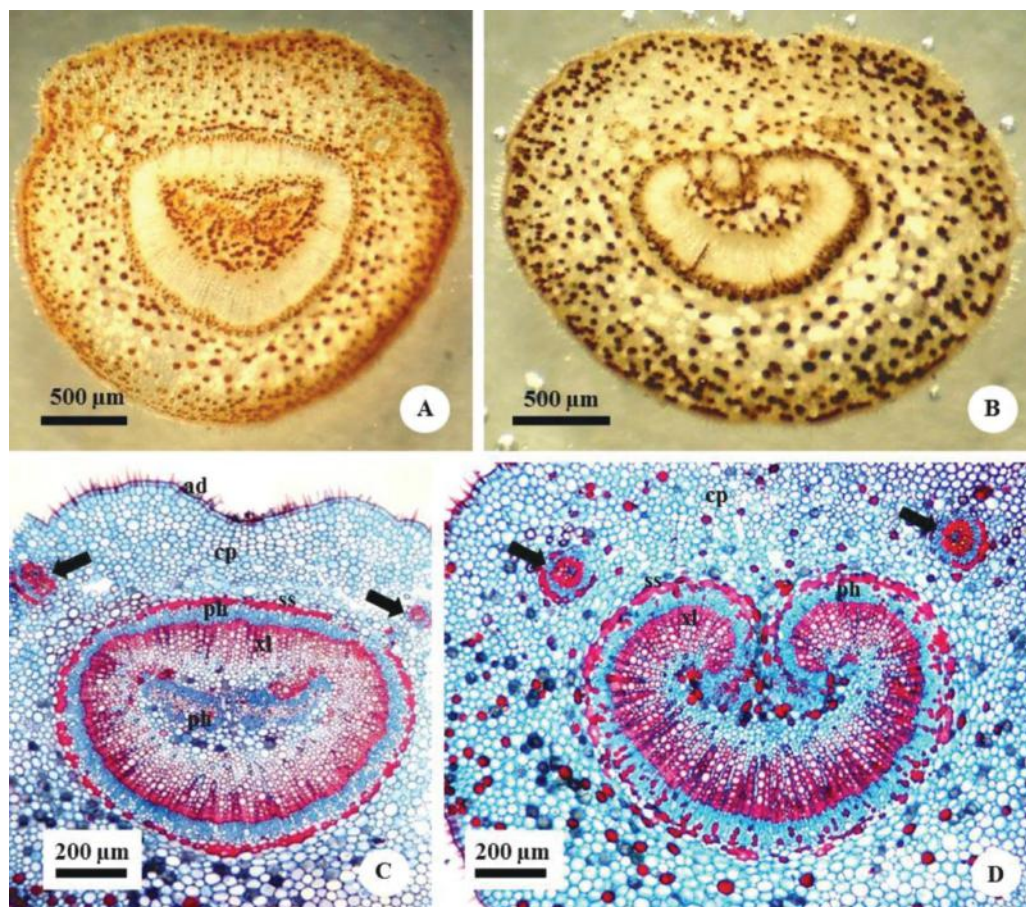
Figure 3. Stereomicrographs of cross-sections of the petiole: (A) Plan-convex petiole; (B) Circular petiole. Photomicrographs of cross-sections of the petiole: (C) Cylindrical collateral bundles in plan-convex petiole; (D) Collateral bundles in U-shape in circular petiole. Arrow: Isolated bundles amphicribal; cp = cortical parenchyma; ss = sclerenchyma sheath; ph = phloem; xl = xylem.

The sinuous and straight shape found in the anticlinal walls of epidermal cells was also described for other species of Rubiaceae as *Psychotria longepedunculata* and *Psychotria glaziovii* (Moraes et al., 2011), *Simira grazielae* and *Simira pikia* (Moraes et al., 2009) and *Rondeletia odorata* (Kocsis, Darók, & Borhidi, 2004). Structural aspects of the epidermis, such as the outline of the anticlinal wall and the shape of the external periclinal wall of the epidermal cells may be related to light intensity. However, other factors, such as water availability and temperature may influence to the expression of these characteristics (Moraes et al., 2011).

Simple non-glandular trichomes are frequent in Rubiaceae (Pereira, Meira, & Azevedo, 2003) and according to Gomes and Neves (2009), the trichomes have great value in taxonomical, ecological and evolutionary studies. Trichomes are usually found in the leaves, protecting the plant from phytophagous animals (Bieras & Sajo, 2009), and may function as a mechanical protection against extreme temperatures, high light intensity, excessive water loss and other factors (Werker, 2000).

According to Bieras and Sajo (2009), the presence of waxes, cuticle and trichomes protect the leaf from heat and excessive radiation due to the high luminosity that prevails in this biome.

Hypostomatic leaves are common in Rubiaceae (Moraes et al., 2011). According to Mantovani, Gomes, Gomes, and Vieira (1995), the stomata exclusively on the abaxial surface are a protection against the obliteration of stomatal slits for small mosses and other flora components that constantly coat the adaxial surface of different species. The paracytic stomata are the most reported type in the Rubiaceae species. *C. sessilis* exhibited a stomatal density which was relatively higher when compared to that of other Rubiaceae species as *Tocoyena bullata* (220 stom. mm^{-2}) (Vieira, 1986/88), *Rudgea decipiens* (38 stom. mm^{-2}), *Rudgea macrophylla* (130 stom. mm^{-2}) (Mantovani et al., 1995) and *Chicocca brachiata* (353 stom. mm^{-2}) (Gusmão, Souza, Silva, & Silva, 1992). According to Silva, Alquini, and Cavallet (2005), the number, frequency, size, distribution, shape and mobility of the stomata vary from different plants, interfering with photosynthetic ability.
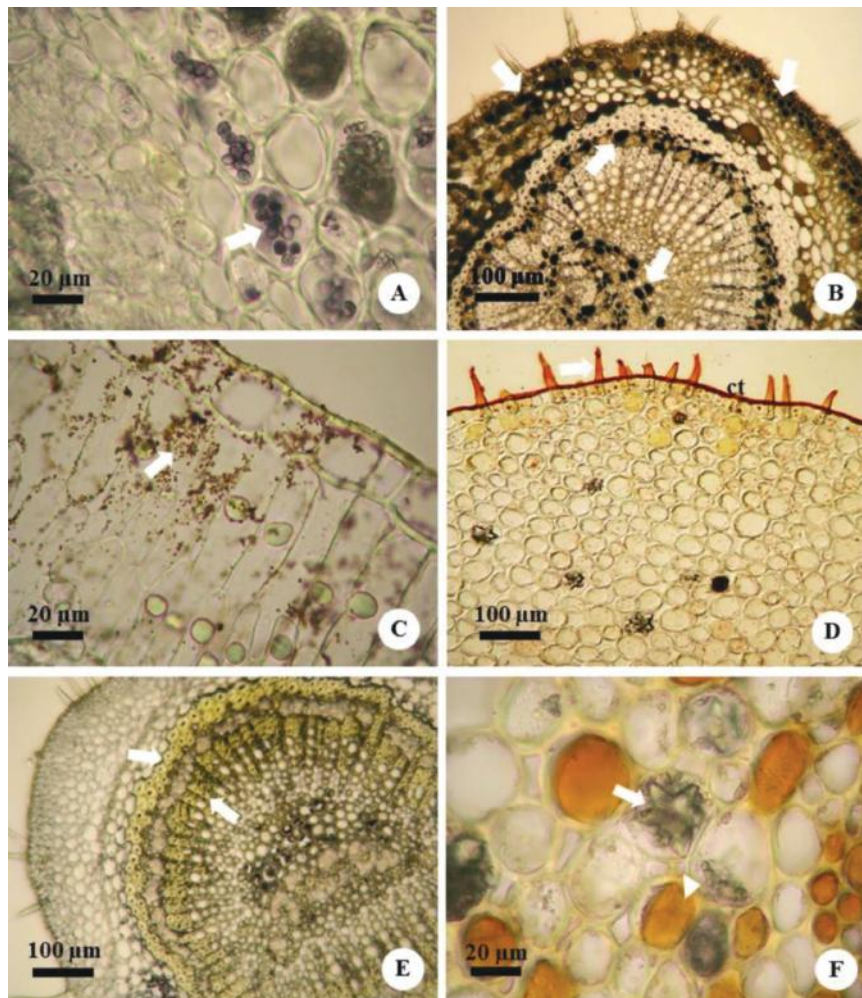
Figure 4. Photomicrographs of histochemical characteristics of *Cordiera sessilis*. (A) Presence of starch sheath (arrow) in the petiole; (B) Phenolic compounds in the midrib (arrow); (C) Alkaloids (arrow) in the mesophyll; (D) Lipid substances in the epidermal cells and on the surface of trichomes (arrow) in the petiole, (E) Lignin in the midrib (arrow); (F) Idioblasts with druses (arrow) and clusters of single crystals (arrowhead) in the petiole. ct = cutin.

Features such as one-layered epidermis, thick cuticle and dorsiventral mesophyll were also described for the leaves of *Alibertia myrcifolia* (Silva, Alves, & Braga, 2004). However, the epidermal cells in this species are rectangular, with a thicker cuticle on the adaxial surface. The dorsiventral mesophyll is reported often for Rubiaceae species and cylindrical vascular bundles described for *C. sessilis* were also observed in *Bathysa stipulate* (Nascimento, Gomes, & Vieira, 1996). The midrib of the leaves of *C. sessilis* showed very similar characteristics to *Alibertia myrcifolia* where the phloem spreads concentrically to the xylem and presence of phloem is observed in the medullary region (Silva et al., 2004).

The presence of crystals on the leaf and petiole tissues is well described for Rubiaceae and may be used as a taxonomic characteristic (Raman & Horner, 2014; Moraes et al., 2009). They include crystal sand, raphides, druses and styloids. According to Evert (2013), the calcium oxalate crystals are insoluble salts formed from the combination of oxalic acid resulting from the metabolism of the plant with calcium salts from soil. The presence of small crystals in higher plants is related to physical protection, removal of oxalate from the metabolic system, storage of calcium, and regulation of light during photosynthesis in plants that grow in the shade (Moraes et al., 2011). They also promote the defenses against attacks by herbivores (Mantovani et al., 1995). Calcium oxalate crystals are of great importance in plant diagnosis. The shape of crystals, their location and their frequency in certain plant organs are singular elements for the identification of the plant. For instance, the presence of crystal sand in leaf mesophyll helps to identify *Rondeletia* species (Kocsis et al., 2004); druses may be used to identify *Genipa americana* (Erbano & Duarte, 2010) and raphides in leaf mesophyll and cortical parenchyma

of petiole were observed in *Palicourea longepedunculata* (Pereira et al., 2003). Andersson and Antonielli (2005) reported that the presence of raphides-type crystals is common in species of Rubioideae subfamily and the absence of this type and presence of druses and/or crystal sand characterize species of Cinchonoideae, subfamily that belongs the *C. sessilis*. Thus, this feature may be considered as an important diagnostic characteristic for *C. sessilis*.

The arrangement of the vascular system in the petiole and the midvein are useful in the diagnosis of some plant species. Their morphological patterns have been established for taxonomic purposes when dealing with several plant families (Arruda, Gomes, Azevedo, Magalhães, & Gomes, 2010). According to Martínez-Cabrera, Terrazas, and Ocholerena (2009), the organization of the vascular system have a higher taxonomic value in Rubiaceae. The vascular tissues in the leaves of many Rubiaceae may be arranged in U, O or V-shapes (Kocsis et al., 2004).

The structural organization of the petiole of *C. sessilis* is similar to that described for *Genipa americana* (Erbano & Duarte, 2010) and *Gardenia spathulifolia* (Metcalfe & Chalk, 1950), particularly with regard to the occurrence of accessorial lateral bundles. However, the presence of accessorial lateral bundles are not important for the Rubiaceae family from the taxonomic point of view because many species exhibit it (Kocsis et al., 2004). According to Metcalfe and Chalk (1950), Martínez-Cabrera et al., (2009) and Moraes et al. (2011), the petiole has a considerable taxonomic importance because it is not heavily influenced by environmental changes. In *C. sessilis*, the organization of vascular bundles in arc is similar to *Palicourea longepedunculata* (Pereira et al., 2003), *Psychotria viridis* (Quinteiro, Teixeira, Moraes, & Silva, 2006) and several representatives of *Rondeletia* analyzed by Kocsis et al. (2004). Petiole with circular shape consisting of collateral vascular bundles arranged in U-shape was also observed for *Psychotria viridis* (Quinteiro et al., 2006), *Tocoyena bullata* (Vieira, 1986/88) and *Rudgea macrophylla* (Mantovani et al., 1995). This result suggests that the organization of the vascular system in a U-shaped arc, curved ends of the primary vein, has not diagnostic value for *C. sessilis*.

Idioblasts containing phenolic compounds and crystals are frequent in Cerrado species (Bieras & Sajo, 2009). According to Varanda, Ricci, and Brasil (1998), plants growing on poor soils, like those of the savanna, usually deviate the biosynthetic ways to produce defense compounds, such as phenolic compounds and alkaloids, due to the high cost to replace the material lost by activities of herbivores. The alkaloids identified in mesophyll cells through the positive reaction to the Dragendorff reagent were also observed in *Simira* and *Psychotria* species (Moraes et al., 2009, 2011). However, in the case of the *Simira* species, the alkaloids were identified in mesophyll cells and in *Psychotria* the metabolite also appears in the parenchyma and epidermal cell of the midrib. Indolic, quinolinic and isoquinolinic alkaloids were reported by Robbrecht (1988) in tribes of Rubiaceae. However, further phytochemical studies are necessary to determine the chemical structures of these alkaloids in *C. sessilis*.

Conclusion

The presence of druses and crystal sand, the absence of raphids and the presence of alkaloids may be of taxonomic relevance for *C. sessilis*. Cuticle ornamentation, epicuticular wax type, the pattern of anticlinal cell walls, and the presence or absence of trichomes, as well as the type and localization of crystals may be used to identify the *C. sessilis*. However, these characteristics should be considered together since singly they have low specific diagnosis value, due to their occurrence in different species of the family. The results encourages an in-depth comparative study of the *Cordiera* genus for provide support for identification.

Acknowledgements

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