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Original Article

Morpho-anatomy of the leaf of *Myrciaria glomerata*

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ABSTRACT

Myrciaria glomerata O. Berg., Myrtaceae, popularly known as "cabeludinha", has high content of ascorbic acid and anti-inflammatory property and is used in folk medicine. The objectives of this study were the morphological, anatomical and histochemical characterization of the leaves. Leaf studies were made with optical, scanning electron and confocal microscopy. The collection of botanical material was held at the Tijuca Forest, Rio de Janeiro, RJ. Histochemical tests aimed the identification of lipids, starch grains, phenolic compounds and crystals. The leaves are simple, opposite, lanceolate, pinnate, hairy, with involute margins, hypostomatic and dorsiventral. The stomata are anomocytic. The epidermis presents simple trichomes. Epidermal cells show uneven thickening of their periclinal outer walls, mainly on the adaxial side of the leaf. Secretory cavities of essential oils are subepidermal and exceed, in height, the palisade parenchyma, formed by one cell layer. Four to five cellular layers, rich in phenolic compounds and lipids form the spongy parenchyma. The bundles are collateral and there are many crystals of calcium oxalate spread throughout the mesophyll. In the midrib and petiole the bundles are bicollateral. Analysis by scanning electron revealed epicuticular wax rod-shaped and as grains. In confocal microscopy, the adaxial epidermis, the fibers and the secretory epithelium of the cavities show autofluorescence. The data obtained are important in quality control exams of samples of this species.

Introduction

Contemporary therapeutic uses drugs obtained from various sources, which may be synthetic or natural. Natural sources are plants, animals or mineral (Kviecinski, 2013). Since the beginning of humanity, man has used plants for relief and healing to many diseases, thus constituting one of the oldest forms of medical practices (Serafin, 2006; Fischer, 2007; Salvagini et al., 2008; Lopes, 2008; Niehues et al., 2011). The use of medicinal plants has been encouraged by the World Health Organization since it realized that about 80% of the population turns to the popular culture to get rid of diseases (Salvagini et al., 2008; Niehues et al., 2011). Many factors have collaborated in the development of health practices using medicinal plants, including economic and social.

The allopathic therapy showed to be effective against various diseases, but in many, it appears a low survival time, as in the case of lung cancer. Several therapies are associated in order to reach better results (Begalli, 2013), such as chemotherapy, which is the use of drugs that aim to destroy the cancerous cells by blocking their development. According to Mesquita (2009), the majority of these medicines are obtained from plants, microorganisms and marine organisms in which there is a wide variety of compounds that act by different mechanisms.

In this sense, the use of plants with medicinal properties as extracts, essential oils and phytochemical have grown in importance in the current therapy (Franco et al., 2005; Pereira et al., 2006; Mesquita, 2009; Kviecinski, 2013).

The object of this study was *Myrciaria glomerata* O. Berg., popularly known as "cabeludinha". It belongs to Myrtaceae family, which stands out in the plant kingdom because presents a wide range of species with medicinal potential (Fevereiro, 1996). Besides the high ascorbic acid content in *M. glomerata* (Malavolta et al., 1956) and positive antimicrobial property (Serafin et al., 2007), recent studies are highlighting its analgesic properties, which would be much more powerful than the leaders of market in this category (Fischer et al., 2008; John, 2010). In this scenario, the use of *M. glomerata* as a natural drug is highly encouraged. This plant is also known by the synonyms *Plinia glomerata*, *Eugenia cabelluda* and *Eugenia tomentosa* (Serafin et al., 2007; Fischer et al., 2008).

The objectives of this work were to study the morphological and anatomical characteristics of the leaves of *Myrciaria glomerata* to subsidize quality control tests.

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Material and methods

Morphological and anatomical study

The material studied was collected in the Tijuca Forest, Estrada das Furnas, n° 1984, Alto da Boa Vista (collection permit ICMBIO 43972-1) inserted in the Atlantic Forest, which is characterized by having high rainfall and mild temperatures. Machado (1992) refers to the forest of the Tijuca National Park as dense tropical rain forest. *Myrciaria glomerata* O. Berg., Myrtaceae, has approximately 3–4 m in height and 10 cm in diameter. The identification of the material was performed by Carlos Alberto Leal de Oliveira and confirmed in comparative analysis at the Herbarium Alberto Castellanos – GUA. Exsicatas have been deposited in the Herbarium of the University of the State of Rio de Janeiro under the registration number HRJ 012450.

The morphological study was held by examining the samples with naked eye and a ruler was used to make the measurements of the leaf blade and petiole. Thirty leaves were used and the arithmetic mean was calculated, considering the width and length of the blade and the length of petiole. The venation pattern was established based on Oliveira and Akisue (1989).

The anatomical study was performed using fully developed leaves from the 3rd to 5th node obtained in the periphery of the crown, which is well exposed to light. The samples were fixed in FAA 70 and stored in 70% alcohol (Johansen, 1940). Histological slides were made by free hand sections from the median third of the leaf blade and petiole, according to the usual techniques (Kraus and Arduin, 1997). Epidermal fragments were removed for surface analysis, using Jeffrey's solution (Johansen, 1940). The stain was a mixture of safranin and astra blue (Bukatsh, 1972).

Histochemical tests to highlight the occurrence of starch grains, lipids and phenolic compounds were performed in fresh sections of the material, using lugol, Sudan IV and solution of iron salts, respectively (Johansen, 1940). The chemical nature of the crystals was analyzed by its solubility in acids (Howarth and Warne, 1959).

The registration of anatomical sections was held in photomicrographs obtained with the aid of an optical photomicroscope Primo Star Zeiss coupled to a computer. Slides used for the optical microscopy (OM) were also analyzed and photographed in confocal microscope (CM) Zeiss 510 META with ZEN 2009 software.

For examination with JEOL scanning electron microscope (SEM), leaf fragments were dehydrated in ascending ethanol series, taken

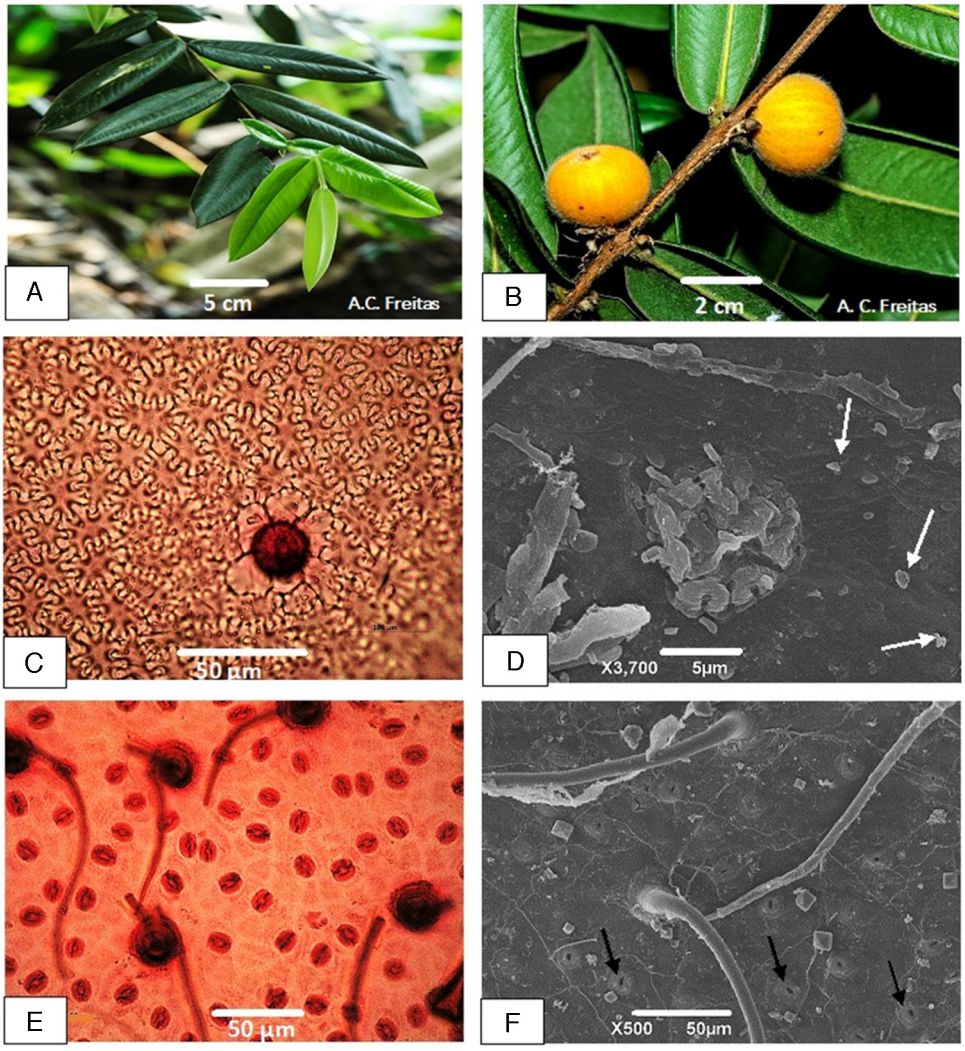

Fig. 1. *Myrciaria glomerata* – (A) Branch with opposite leaves; (B) Detail of the abaxial side of the leaves and ripe fruits. Note the incurved margins. (C) Epidermal surface of the adaxial side of the leaf in OM. Note the intense sinuosity of anticlinal walls and the cover cells of the secretory cavity (darker color). (D) Surface of the adaxial side of the leaf in SEM. Note the region of the secretory cavity (clustered) and the granules of epicuticular wax (white arrows). (E) Epidermal surface of the abaxial side of the leaf, in OM, showing stomata and trichomes. (F) Epidermal surface of the abaxial side of the leaf in SEM. Note the simple trichomes, stomata (black arrow) and wax in the form of prismatic crystals and grains.

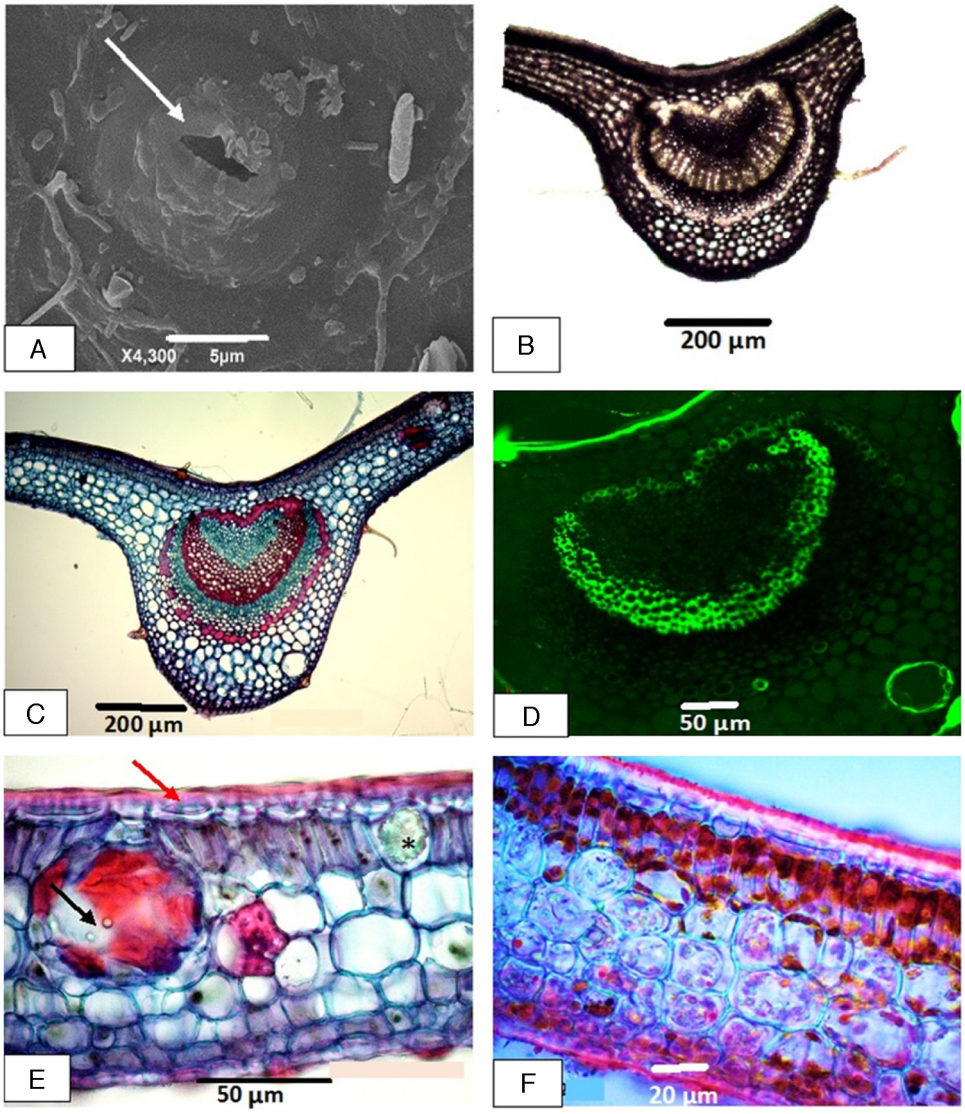

Fig. 2. *Myrciaria glomerata* – (A) Detail of the abaxial surface in SEM. Stomata (white arrow), epicuticular wax rod-shaped and as grains. (B) Cross section of midrib. Histochemical test for phenolic compounds. Positive reaction on darker cells. (C) Cross section of midrib stained with astra blue and safranin. Note the vascular system. (D) Confocal microscopy, showing the midrib. It is visible the autofluorescence of the fibers, cuticle and the epithelium of the secretory cavity. (E) Cross section of leaf blade, showing the dorsiventral mesophyll. Note the wall projections on the epidermal cells (red arrow), the secretory cavity (black arrow) and a druse (*). (F) Positive histochemical test of lipids in leaf blade (droplets and cuticle red colored).

to the critical point, using the CPD-30 dryer, and then, plated with gold.

Results

Morphology

The leaves of *M. glomerata* are simple, opposite, lanceolate, pinnate with a collecting marginal vein. The texture is chartaceous. The color is bright dark green on the adaxial surface and mate light green on the abaxial one. This side is strongly hairy. The median rib is prominent on the abaxial surface and the edges of the leaves are curved down (Fig. 1A and B). The blade measures 3–4 cm wide and 9–11 cm long. The petiole is 1 mm long and is twisted.

Leaf anatomy

The leaf of *M. glomerata* is hypostomatic and dorsiventral. The epidermis is uniseriate and presents significant thickening of the cuticle. The mesophyll has oil secretory cavities and calcium oxalate crystals in the form of druses.

In surface view, the adaxial cells of the leaf present markedly sinuous walls (Fig. 1C). The epidermal regions located over the subepidermal secretory cavities can be recognized by the pairs of cover cells or by the single one with spherical contour (Fig. 1C). The adaxial surface observed with SEM reveals granules of epicuticular wax and the aspect of a cover region of a secretory cavity (Fig. 1D). The thickness of the outer walls of the epidermal cells is uneven. Examining the epidermal surface, with OM, this cell characteristic is detected by bright and darker regions that alternate in the periphery of the periclinal walls. In cross section, this irregularity of the thickness is noted by numerous projections of the walls toward the cell (Fig. 2E). This feature also occurs in the cells of the abaxial epidermis, but in an attenuated mode. The abaxial side of the epidermis has simple and long trichomes (Fig. 1E and F) and anomocytic stomata. In SEM it is possible to note the epicuticular wax as grains and rod-shaped and the stomata (Fig. 2A). In CM, the autofluorescence of the adaxial side of the leaf blade becomes visible (Figs. 2D and 3B).

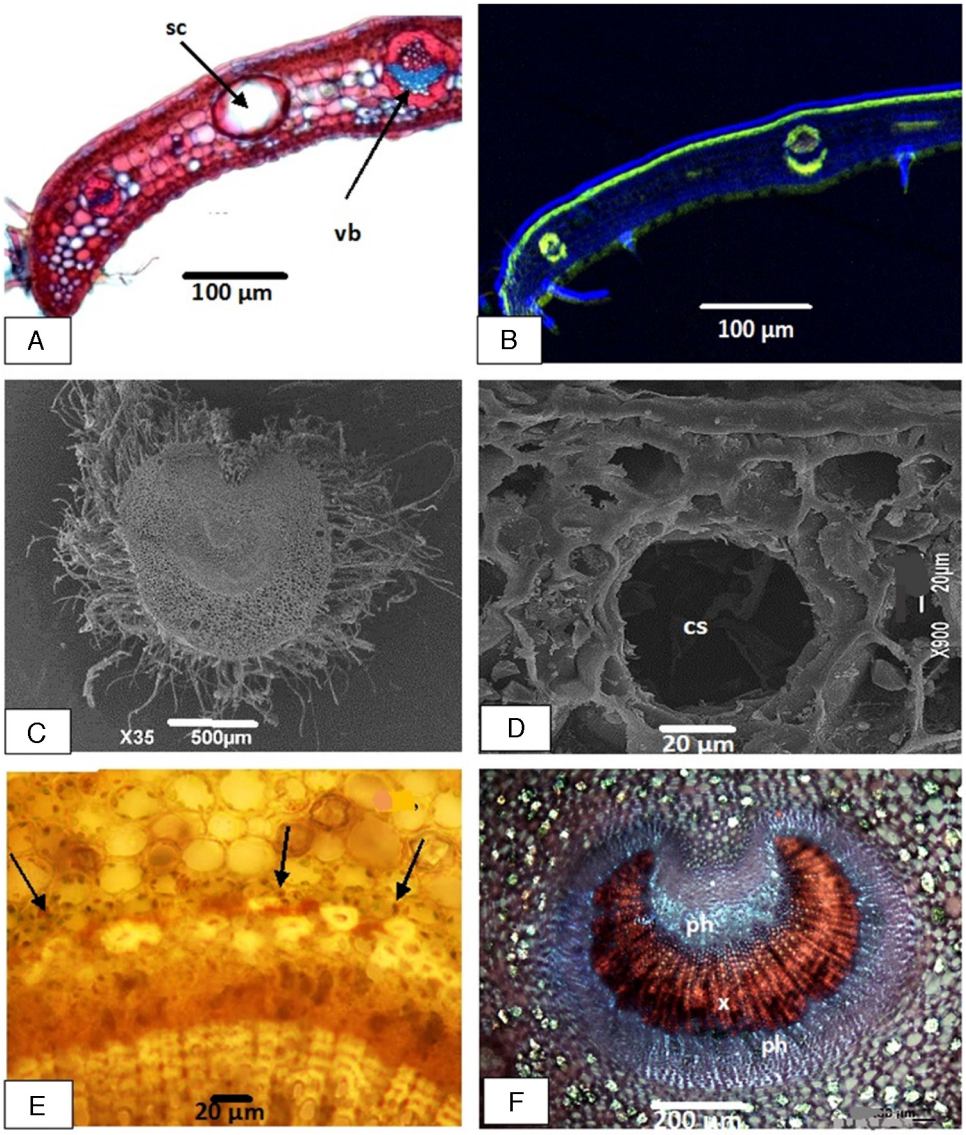

Fig. 3. *Myrciaria glomerata* – (A) cross section of leaf margin: sc = secretory cavity; vb = vascular bundle. (B) Idem, in confocal microscopy. Note the autofluorescence of the fibers and adaxial epidermis. (C) Cross section of the petiole in SEM. (D) Detail of cortical region of petiole: sc = secretory cavity. (E) Positive histochemical test of starch grains in the petiole (arrows). (F) Middle region of petiole, in polarized light, showing druses in parenchymatous cells (bright) and vascular system: x = xylem; ph = phloem.

The midrib, in cross section, shows a slight concavity in the adaxial side and is convex in the abaxial surface. The epidermal cells are smaller than those of the remaining leaf blade. There are 2–3 layers of collenchyma in subepidermal position, interrupting the row of palisade parenchyma present all over the extension of the leaf blade. Centraly the vascular system presents phloem on both sides of xylem, surrounded by fibers (Fig. 2C). In the dorsal region of the midrib occur about six parenchyma cell layers. Histochemical tests reveal a strong positive reaction to phenolic compounds (Fig. 2B) and lipids. In CM the fluorescence of the epithelium of the secretory cavities can be observed, and, in addition, the fibers and cuticle (Fig. 2D).

The leaf blade is dorsiventral with a single layer of palisade (Fig. 2E and F), which is interrupted by secretory cavities whose dimensions far exceed the height of this parenchyma, approaching the abaxial surface (Figs. 2E and 3A). The spongy parenchyma is formed for about 4 cell layers. Calcium oxalate druses occur all over the mesophyll. The bundles are collateral surrounded by fibers. The histochemical tests denoted plenty of phenolic compounds throughout the mesophyll. In addition, oil droplets were detected in the epidermal and parenchymatous cells (Fig. 2F) and in the secretion of the secretory cavities. Starch grains were detected in the parenchyma.

The leaf margin is curved toward the abaxial surface (Fig. 3A and B) and presents anatomical features similar to those described for the leaf blade. At the edge there is mechanical tissue. With CM, the autofluorescence of the perivascular fibers and of the cuticle is highlighted (Fig. 3B).

The petiole, in its middle region, has an approximately circular shape. The epidermis is simple and presents many trichomes (Fig. 3C). The central portion of the petiole is formed by the vascular system, which is constituted by an arc shaped bundle (Fig. 3F). The xylem elements are arranged in rows and are completely surrounded by the phloem. The cortical region is formed by 16–20 layers of compact parenchymatous cells whose walls show significant thickening. In the cortical region there are secretory cavities (Fig. 3D) and many druses of calcium oxalate. Starch grains were detected in the periphery of the vascular system (Fig. 3E).

Discussion and conclusions

The great hairiness of *Myrciaria glomerata* leaves gives a velvety texture that helps in species identification in the field. The dimensions and shape of the blade, besides the typical opposite phyllotaxy also contribute to the recognition of the species.

Histological study of *M. glomerata* leaf show several features highlighted by Solereder (1908), Metcalfe and Chalk (1950), Fiuza et al. (2008), Döll-Boscardin et al. (2010), Nunes and Martins (2010), as representative of the Myrtaceae family, for example the presence of secretory cavities, located below the epidermis, producing essential oils. The secretion of these cavities has a complex nature because beyond the lipids, there are, in addition, phenolic compounds. Essential oils, according to Gotlieb and Salatino (1987), are formed by a large number of substances whose biodynamic activity can lead to drug discovery. These authors state, further, that the presence of defined anatomical structures as secretory cells, secretory cavities and glandular trichomes are more important for the recognition of essential oils than the odor itself, since other plant materials can also be aromatic. Lipid droplets occur all over the leaf blade of *M. glomerata*, as well as starch grains and phenolic compounds, these, in large amounts. Serafin et al. (2007) make a link between the phenolic compounds and the analgesic potential of the species. Costa (1986) states that the phenolic compounds are very abundant in dicotyledons, particularly in some families, citing, among these, Myrtaceae. Other important anatomical features, confirmed in *M. glomerata*, are the internal phloem and the anomocytic stomata restricted to the abaxial face of the leaf (Defaveri et al., 2011; Armstrong et al., 2012).

The mesophyll of *M. glomerata* resembles that species of Myrtaceae as *Syzygium cumini* (Nunes and Martins, 2010), *Eugenia umbeliflora* (Medeiros, 2000), *Eugenia uniflora* (Alves et al., 2008; Fiuza et al., 2008), *Eugenia brasiliensis* (Donato and Morretes, 2007), *Campomanesia adamantium*, *Myrcia cordiifolia*, *Myrcia decrescens*, *Myrcia torta* (Gomes et al., 2009). The mesophyll of *M. glomerata* is dorsiventral and the palisade parenchyma has one single layer interrupted by secretory cavities, as are very common in Myrtaceae (Sudgen, 1985; Jorge et al., 2000). However, *M. glomerata* differs from the others species by the large dimensions of the secretory cavities, which can go far beyond the height of palisade parenchyma, approaching the abaxial face. The spongy parenchyma has about four cell layers, while in *Psidium widgrenianum*, occur about seven layers (Donato and Morretes, 2005) and in *Eugenia brasiliensis* there are, approximately, nine layers (Donato and Morretes, 2007). The organization of the median rib of *M. glomerata* is similar to the others species of Myrtaceae, i.e., it is of bicollateral type surrounded by sclerenchyma fibers, resembling an arc (Machado et al., 1988; Donato and Morretes, 2005, 2007; Fiuza et al., 2008; Döll-Boscardin et al., 2010; Nunes and Martins, 2010). Other species show the vascular system of the midrib as an arc very open, almost flat, as in *C. adamantium* or, in addition, as an arc strongly closed as in three species of *Myrcia* (Gomes et al., 2009).

Regarding the epidermis, it is noteworthy the intracellular wall projections that occur at the external periclinal walls of the adaxial cells, which greatly increase its surface. This feature was recorded in several species of the Myrtaceae family, such as some *Gomidesia* (Fontenelle et al., 1994), *Myrcia* (Gomes et al., 2009) and *Eugenia* (Machado et al., 1988; Fontenelle et al., 1994; Donato and Morretes, 2007) and possibly correlates with the transport of essential oils that are released to the atmosphere through the coverage cells of the secretory cavities. This inference arises from the analogy between this feature and that named by Gunning and Pate (1969) as "wall ingrowths" when these authors described the transfer cells associated to the transport of solutes within short distances. Analysis of the leaf surface reveals whether the anticlinal walls of epidermal cells are straight or wavy, noting that in *M. glomerata* the walls are winding as well as in *Myrcia cordifolia*, *M. decrescens*, *M. guianensis* and *M. racemosa* (Gomes et al., 2009). The epidermal cells located up the secretory cavities are also important in the set of diagnostic features. In *M. glomerata* these regions of the epidermis can be recognized by a rounded central cell which is surrounded by 5–7 cells disposed radially or by a single pair of larger cells. The appearance of the coverage cells of the secretory cavities is variable, in shape and amount of cells, within the species of Myrtaceae. Gomes et al. (2009) refer that the presence of pairs of cells on the top of the secretory cavities is quite common as can be seen in *M. decrescens* and *M. torta*.

No reference was found in the literature about the autofluorescence observed in the present study, in the cuticle, in the fibers and in the epithelium of the secretory cavities. Probably, this property can be associated with the typical waterproofing of these structures. This idea is supported by Ascenção (2007) that states that the walls of oil cells contain suberin, so that the secretion produced becomes isolated, protecting the adjacent cells of the toxic components.

The morphological and anatomical characteristics contribute to the identification of this species and support quality control tests. Some features may be standed out as hairy leaves with curved edges; epidermal cells with ripples and wall projections and, in addition, the secretory cavities with dimensions that go beyond the height of palisade parenchyma.

Authors' contributions

NVPS assisted in collecting and identifying plant samples, prepared the exsiccates, was responsible for preparing the slides, data analysis and drafting the paper. AMD drawed the experiment, conducted and supervised laboratory experiments, data analysis, microscopy and drafted the paper. Both authors undertook a critical reading of the manuscript and agreed to its submission for appraisal.

Conflicts of interest

The authors declare no conflicts of interest.

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