

Plinia edulis - leaf architecture and scanning electron micrographs

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Article

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Abstract: Many species of Myrtaceae, including *Plinia edulis* (Vell.) Sobral (cambucá), have pharmacological properties and are used as hypoglycemiants and therapeutic agents against stomach problems and throat infections. Samples were collected from Tijuca Forest in Rio de Janeiro, and the morpho-anatomical data were compared with other specimens obtained from Trindade, Paraty, found in the literature. Variations in leaf anatomy were observed, and the possible causes for these effects are discussed. The plant material collected from Tijuca Forest was analyzed using scanning electron and optical microscopy. Histochemical tests were applied to identify starch, lipids, phenolic compounds and lignin. The epidermal cells exhibit straight or slightly sinuous anticlinal walls covered by a smooth cuticle with granules of wax. Simple trichomes are restricted to the midrib region, and paracytic stomata are only observed on the abaxial leaf surface. The mesophyll is dorsiventral, with conspicuous intercellular spaces in the spongy parenchyma. Intercalated columns of crystalliferous cells and subepidermal secretory cavities are observed in the single layer of palisade parenchyma. The samples obtained from Trindade, Paraty, show larger leaves, anomocytic stomata and trichomes scattered throughout the leaf surface. This plasticity might reflect leaf adaptations to environmental factors or different stages of leaf development.

Introduction

Myrtaceae is one of the most representative families of the Brazilian flora, particularly in the Atlantic Forest and Restingas (Mori et al., 1983; Barroso & Peron, 1994; Fabris & Cesar, 1996; Souza & Lorenzi, 2005). This family includes trees or shrubs with simple, opposite leaves, with oil glands, which appear as translucent points, and entire margins. Menezes-de-Lima Jr. et al. (1997) showed that several species of Myrtaceae exhibit significant anti-inflammatory activity associated with the essential oils produced in these plants. Siani & Branquinho (1997) described the essential oils of a plant as defensive chemical reagents against herbivorous predators and microorganisms. According to Souza et al. (1998), these oils have a broad spectrum of biological effects, including anticancer, anti-malarial, anti-inflammatory, antiviral and microbicidal activity. Bragança (1996) and Maciel & Cardoso (2003) reported that the leaves of *Plinia edulis* (Vell.) Sobral, known as “cambucá,” exhibit anti-diabetic activity and are useful for the treatment of stomach problems and throat infections. Cruz (1995)

referred to the delicious fruit of this species as boosters. The ripe fruits are yellow and represent an important food source for wildlife. The flowering period is short, from approximately 7-12 days, starting in mid-December to late January, followed by fructification from mid-January until early March. The flowers grow directly on the stem (Figure 1B), and the trunk of this plant is typically “marbled” due to the exfoliation of the rhytidome, which gives cambucá a distinctive and ornamental quality (Figure 1A). The morpho-anatomical aspects of this species, collected from Tijuca Forest, have been previously described (Donato, 2003). In addition, Ishikawa et al. (2008) described the anatomy of cambucá based on samples obtained from Trindade, Paraty. The aim of the present study is to analyze the foliar structure of *P. edulis* in order to contribute to the knowledge concerning this medicinal plant. According to Farmacopeia Brasileira (2010), “the identity, purity and quality of a plant material should be established through detailed macroscopic and microscopic examination”; therefore, any variations in the leaf structure of *P. edulis* must be considered in diagnostic studies of this species.

Materials and Methods

Plant material

The leaves of *Plinia edulis* (Vell.) Sobral, Myrtaceae, were collected at Vista Chinesa Road, km 2, Tijuca Forest, Rio de Janeiro City, Rio de Janeiro State, Brazil (ca. 22 ° S, 43 ° W and 330 m elevation). This area is part of the Atlantic Forest and is characterized by mild temperatures. *P. edulis* typically grows under conditions of light partially filtered through the upper canopy. Machado (1992) referred to this vegetation as a dense, shadowed forest. The collected plant material was identified by comparison with herbarium specimens at the Alberto Castellanos Herbarium (GUA). Samples of the botanical material, with flowers, were used to prepare exsiccates and deposited in the Herbarium of the University of São Paulo, USP (SPF 155.532), and the Herbarium of the University of the State of Rio de Janeiro, UERJ (HRJ 9.940).

The description presented in the present study was based on leaves collected from five plants during the vegetative stage at Tijuca Forest. Light could reach the inside of the crown in a similar intensity because the leaves were not too congested and thus, no separation between sun and shade leaves was made.

Assays

For scanning electron microscopy (SEM), the leaf fragments were dehydrated twice in an increasing ethanol series and subsequently subjected to critical-point drying using a CPD-30 dryer. The samples were coated with gold for examination and photographic registry using SEM.

The leaf shape was determined through a comparison with the models of Oliveira & Akissue (1989). Whole leaves were clarified and stained using the technique of Foster (1949), and the venation pattern was based on Hickey (1979).

For the anatomical analysis, completely developed leaves, from the third to fifth node, were fixed in FAA 70° for 48 h and subsequently stored in ethanol 70° GL (Johansen, 1940). To prepare the semi-permanent slides, transverse freehand sections were produced and processed according to typical plant anatomy methods using astra blue and safranin stains (Bukatsch, 1972). Epidermal peels were obtained through maceration in Jeffrey's solution and stained with safranin (Johansen, 1940). The anatomic sections were recorded using an Axiostar Plus Zeiss photomicroscope equipped with the Axiovision 4.5 software. The diagrams were obtained using a stereoscopic microscope (Zeiss, model Stemi SV6) equipped with a camera lucida.

The following reagent solutions were used for the histochemical tests: ruthenium red for pectic compounds, potassium hydroxide for suberin, ferrous

sulfate for phenolic compounds, Lugol's solution for starch (Johansen, 1940), Sudan III for oil (Sass, 1951) and phloroglucin for lignin (Foster, 1949). The chemical nature of the crystals was analyzed using acid solubility tests (Howarth & Warne, 1959).

Results

Macroscopic diagnosis

The leaves are opposite, simple and lanceolate to oblong-lanceolate, with entire margins, a chartaceous texture and an acuminate base and apex (Figure 1C). The foliar blade is 9-12 cm long and 2.7-3.5 cm wide. The petiole is velvety in texture, measuring 1.2-1.8 cm in length and 1.5-2 mm in diameter. The leaf is dark green on the adaxial side and clearer on the abaxial surface. The venation pattern is camptodromous to brochidodromous, as the secondary veins form connecting arcs without reaching the margin of the leaf. The terminal veins are typically branched in the areoles (Figures 1D, E).

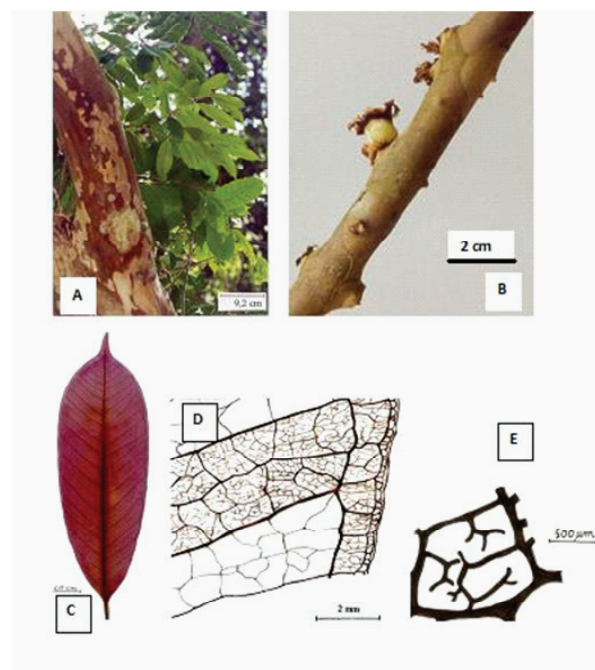


Figure 1. *Plinia edulis* (Vell.) Sobral, Myrtaceae. A. “Marbled” trunk due to the exfoliation of the rhytidome; B. Detail of the flowers growing directly on the stem; C. Clarified leaf stained with safranin; D. Detail of an areola.

Microscopic diagnosis

SEM revealed that the adaxial side of the leaf of *P. edulis* has a smooth cuticle with numerous granules of wax along the entire leaf blade (Figure 2A) and numerous unicellular and long trichomes in the region of the midrib (Figure 2B). Paracytic stomata and several trichomes

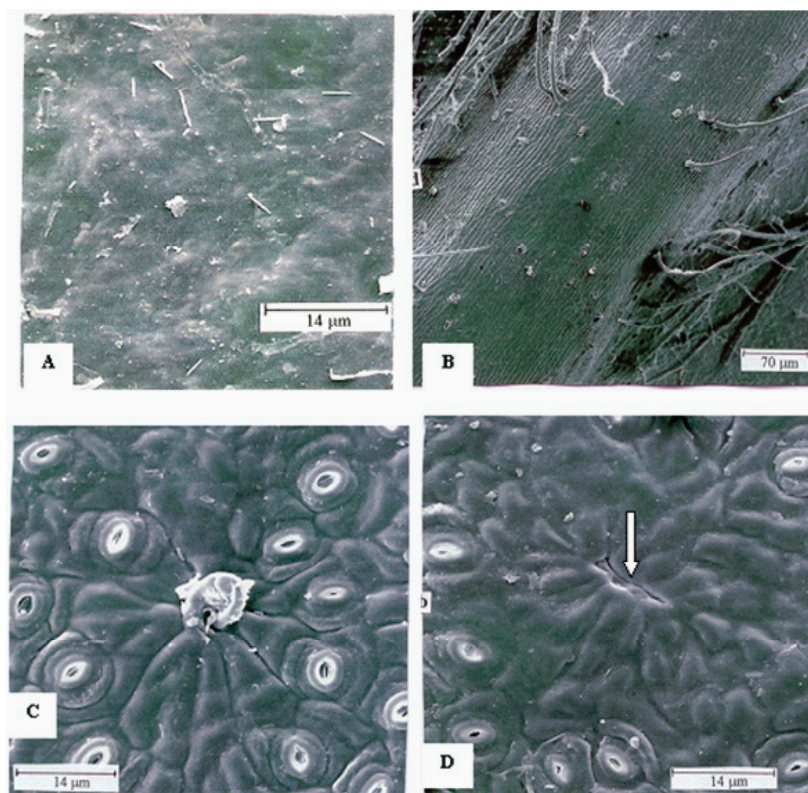


Figure 2. *Plinia edulis* (Vell.) Sobral, Myrtaceae. Scanning electron microscopy. A. Adaxial surface of the leaf blade, in the intercostal region, showing epicuticular wax granules; B. Non-glandular trichomes and scars resulting from abscission are visible on the midrib region; C. Abaxial surface, showing paracytic stomata and other epidermal cells surrounding a trichome scar; D. Top cells of a secretory cavity, showing a median elongated slot (arrow).

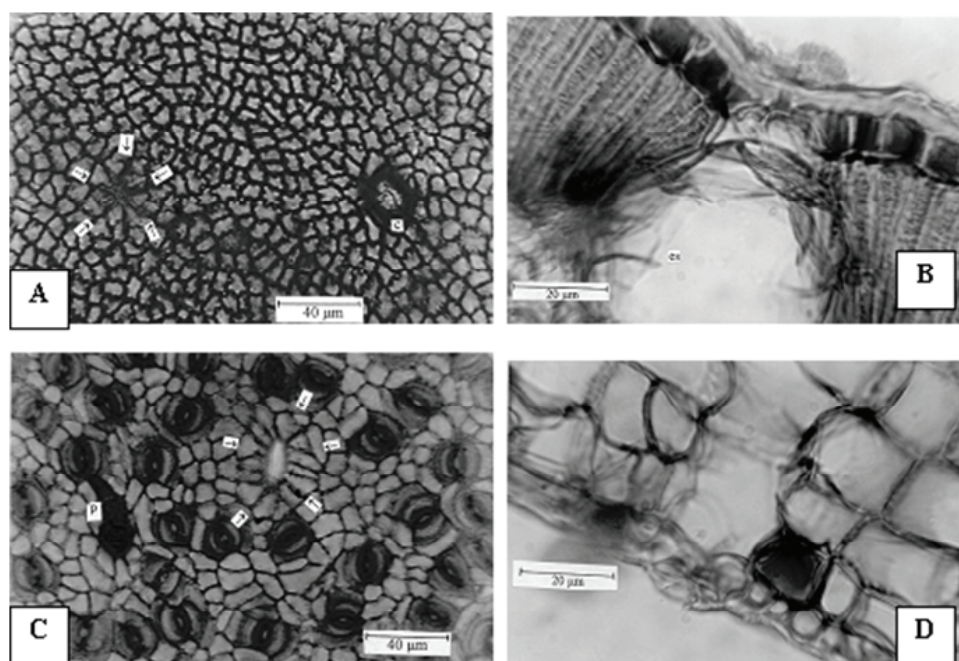


Figure 3. *Plinia edulis* (Vell.) Sobral, Myrtaceae. A. Frontal view of adaxial surface: top cells of secretory cavity (arrows) and a trichome scar (c). B. Adaxial epidermis in transverse section showing part of a secretory cavity interrupting the palisade parenchyma. Notice the secretory epithelium with slightly disaggregated cells; C. Frontal view of the abaxial surface, with numerous paracytic stomata and a region with top cells of the secretory cavity (arrows). D. Transverse section of the abaxial region of the foliar blade showing a stomata placed at the same level of the common epidermal cells.

scars were observed on the abaxial side (Figure 2C). The epidermal cells overhead the secretory cavities exhibit a radial arrangement (Figure 2D).

Optical microscopy (OM) showed that the epidermal cells on the adaxial surface of the leaf, from a frontal view, exhibit a polygonal or slightly sinuous shape with thick walls (Figure 3A). Numerous scars resulting from pre-existing trichomes were detected by the radial arrangement of the surrounding cells and the suberization of the walls. Simple, unicellular, long and persistent trichomes are located on the midrib. Central, elongated epidermal cells were observed on top of the secretory structures surrounded by 7-9 adjacent cells, arranged radially (Figure 3A). In the transverse section, the epidermis is uniseriate, revealing rectangular or square adaxial cells (Figure 3B), and the cuticle exhibits extensions between the anticlinal walls, forming cuticular pegs.

The epidermis of the abaxial surface, from the frontal view, exhibits cells with straight or slightly sinuous anticlinal walls and numerous paracytic stomata

with a density of 720 per mm². The cells on the top of the secretory structures are distinguished by the arrangement of the elements radiating from a central, clearer and elongated cell (Figure 3C). The transverse section showed that the epidermis is uniseriate like the adaxial side, but with smaller cells. Stomata are placed at the same level as the other epidermal cells (Figure 3D).

The mesophyll is dorsiventral (Figure 4A). The palisade parenchyma is unistratified and, in some regions, is interrupted by crystalliferous cells forming columns of a height equaling that of the palisade cells (Figure 4A) and by secretory cavities with disaggregated epithelium cells (Figure 3B). These cavities were also observed in contact with the lower epidermis, and these structures were found to be positive for lipid contents. The spongy parenchyma comprises approximately 9 layers of cells, with conspicuous intercellular spaces (Figure 4A). Some cells exhibit large prismatic crystals of calcium oxalate. The palisade layer is connected to the spongy parenchyma through collecting cells. A parenchymatous sheath surrounds the collateral

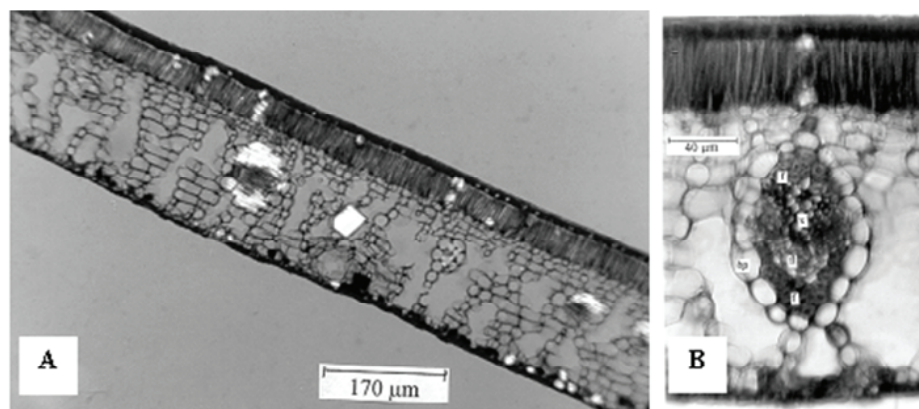


Figure 4. Transverse section of foliar blade of *Plinia edulis* (Vell.) Sobral, Myrtaceae. A. General view, with polarized light, emphasizing the anisotropy of crystals and fibers. Notice the columns of crystalliferous cells intercalated with palisade elements; B. Detail showing a vascular bundle outlined by a parenchymatous sheath and the well-developed intercellular spaces of the spongy parenchyma: f: fibers; x: xylem; fl: phloem; bp: parenchymatous sheath.

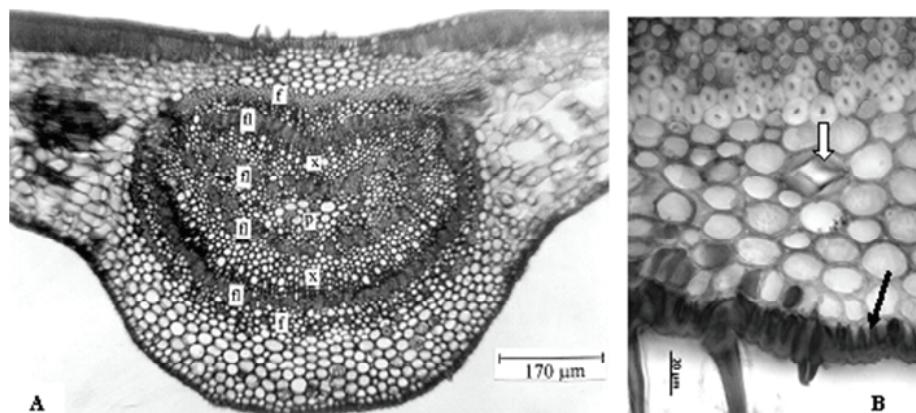


Figure 5. Transverse section of midrib of *Plinia edulis* (Vell.) Sobral, Myrtaceae. A. General view: f: fibers; fl: phloem; x: xylem; p: parenchyma; B. Detail of the abaxial region, emphasizing a crystalliferous idioblast (white arrow) and cuticular flanges on the epidermis (black arrow).

vascular bundles (Figure 4B), and the larger bundles contain internal and external phloem, characterizing the bundle as bicollateral.

The midrib, in the transverse section, is flat on the adaxial side and convex on the abaxial side, with narrower epidermal cells than those of the intercostal regions and deeper cuticular flanges (Figure 5B). Numerous simple, unicellular and non-glandular trichomes are visible in the midrib region. The palisade parenchyma cells are gradually shortened until these cells are replaced by collenchyma in the middle region of the midrib (Figure 5A). Several prismatic crystals were observed in the cells on the abaxial side (Figure 5B). Two arcs of xylem, each with internal and external phloem, delimiting the parenchymatous pith, form the vascular system. Fibers surround the entire vascular system.

The leaf margin is slightly curved toward the abaxial surface. The epidermal cells are gradually reduced, and the cuticular pegs are deepened. Collenchyma replaces the palisade parenchyma in the distal portion, and a small vascular bundle was observed in a nearby region (Figure 6).

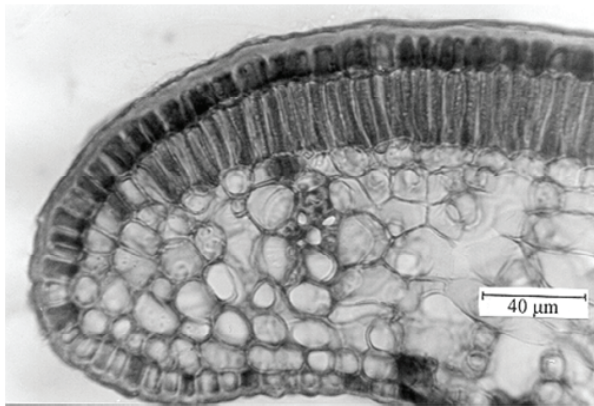


Figure 6. Transverse section of leaf margin of *Plinia edulis* (Vell.) Sobral, Myrtaceae. Notice the gradual shortening of palisade parenchyma cells until replacement with collenchyma. The cuticular flanges and vascular bundle are visible.

The petiole, in the transverse section, is rounded, with slight flattening on the adaxial side (Figure 7A). The epidermis is uniseriate and covered with a thickened cuticle. Numerous non-glandular, unicellular simple trichomes are distributed over the entire surface of the petiole (Figures 7A, B). The cortex comprises approximately 13 layers of parenchyma cells with crystalliferous idioblasts. Several secretory cavities were observed below the epidermis (Figures 7A, B), similar to those of the foliar blade. The subepidermal cells stained positively for phenolic compounds. A parenchymatous pith and surrounding vascular system was observed in the middle region of the petiole, whose transverse section resembles an arc,

with the edges sharply curved toward the center (Figure 7C). The walls of the surrounding fibers of the vascular system are not lignified.

Discussion

Herbal drugs must be correctly identified, and microscopic analysis provides further information concerning organoleptic and macroscopic features. However, potential variations in the structure of an organ, particularly the leaves, must be registered and analyzed as characteristics that support the authentication of these drugs.

The comparison between the anatomical data obtained from the samples collected at Tijuca Forest and the description of the material obtained from Trindade, Paraty (Ishikawa et al. 2008), shows different features, such as the width and length of the foliar blade, the type of stomata, the distribution of trichomes and the localization of the secretory cavities.

Measures of the width and length of the foliar laminae in the sample obtained from Paraty were larger than those obtained from Tijuca Forest. The size of the leaves is a characteristic that might vary in response to environmental factors. According to Esau (1977), plants growing in different habitats exhibit structural differences commonly interpreted as evolutionary adaptations to the conditions of the specific habitat. For example, leaves developing in direct sunlight are smaller than leaves developing in the shade. *Plinia edulis* individuals growing in Tijuca Forest can be considered mesophytic plants, but no information concerning the environmental conditions of the specimens collected from Trindade, Paraty is available. Thus, assigning the morphological differences observed between the two populations to their environmental conditions remains an untested hypothesis.

Paracytic stomata were observed in the samples collected at Tijuca Forest, presenting a high density of 720 mm², whereas on leaves of the samples obtained from Trindade, Paraty, the stomata were anomocytic. This difference may reflect a genetic variation in the species. Larcher (2000) stated that a high number of stomata is associated with water stress. The material examined in the present study was collected in a mesophytic area, i.e., the Tijuca Forest; therefore, the high density of stomata can be interpreted as a characteristic that would enable these plants to live in drier habitats as well.

Non-glandular, unicellular simple trichomes are common in Myrtaceae (Barroso et al., 1984). The material investigated in the present study, collected from Tijuca Forest, showed that this trichome type was restricted to the region that overlies the midrib of the *P. edulis* leaf, whereas in the samples from Trindade, Paraty, the trichomes are scattered throughout the entire the leaf surface (Ishikawa et al., 2008). This difference might reflect the stage of

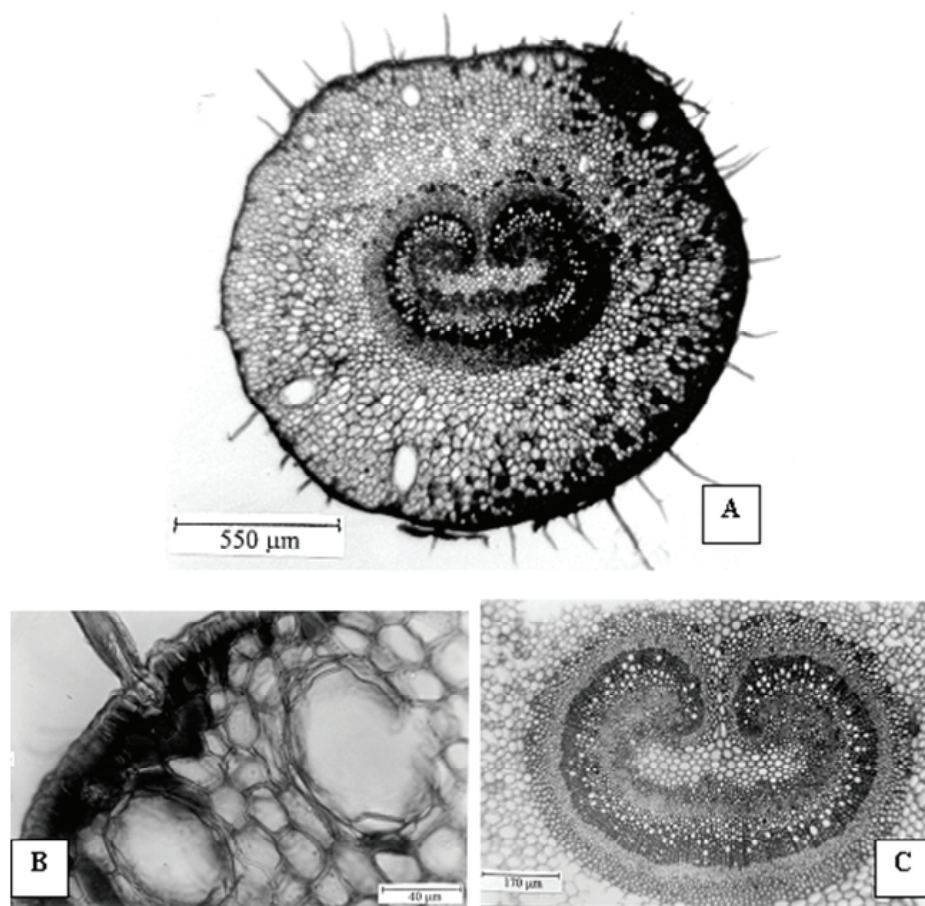


Figure 7. Transverse section of petiole from *Plinia edulis* (Vell.) Sobral, Myrtaceae. A. Overall aspect. The cells of darker color present higher content of phenolic compounds; B. Detail of a peripheral region, showing the epidermis with a non-glandular trichome and subepidermal cells with phenolic compounds; C. Vascular system forming an arc with pronounced curved edges.

development of the leaves because examining a completely developed leaf from Tijuca Forest it is possible to observe many remaining scars from the caducity of trichomes present at juvenile stages.

Ishikawa et al. (2008) reported differences in the oil composition of the specimens collected at Paraty and Porto Alegre (South Brazil) (Apel et al., 2006), suggesting that these differences might reflect distinct preparation procedures, geographical or seasonal variations or, alternatively, might indicate the occurrence of chemotypes. In addition, these differences might indicate different responses of the leaves to environmental factors, particularly light (Esau, 1977; Donato & Morretes, 2007; Moreira et al., 2012), or different stages of leaf development.

The localization of the secretory cavities is typically subepidermal, and the secreted substances are released through epidermal cells located on the apex of the cavity. Ishikawa et al. (2008) reported that these secretory cavities were distributed throughout the leaf lamina. However, these structures are globular, and an anatomical

section obtained away from their middle plan might induce to a misinterpretation of the actual localization of these cavities.

The analysis of the leaf surface of *P. edulis* by SEM revealed a smooth cuticle with epicuticular granular wax. Evert (2006) stated that the wax is a protective coating on the cuticle of the aerial parts of plants that forms a barrier to prevent water loss through the surface and reduces the capacity of the leaves to remain wet, thereby reducing the incidence of fungal spore germination and bacterial growth.

The morphological, histochemical and anatomical study of *P. edulis* confirmed the occurrence of various characteristics previously reported (Solleder, 1908; Metcalfe & Chalk, 1950) as characteristic of the Myrtaceae family, such as the presence of secretory cavities. In *P. edulis*, these cavities are scarce and occur preferentially in the adaxial region of the mesophyll. The material secreted through these cavities comprises lipids, glucose and phenolic compounds. According to Castro & Machado (2006), a secretion containing substances of different

chemical natures is heterogeneous. The epidermal cells of *P. edulis* exhibit well-marked cuticular flanges, reinforcing the intercellular union of the coating system. The external walls of the epidermal cells present internal projections similar to ripples facing the cell lumen. This anatomical feature has previously been reported in other species of the family (Machado et al., 1988; Arruda & Fontenelle, 1994; Fontenelle et al., 1994; Gomes & Neves, 1997; Barros, 1998; Donato & Morretes, 2007; 2009). Considering that the surface of the cell membrane increases when these ripples are present, Evert (2006) suggested that there is also an increase in the flow of substances in these cells. Thus, it is reasonable to conclude that this anatomical feature might improve the release of the essential oils produced in the secretory cavities. The top cells of secretory cavities are also an important distinguishing feature when analyzed from a frontal view. Generally, the top cells occur in pairs with a winding median wall, as in *Eugenia nitida* (Pereira, 1985), *Eugenia uniflora* (Neves & Donato, 1989), *Psidium cattleianum*, *Psidium guajava* (Jorge, 1992) and *Psidium widgrenianum* (Donato & Morretes, 2005). Sometimes, the top region of secretory cavities is characterized by a single cell with a polygonal, elliptical or rounded shape, with the adjacent cells arranged radially. *Eugenia florida* and *Myrcia multiflora* are examples of this case (Donato & Morretes, 2009; 2011). In *P. edulis*, a single top cell was observed, but not significantly different from other epidermal cells except for the lighter color and the arrangement of the surrounding cells.

P. edulis presents a dorsiventral mesophyll, another common feature of Myrtaceae (Solereider, 1908; Metcalfe & Chalk, 1950), and the palisade parenchyma exhibits columns of crystalliferous cells containing prisms or druses of calcium oxalate. Barros et al. (1997) observed the same features for *Plinia martinelli*. Crystals are common in this family, as observed by Cardoso & Sajo (2004), who reported crystalliferous idioblasts in seventeen species of *Eugenia*. The spongy parenchyma of *P. edulis* has remarkable intercellular spaces, consistent with the observation of Ishikawa et al. (2008). Another outstanding feature is the occurrence of internal phloem in the midrib and larger vascular bundles. Solereider (1908), Metcalfe & Chalk (1950) and Costa (1986) also reported the abundance of phenolic compounds as a characteristic of the Myrtaceae family, which was confirmed in the present study. Costa (1986) stated that the phenolic compounds acquire astringent properties through the precipitation of the proteins at the mucosal cell surface, forming protective coatings, reflecting the medicinal properties common to many species of this family.

The transverse section of the petiole of *P. edulis* showed the shape of the vascular system similar to an arc with markedly involuted margins. According to Cardoso & Sajo (2004), the configuration of the vascular system of the petiole has a taxonomic value and might present a contour

like an arc with attenuate or erect margins or, in addition, margins curved toward the middle of the structure, as observed in the material examined in the present study. These authors also suggest that the perivascular sheath of the petiole might be parenchymatous, sclerenchymatic or even mixed, according to the species. *P. edulis* presents a perivascular sheath composed by fibers with non lignified cell walls, consistent with Donato & Morretes (2009), who described the same characteristics for *Eugenia florida*. These fibers are more malleable than those with lignified walls and enable the repositioning of the leaf blade in order to optimize the exposition of this organ to light. The petiole of *P. edulis* exhibits a twisted basal region, suggesting the changing of the leaf position.

In view of all these considerations, it can be said that morphological, anatomical and environmental data provide important information for the diagnosis of medicinal plants.

Authors contributions

Both authors designed the study. AMD contributed in collecting plant samples, confection of herbarium exsiccates, running the laboratory work, analysis of the data and drafted the paper. BLM contributed to data analysis, discussion and to critical reading of the manuscript. Both authors have read the final manuscript and approved the submission.

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