

***Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae): stem and leaf anatomy of a medicinal plant**

***Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae): anatomia do caule e da folha de uma planta medicinal**

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Abstract

The study of medicinal plants involves several areas of science. Anatomy contributes to species identification and consequently, with quality control of plant product. This paper describes the leaf and stem anatomy of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae), collected in Seasonal Semideciduous Forest. The studied organs presented uniseriate epidermis covered by a thick cuticle and secretory cavities. The stem showed a continuous ring of vascular tissues around the pith, with phloem on both sides of the xylem. The leaf was hypostomatic, with trichomes on the abaxial face, with bifacial mesophyll and amphicrival vascular bundle, surrounded by a sclerenchymatous pericycle in the petiole and in the midrib. Among the histochemical tests, positive results were obtained for lipids, phenolic compounds, starch and calcium oxalate (druses). The species had anatomical features typical of the family and the secretory cavities present in leaves and stems were related to the secondary metabolites detected.

Keywords: Secondary metabolites. Secretion. Secretory cavities. Tropical tree.

Resumo

O estudo de plantas medicinais envolve várias áreas da ciência. Neste contexto, a anatomia contribui para a identificação da espécie e conseqüentemente, com a qualidade do produto da planta. Este trabalho descreve a anatomia do caule e da folha de *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae), coletada em Floresta Estacional Semidecidual. Os órgãos estudados apresentaram epiderme unisseriada, recoberta com cutícula espessa, e cavidades secretoras. O caule apresentou anel contínuo de tecido vascular ao redor da medula, com o floema em ambos os lados do xilema. A folha foi hipoestomática com tricomas na face abaxial, com mesofilo bifacial e feixe vascular anficrival circundado por periciclo esclerenquimático no pecíolo e na nervura principal. Entre os testes histoquímicos, resultados positivos foram obtidos para lipídeos, compostos fenólicos, amido e oxalato de cálcio (drusas). A espécie apresentou aspectos anatômicos típicos da família e as cavidades secretoras presentes nas folhas foram relacionadas aos compostos secundários detectados.

Palavras-chave: Cavidades secretoras. Compostos secundários. Espécies tropicais. Secreção.

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Introduction

The anatomical study of medicinal plants contributes to the correct species identification and consequently, with quality control of plant product. The diagnosis of plants is made initially by morphological analysis of their vegetative and reproductive organs and their organoleptic characteristics. However, the knowledge of their micro-morphology is very important for the establishment of patterns, in order to compare samples of plant specimens used as drugs, proving their authenticity. Make use of plants, erroneously identified, is a risky attitude and may lead to the failure of the expected pharmacological effects or to undesirable reactions due to the presence of toxic substances in plants (DONATO; MORRETES, 2005, 2007).

Among the anatomical characters, secretory structures and secreted material are very interesting to anatomists and taxonomists, because they often give a distinctive appearance to cellular patterns in the plants where they are present (METCALFE; CHALK, 1989). This affirmation is corroborated by many studies in the literature. For example, Ciccarelli, Andreucci and Pagni (2001) studying *Hypericum perforatum* (Hypericaceae), a species traditionally used as tranquilizer, antidepressant and anti-inflammatory, found translucent glands on leaves, sepals and petals; and three different types of canals, considering the whole body of this plant. Sant'Anna-Santos et al. (2006) evaluated the anatomy and histochemistry of secretory structures of the stem of *Spondias dulcis* (Anacardiaceae) and found tanniniferous idioblasts and canals with a complex secretion composed by essential oils, polysaccharides and phenolic compounds. Silva; Machado (1999) studied the structure and the development of secretory trichomes on leaves of *Piper regnellii* var. *regnellii* (Piperaceae). They found two kinds of secretory trichomes: pearl glands and sac-like trichomes. In stem and leaves of *Maytenus ilicifolia* (Celastraceae), used for gastritis and ulcers, were found prismatic crystals and

phenolic compounds (DUARTE; DEBUR, 2005). In an anatomical and chemical study of *Rustia formosa* (Rubiaceae), Vieira et al. (2001) observed secretory cavities with lipids between the palisade and the spongy parenchyma in foliar lamina and in the cortical region of the petiole.

According to Metcalfe; Chalk (1979), secretory cavities are common in Myrtaceae species. Some of them are used as medicinal plants by the population. For example, in *Psidium widgrenianum*, which has anti-inflammatory and antineoplastic activities, Donato; Morretes (2005) found secretory cavities with essential oils distributed in both faces of its leaves and calcium oxalate druses near the vascular bundles. Donato; Morretes (2007) evaluated the leaf anatomy of specimens of *Eugenia brasiliensis*, from two localities, and found numerous secretory cavities with essential oils in both faces of the leaf; the number of observed cavities was different between the localities studied. In *Eugenia dysenterica*, used to combat diarrhea, Palhares (2003) found a large number of glands scattered in the mesophyll. The author also noted the occurrence of druses and idioblasts containing rhomb crystals of calcium oxalate.

Pimenta pseudocaryophyllus (Gomes) L. R. Landrum (Myrtaceae), known as "craveiro", has its leaves used by population for production of tea for flu and in the culinary, besides of its use as wood (LORENZI, 2002). It is a Brazilian endemic species found mainly in the Cerrado and Atlantic Forest (LANDRUM; KAWASAKI, 1997). In pharmacognostic studies of *P. pseudocaryophyllus*, Paula et al. (2006) and Paula et al. (2008) collected stems and leaves, respectively, from individuals growing in Cerrado biome, and detected the presence of phenolic compounds, tannins and flavonoids. Antimicrobial (PAULA, 2006) and antifungal (EL ASSAL, 2012) activity has been suggested to the extract of this species. Because of its importance, it is essential to provide detailed information about the anatomy and the histochemistry of stems and leaves of *P. pseudocaryophyllus*. The leaf anatomy

was described by Paula et al. (2008) and Farias et al. (2009) from material collected in Cerrado and Mixed Ombrophilous Forest, respectively. Considering that environmental conditions can affect the leaf anatomy, it is necessary to evaluate plants in other vegetation formations where the species occurs naturally, as the Seasonal Semideciduous Forest. These studies may contribute to the correct identification of the species and to obtain parameters for quality control of plant material.

In this context, this study aims to describe the leaf and stem anatomy of *P. pseudocaryophyllus*, collected in Seasonal Semideciduous Forest fragment, comparing our results with others described in the literature, both, for this species and others from the same family. These organs were studied because they are the only ones used in popular medicine and more used in phytochemical studies. The results will allow to evaluate if the species presents anatomical plasticity and to establish a description pattern for the correct identification of the species.

Material and Methods

Plant material

Pimenta pseudocaryophyllus is found in several states of Brazil: from Minas Gerais to Rio Grande do Sul (SOBRAL et al., 2013). This species is a shrub or a tree of 1-10m high and its planting is recommended for urbanization and reforestation. It is considered pioneer, semideciduous, selective xerophyte and exclusive of Montane Forests and Brazilian "Caatinga" (LEGRAND; KLEIN, 1978; LORENZI, 2002). The leaves are elliptic, obovate, oval, oblong-elliptic, or elliptic-oblongate, 4-22 cm long, 1-7.3 cm wide, glabrous to densely sericeous; petiole of 3-20 mm long, 1-2 mm thick, glabrous to densely covered with hairs. The inflorescence is a dichasium or a panicle up to 12 cm long, with 3-15 flowers, sparsely to densely

covered with hairs. The fruits are subglobose, 0.8-1.5 cm long and the seeds are hard, shiny, sometimes angular, 0.4-0.8 cm long (LANDRUM, 1986).

Samples of *P. pseudocaryophyllus* were collected in a semi-deciduous seasonal forest fragment (23°43'30" S e 50°43'47" W), localized in São Jerônimo da Serra, Paraná State, Brazil. The climate is Köppen's Cfa with a mean annual rainfall between 1400 to 1600 mm and mean annual temperature between 18 to 20°C. In the summer rains from 500 to 600 mm and the average temperature is between 25 to 27°C, whereas in the winter precipitation is lower (225 to 250 mm) and temperature is between 13 to 16°C. The region has acid and low fertility soils (SÁ, 2004).

The voucher was deposited in the Herbarium of Universidade Estadual de Londrina (FUEL 43025). Samples were collected from 10 randomly chosen individuals in the edge of the remnant. For each of them, we sampled five leaves (all from second node) and two terminal segments of stems. Fragments of these organs were fixed and stored in 70% ethanol. These materials were used for mounting permanent and semi-permanent slides. For histochemical tests were used fresh materials. For scanning electron microscopy, we collected five leaves (n=1 per individual, second node). Leaf fragments were fixed in primary fixative (2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2) for 24 hours at room temperature.

Stem and leaf anatomy

Permanent and semi-permanent slides of stems and leaves were prepared by usual techniques described by Kraus; Arduim (1997). For this, we used segments of stem (first, second and fourth internode), petiole (medium region) and middle fragments of leaf blade (midrib, internodal region and margin). The permanent slides were stained with astra-blue and basic-fuchsin.

The anatomical study of the stem was completed with the maceration of the xylem, using Jeffrey's solution. Stem samples were placed in this solution

until their softening. Then, samples were washed in distilled water and later in 50% ethanol. These materials were maintained in 1% safranin in 50% ethanol (KRAUS; ARDUIM, 1997).

Besides the cross-sections, leaves were used for diafanization and epidermis dissociation. For diafanization, leaves were placed in 5% sodium hydroxide and kept in oven at 37°C until their clearance. Then, leaves were washed in distilled water several times and transferred to Chloral hydrate (1.6:1) for one week. After this time, leaves were washed in water for 12 hours and placed in 70% ethanol, stained in 1% safranin in 50% ethanol, dehydrated in alcohol series and mounted in balsam-of-canada (KRAUS; ARDUIM, 1997). The epidermis dissociation was made by Jeffrey's method. Segments of 1 cm² of internodal region were kept in Jeffrey's solution until the epidermis dissociation. After cleaning and washing, the epidermis was stained with diluted aqueous safranin and mounted in glycerin jelly.

Stems and leaves slides were photographed with a MOTIC B1-SERIES light microscope coupled to a MOTICAM Live 2300 3.0 MP resolution camera, using the Motic Images Plus 2.0 ML version software. The cleared leaves were analyzed and photographed with a LEICA MZ 12.5 stereoscopic microscope.

Scanning electron microscopy was performed at the Laboratory of Microscopy and Microanalysis of the Universidade Estadual de Londrina. The leaf samples were fixed in primary fixative and washed three times in 0.1 M phosphate buffer (pH 7.2) for 10 minutes each. Materials were post-fixed in 1% osmium tetroxide for 2 hours at room temperature. Again, materials were washed in 0.1 M phosphate buffer (pH 7.2) three times, 10 minutes each. After this stage, materials were dehydrated in an ethanol series followed by critical-point drying in a Bal-Tec Critical Point Dryer CPD 0.30 apparatus; the specimens were mounted on stubs and coated with a film of gold (40nm) in a Bal-Tec SCD 050 sputter

coater. The samples were observed with a FEI-QUANTA 200 scanning electron microscope.

Histochemical tests

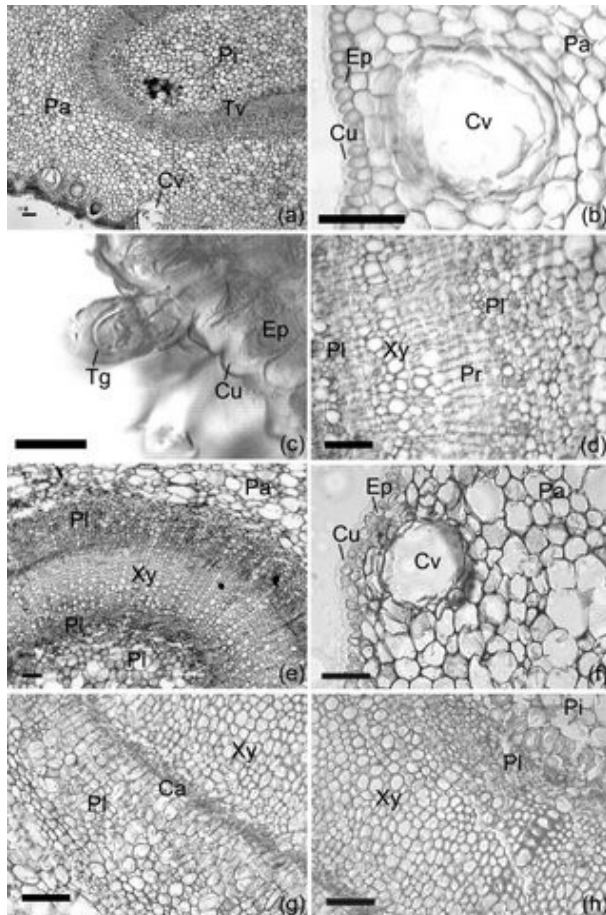
Histochemical tests were performed on free hand sections from fresh organs. For this, sections were submitted to the following tests: presence of starch with Lugol, of calcium carbonate crystals with glacial acetic acid, of calcium oxalate crystals with 10% HCl, of phenolic substances with ferric chloride III and lipids with Sudan III (KRAUS; ARDUIM, 1997).

Results

Stem anatomy

The first (Fig. 1a) and the second internode of *P. pseudocaryophyllus* were similar in structure. These internodes presented one-layered epidermis with a thick cuticle, and glandular (Fig. 1c) and tector trichomes. The cortex was parenchymatous and exhibited several schizogenous secretory cavities, of circular section, located in its external region (Fig. 1a, b). The vascular tissues formed a continuous ring around the pith with the xylem surrounded by the phloem in its internal and external face (Fig. 1d). Both internodes did not present secondary growth. The fourth internode had similar structure when compared to the first and second ones (Fig. 1e, f), however exhibited evident secondary growth in vascular tissues (Fig. 1g, h).

Figura 1 - Stem transections of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR. a, c, d - first internode; b - second internode; e - h - fourth internode. Bars = 50µm (a, b, d - h) and 25µm (c). Abbreviations - Ca: vascular cambium; Cv: secretory cavity, Cu: cuticle; Ep: epidermis; Pa: parenchyma; Pi: pith; Pl: phloem; Pr: procambium; Tg: glandular trichome; Tv: vascular tissue, Xy: xylem.



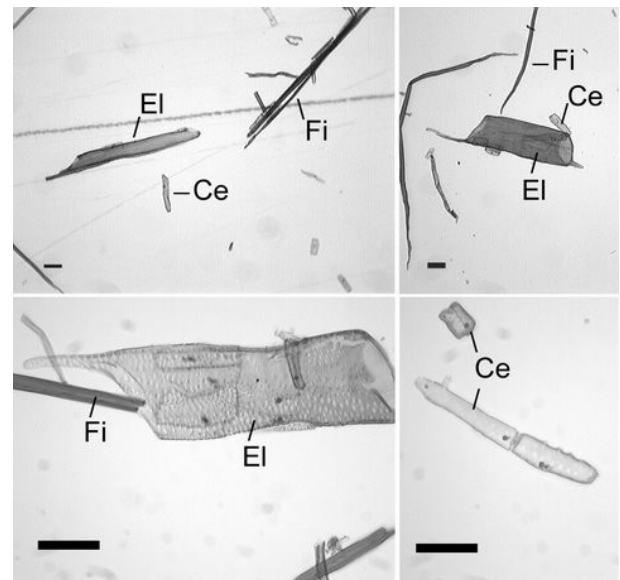
Fonte: autores

The xylem showed parenchymatous cells, many libriform fibres and different types of vessel elements. Some of them were longer and narrower and others shorter and wider, but all presented slightly oblique end walls and simple perforation plates (Fig. 2).

Leaf anatomy

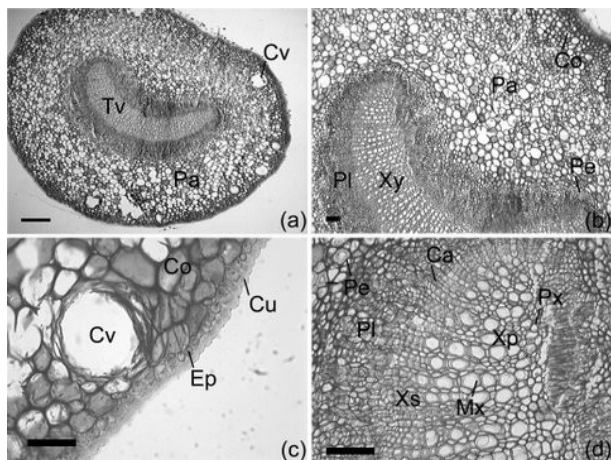
In the cross-section, the petiole adaxial surface was flat to slightly concave and the abaxial surface was convex (Fig 3a). It showed uniseriate epidermis with thick cuticle, and cortex formed by collenchyma and parenchyma (Fig. 3b, c). The secretory cavities occurred in the external part of the cortex and they were similar to those found in the stem (Fig. 3a, c). The vascular system was formed by an amphicribral vascular bundle, surrounded by a sclerenchymatous pericycle (Fig. 3a, b, d). Vascular cambium and secondary xylem were observed in the vascular bundle (Fig. 3d).

Figura 2 - Macerated xylem stem of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR. Bars = 50µm. Abbreviations - Ce: parenchyma cell; Fi: libriform fibre; El: vessel element.



Fonte: autores

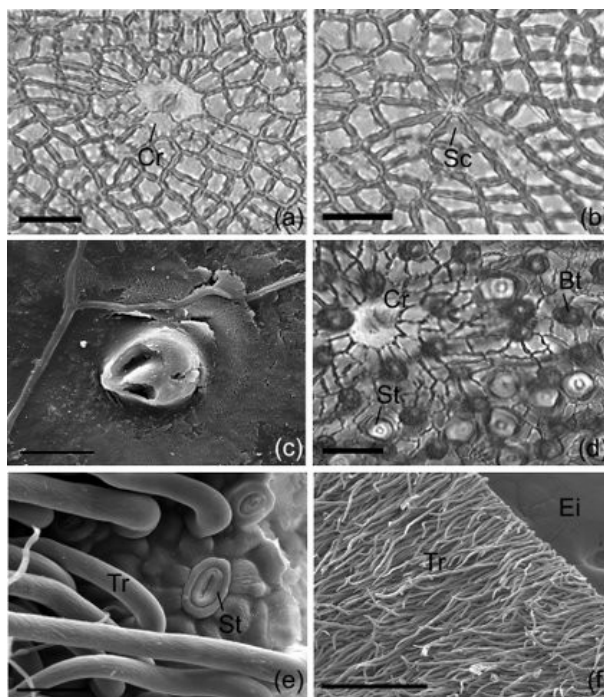
Figura 3 - Petiole transections of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR. (a, b) general appearance (c) secretory cavity (d) vascular bundle. Bars = 250µm (a) and 50µm (b - d). Abbreviations - Ca : vascular cambium; Co: collenchyma; Cv: secretory cavity, Cu: cuticle; Ep: epidermis; Mx: metaxylem; Pa: parenchyma; Pe: pericycle; Pl: phloem; Px: protoxylem; Tv: vascular tissue, Xp: primary xylem; Xs: secondary xylem; Xy: xylem.



Fonte: autores

In frontal view of the leaf blade, the epidermal cells of the adaxial surface showed different sizes and shapes, with thick and wavy anticlinal walls (Fig. 4a, b). This face was glabrous and had no stomata. Radially arranged epidermal cells were observed in this face (Fig. 4b), similar to trichomes bases (Fig. 4c). These structures were identified as scars, once young leaves had abundant trichomes on adaxial face. These trichomes are normally shed during leaf development. Regions formed by two or three cells surrounded by smaller epidermal cells also were observed. These cells overlaid the secretory cavities (Fig. 4a). In the abaxial face, the epidermal cells had also different sizes and shapes with thick and wavy anticlinal walls (Fig. 4d). Many stomata (Fig. 4d, e), predominantly anomocytic, and long unicellular trichomes (Fig. 4e, f) were observed in this face.

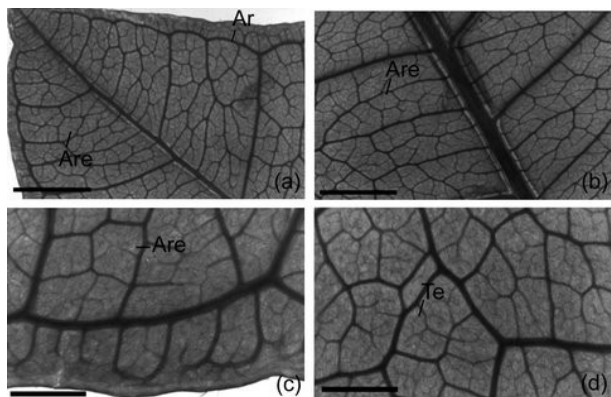
Figura. 4 - Leaf epidermal dissociation and scanning electron microscopy of the adaxial and abaxial faces of the epidermis of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR. (a) adaxial epidermis showing the cells that cover the secretory cavities (b) adaxial epidermis showing scar (c) detail of the base of the trichome on adaxial epidermis (d) abaxial epidermis showing the cells that cover the secretory cavities (e) detail of stomata on the abaxial surface (f) abaxial epidermis showing trichomes. Bars = 10µm (d), 20µm (g), 25µm (a, b, e, f) and 300µm (c, h). Abbreviations - Bt: base of the trichome; Cr: cells that cover the secretory cavity; Ei: abaxial epidermis; Sc: scar; St: stoma; Tr: trichome.



Fonte: autores

Pimenta pseudocaryophyllus presented camptodromous-brochidromous venation pattern, with veins from first to fourth order, areolas of irregular shape, distributed without preferential orientation in the leaf (Fig 5).

Figura 5 - Diaphanized leaves of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR. (a) apical region (b) median region (c) leaf margin (d) internerval region. Bars = 1.25 mm (a e b), 525 µm (c) e 325µm (d). Abbreviations - Ar: arches; Are: areolas; Te: vascular endings.



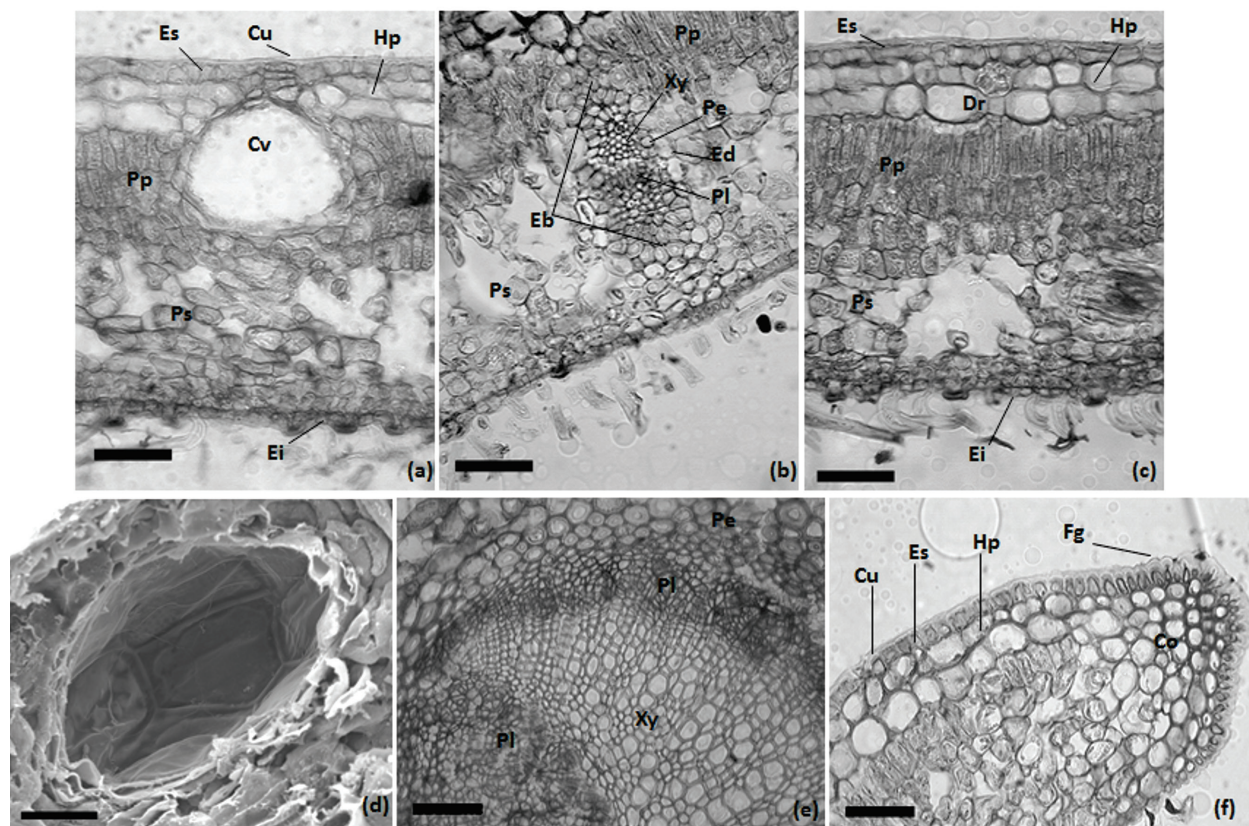
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In the cross-section, the leaf blade is bifacial, characterized by a heterogeneous mesophyll with palisade parenchyma, consisting from two to three layers, towards the adaxial face and spongy parenchyma, formed by seven to nine layers, towards the abaxial face (Fig. 6a, c). The one-layered epidermis was formed by square to rectangular cells, covered by thick cuticle. The epidermal cells were bigger on the adaxial than on the abaxial surface. Stomata were raised above the level of the neighboring ordinary epidermal cells (Fig. 6a, c). Adjacent to the adaxial surface, there was two or three layers of cells slightly bigger than the epidermal ones (Fig. 6a, c), being similar to a hypodermis. The vascular bundles were collateral and surrounded by a sclerenchymatous bundle sheath (endodermis and pericycle), which exhibited extensions to both surfaces (Fig. 6b). In the mesophyll were found many schizogenous secretory cavities similar to those found in stems (Fig. 6a, d), all in palisade parenchyma, and crystals of calcium oxalate (druses) (Fig. 6c).

An amphicribal bundle formed the midrib, similar to that found in the petiole, also surrounded by a sclerenchymatous pericycle (Fig. 6e). The cortex of the midrib was formed by parenchyma and in some regions by collenchyma. The epidermis was uniseriate, with glabrous adaxial surface and densely hairy abaxial surface. Subepidermal layers, similar to a hypodermis, were present in the adaxial face.

The leaf margin was slightly bent towards the abaxial surface and was similar in appearance to the internerval region, showing collenchyma in its extremity, with thicker cuticle forming cuticular flanges (Fig. 6f).

Figura 6 - Optical and scanning electron microscopy of cross sections of *Pimenta pseudocaryophyllus* leaves collected in São Jerônimo da Serra, PR. (a - c) general appearance of blade (d) details of the secretory cavity (e) detail of the amphicribral bundle in the midrib (f) leaf edge. Bars = 25µm (d) and 50µm (a - c, e, f). Abbreviations - Co: Collenchyma, Cv: secretory cavity, Cu: cuticle; Dr: druse; Eb: extension of the sheath; Ed: endodermis; Ei: abaxial epidermis; Es: adaxial epidermis; Fg: cuticular flange; Hp: subepidermal layers; Pe: pericycle; Pl: phloem; Ps: spongy parenchyma; Pp: palisade parenchyma; Xy: xylem.



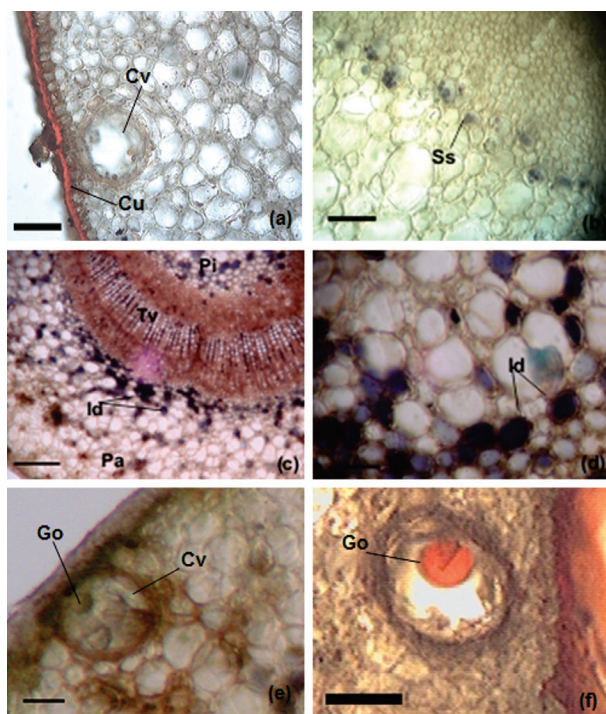
Fonte: autores

Histochemical tests

Lipids were detected in the cuticle of stems (Fig. 7a) and leaves. A starch sheath around the vascular system (Fig. 7b) was observed in the stem and in the petiole. Calcium oxalate crystals (druses) were present in stem (pith and cortex, especially in its inner region), blade leaf (mainly in spongy parenchyma, but also present in palisade

parenchyma, subepidermal layers and midrib parenchyma) and petiole (cortex) (Fig. 6c). Also idioblasts with phenolic compounds were observed in these organs (Fig. 7c, d). Secretory cavities in stems and leaves showed positive results for phenolic compounds and lipids (Fig. 7e, f). The test was negative for the presence of calcium carbonate crystals for this species.

Figura 7 - Stem transections of *Pimenta pseudocaryophyllus* collected in São Jerônimo da Serra, PR, showing positive results of histochemical tests. (a) Sudan III (b) Lugol - showing the starch sheath (c) ferric chloride III (d) ferric chloride III - detail showing idioblasts (e) secretory cavity with material stained by ferric chloride (f) secretory cavity with material stained by Sudan III. Bars = 50µm (a, b, d - f) and 250µm (c). Abbreviations - Cv: secretory cavity, Cu: cuticle; Go: drop of material secreted by secretory cavity; Id: idioblast, Pi: pith; Ss: starch sheath; Tv: vascular tissue.



Fonte: autores

Discussion

The species *P. pseudocaryophyllus* exhibited typical anatomical features of Myrtaceae, such as the presence of phloem internal to the xylem, hypostomatic leaves, anomocytic stomata, tector trichomes and secretory cavities (METCALFE; CHALK, 1979, 1989). This arrangement of vascular tissues was observed in *Psidium guajava* (DUARTE; PAULA, 2005), while hypostomatic leaves, with anomocytic stomata, were observed in *Psidium*

widgrenianum (DONATO; MORRETES, 2005), *Eugenia dysenterica* (PALHARES, 2003) and in *Eugenia brasiliensis* (DONATO; MORRETES, 2007).

Scars of trichomes in *P. pseudocaryophyllus* were first described by Landrum (1986). According to Gomes et al. (2009), the absence of the foot cell and a narrow base render fragile the insertion of the trichome into the epidermis, contributing to the occurrence of glabrescent leaves. These authors showed the natural fall of trichomes in about 74% of the species, analyzing the indument of leaf primordia and mature leaves of 46 species of Myrtaceae.

Cuticular flanges were described in *Psidium widgrenianum* (DONATO; MORRETES, 2005) and in *Eugenia brasiliensis* (DONATO; MORRETES, 2007); however, they are not common in Myrtaceae species. Thus, these structures can be used to delimit certain species, which are similar morphologically (FARIAS et al., 2009).

The epidermal cells, which cover the secretory cavities, were called of overlying cells (OLC) by Fontenelle; Costa; Machado (1994). In *Eugenia* species, studied by these authors, they were present in both epidermis and were found isolated, in pairs or in trios. Sets of OLC were also reported for *Eugenia dysenterica* (PALHARES, 2003), *Eugenia brasiliensis* (DONATO; MORRETES, 2007), and *Gomidesia nitida* (GOMES; NEVES, 1993/1997). Similar structures were also found in adaxial surface of leaves of *Psidium widgrenianum*, which were formed by a pair of reniform cells reminding butterfly wings (DONATO; MORRETES, 2005).

Ontogenetic studies are needed to clarify whether subepidermal layers can be a multiseriate epidermis or a hypodermis. However, Metcalfe; Chalk (1979) considered that the hypodermis is present in Myrtaceae. In spite of the question about the origin of this tissue, its presence was used to identify species of Myrtaceae (GOMES et al., 2009; HUSSIN; CUTLER; MOORE, 1992; KANTACHOT;

CHANTARANOTHAI; THAMMATHAWORN, 2007), and Feller (1996) suggested that it could protect the photosynthetic tissue, especially in habitats with nutritional deficiency and excessive light, that are similar to the habitat of the plants used in this study.

The venation pattern observed here was similar to that reported by Cardoso; Sajo (2006) for this same species and for the most species of the subtribes Myrtinae, Myrciinae and Eugeniinae that they studied.

The types of vascular bundles found in *P. pseudocaryophyllus* have been reported for other species of Myrtaceae. Thus, the amphicrival bundle was also reported in *Eugenia brasiliensis* petiole (DONATO; MORRETES, 2007) and in *Maytenus ilicifolia* midrib (DUARTE; DEBUR, 2005), while minor vascular bundles of collateral type, surrounded by sclerenchymatous sheath, were reported in *Psidium guajava* (DUARTE; PAULA, 2005) and in *Eugenia brasiliensis* (DONATO; MORRETES, 2007). Also the leaf margin observed in the studied species was similar to that one described for *Eugenia brasiliensis* (DONATO; MORRETES, 2007).

A well-developed palisade parenchyma, like the observed in *P. pseudocaryophyllus* leaves, was mentioned by Fontenelle; Costa; Machado (1994) for some *Eugenia* species. According to these authors, a well-developed palisade parenchyma, together with the presence of tannins and epidermal cells with thick anticlinal walls, are morpho-physiological adaptations to environmental factors, such as high luminosity. These characteristics and the occurrence of hypostomatic leaves, with many trichomes on the abaxial surface, are expected in plants in edges or open areas in Seasonal Deciduous (ESPOSITO-POLESI; RODRIGUES; ALMEIDA, 2011) or Semidecidual Forests, like the individuals sampled in this study. However, these features were also observed by Farias et al. (2009) in *P. pseudocaryophyllus* sampled in Mixed Ombrophylous Forest. These authors

suggested that these features can be adaptations of ancestors and are not necessarily related to current habitat.

Schizogenous secretory cavities observed here in *P. pseudocaryophyllus* were also found in leaves of *Eugenia brasiliensis* (DONATO; MORRETES, 2007), *Eugenia dysenterica* (PALHARES, 2003), *Psidium guajava* (DUARTE; PAULA, 2005) and *Psidium widgrenianum* (DONATO; MORRETES, 2005). However, Farias et al. (2009) reported that secretory structures observed in the leaves of *P. pseudocaryophyllus* were lysogenous ducts, which was not confirmed by this study.

Calcium oxalate crystals, as those observed in *P. pseudocaryophyllus*, have ecological importance, since they are related to defense mechanisms against herbivores (LUCAS et al., 2000), and physiological importance, once the crystals can regulate the level of calcium in the tissues (VOLK et al., 2002). Furthermore, according to Franceschi and Nakata (2005), crystals might contribute for light distribution to the chloroplasts and with the light excess dissipation during high luminosity periods. In *P. pseudocaryophyllus*, crystals could have this role because the sampled individuals had their crowns fully or partially exposed to the sun.

Lipids in the cuticle and a starch sheath were also observed in other members of Myrtaceae, like in *Psidium widgrenianum* (DONATO; MORRETES, 2005) and in *Eugenia brasiliensis* (DONATO; MORRETES, 2007). Idioblasts containing phenolic compounds were reported in leaves and stems of *Psidium guajava* (DUARTE; PAULA, 2005) and in the leaves of *Eugenia brasiliensis* (DONATO; MORRETES, 2007).

In the secretory cavities of *P. pseudocaryophyllus* were detected phenolic compounds and lipids. Fahn (1979) mentioned that these structures could produce a mixture of terpenes or terpenoids with carbohydrates, among other components. In some species, heterogeneous secretions formed by a mixture of essential oils and phenolic compounds can be found

in secretory cavities (CASTRO; MACHADO, 2003). This concept can be applied to *P. pseudocaryophyllus*, considering the positive results obtained for phenolic compounds and lipids. Essential oils were isolated from *P. pseudocaryophyllus* leaves by Custódio et al. (2010) and Paula et al. (2011). According to Castro; Machado (2003), volatile oils may be important for pollinators' attraction or to repel insects, because of their deterrent and insecticide action, reducing herbivory. Phenolic compounds in plants also have a protective function because of the unpalatability, acting as a deterrent and reducing herbivory. These compounds can contribute to cellular maintenance when plants are under water stress (CASTRO; MACHADO, 2003) and can function as solar filter, increasing the protection against ultraviolet radiation (BOEGER; POULSON, 2006).

The occurrence of secretory cavities, the abundance of phenolic compounds as tannins, and the presence of solitary or grouped crystals of calcium oxalate are common characteristics of Myrtaceae (METCALFE; CHALK, 1979). Furthermore, the presence, frequency and distribution of secretory structures are important parameters to species diagnostic ends (METCALFE; CHALK, 1989).

Previous anatomical studies with *P. pseudocaryophyllus* leaves, collected in different vegetation formations (FARIAS et al., 2009; PAULA et al., 2008), showed similar characteristics to the observed in the present study. This suggests that the species present a low leaf phenotypic plasticity. The main difference among the studies refers to the vascular bundle of the petiole and midrib. While Paula et al. (2008) and Farias et al. (2009) observed the bicollateral vascular bundle; in this study we observed an amphicribral vascular bundle.

The species exhibits typical anatomic features of Myrtaceae and the secretory cavities that occur in leaves and stems are related to secretion of the secondary metabolites detected. As the plant is used as medicinal, this study contributes to the establishment of an anatomical and chemical pattern for the species.

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