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Leaf and stem morphoanatomy of *Petiveria alliacea*

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Abstract

Petiveria alliacea is a perennial herb native to the Amazonian region and used in traditional medicine for different purposes, such as diuretic, antispasmodic and anti-inflammatory. The morphoanatomical characterization of the leaf and stem was carried out, in order to contribute to the medicinal plant identification. The plant material was fixed, freehand sectioned and stained either with toluidine blue or astra blue and basic fuchsine. Microchemical tests were also applied. The leaf is simple, alternate and elliptic. The blade exhibits paracytic stomata on the abaxial side, non-glandular trichomes and dorsiventral mesophyll. The midrib is biconvex and the petiole is plain-convex, both traversed by collateral vascular bundles adjoined with sclerenchymatic caps. The stem, in incipient secondary growth, presents epidermis, angular collenchyma, starch sheath and collateral vascular organization. Several prisms of calcium oxalate are seen in the leaf and stem.

Keywords: Petiveria alliacea; Morphoanatomy

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1. Introduction

The genus *Petiveria* belongs to the Phytolaccaceae which are regarded as the most archaic family of the Caryophyllales [1]. The family comprises about 17 genera and 120 pantropical species, often distributed in the American continent [2,3]. It includes herbs and shrubs, rarely trees, with simple and alternate leaves and small flowers usually assembled in racemose or spicate inflorescences [1,3].

Petiveria alliacea L. (Phytolaccaceae) is a perennial herb which attains about 70 cm high and has small flowers forming long racemose inflorescences and cuneate achenes bearing spines for dissemination. It is indigenous to the Amazonian region and largely cultivated for ornamental and medicinal purposes in many tropical areas [4–11]. It is commonly known as guiné or pipi in Portuguese, anamú or zorrillo in Spanish, garlic scented petiveria in English and verbeine puante in French [12]. In folk medicine, leaf, stem and root infusions or decoctions have been employed as diuretic, antispasmodic, emmenagogue, sedative, analgesic and anti-inflammatory [8,9,13,14].

Pharmacological studies have been conducted to confirm the reputed properties of *P. alliacea* and some results have demonstrated its immunomodulatory [15–18] and antimitotic actions [19], analgesic and anti-inflammatory activities [20,21], uterine contraction [22], antinociceptive [23], anxyolytic and gastric protective effects [24].

Few studies have been devoted to the species morphology, therefore this work has aimed to investigate the leaf and stem morphoanatomy of the medicinal plant, in order to contribute to its identification.

2. Experimental

2.1. Plant material

The plant material was collected from specimens of *P. alliacea* cultivated in the city of Curitiba, Paraná State (25° 26′ S and 49° 14′ W, 900 m height), in April 2004. The dried flowering material was equivalent to the voucher Herbarium specimen registered under No. MBM 265731 in the Museu Botânico Municipal de Curitiba.

2.2. Analysis

The external leaf morphology was classified according to Hickey [25]. Fresh mature leaves and stem fragments, collected 5 cm from the apex, were fixed in FAA 70 [26] and kept in 70% EtOH solution [27]. The examined foliar region was the petiole and the lower third of the blade and midrib. Transverse and longitudinal freehand sections were stained either with toluidine blue [28] or with astra blue and basic fuchsine combination [29]. Microchemical reactions were applied with hydrochloric phloroglucin to reveal lignified elements [30], iodine–iodide to starch [27], Sudan IV to lipophilic substances [31], ferric chloride to phenolic compounds [26] and sulphuric acid to calcium oxalate crystals [32].

The results were registered by botanical illustration and photos taken by means of the Olympus BX40 optical microscope equipped with the control unit PM20.

3. Results

3.1. Leaf morphoanatomy

Petiveria alliacea (Fig. 1) has simple leaves, with alternate phyllotaxy, elliptic shape, acute apex and base, slightly wavy margin, measuring 7–12 cm long and 3–6 cm wide. The petiole is short, the foliar texture is membranaceous and the venation is pinnate camptodromous–brochidodromous.

The blade epidermis is single-layered (Fig. 2d) and coated with a thin and smooth cuticle. In face view (Fig. 2a–c), the epidermal anticlinal walls show evident primary fields and are ondulate in the interveinal region and polygonal accompanying the vein direction. In transection (Fig. 2d), the adaxial epidermal cells are higher than the abaxial ones. The leaf is hypostomatic, with paracytic stomata (Fig. 2c) on the same level as the adjacent cells (Fig. 2d). The guard-cells exhibit outer ledges and thick outer periclinal walls. Non-glandular trichomes occur predominantly on the abaxial surface.

The mesophyll is dorsiventral, consisting of about two layers of palisade parenchyma and four strata of spongy parenchyma, the latter representing 60% of the chlorenchyma. Minor collateral bundles encircled with a parenchymatic sheath and idioblasts containing large prismatic crystals of calcium oxalate are seen (Fig. 2d).

The midrib (Fig. 3a and b), in transverse section, is biconvex. The epidermis is uniseriate and has non-glandular trichomes similar to the blade. They are seldom unicellular and often

Fig. 1. Vegetative and reproductive apical branches of *P. alliacea*.

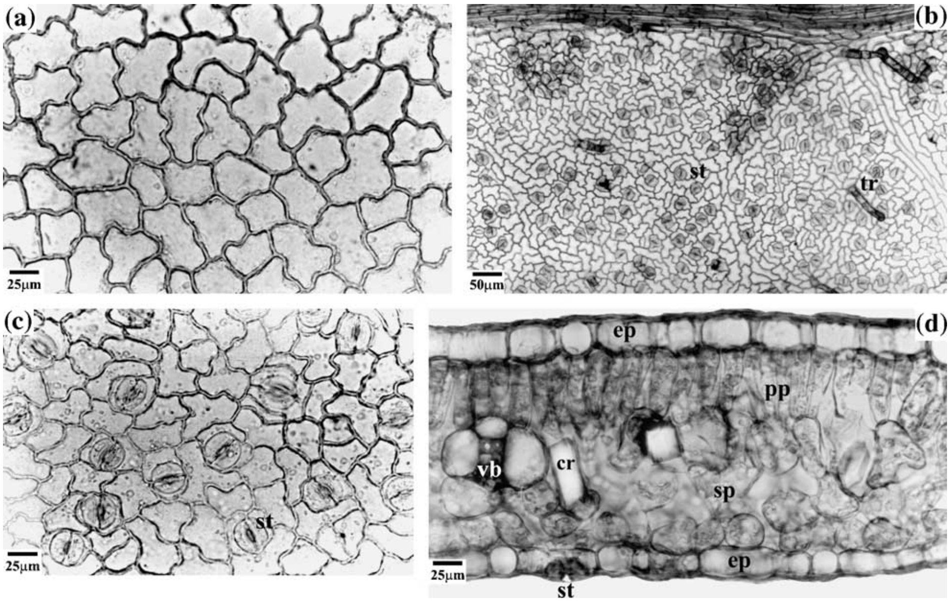

Fig. 2. Leaf structure of *P. alliacea*. (a) Adaxial side of the epidermis, in face view; (b), (c) Abaxial side of the epidermis, in face view; (d) transection of the blade, indicating stomatum even with adjacent cells, dorsiventral mesophyll, minor vascular bundle encircled with a parenchymatic sheath and prismatic crystals. cr: crystal; ep: epidermis; pp: palisade parenchyma; sp: spongy parenchyma; st: stomatum; tr: trichome; vb: vascular bundle.

composed of five to fifteen cells in one rank. The trichome apex may be round and thick-walled (Fig. 3c) and the stalk cells dehydrated. Adjacent to the epidermis, angular collenchyma occur, comprising approximately ten rows on the adaxial side and four on the abaxial one. Embedded in the ground parenchyma, one to five collateral vascular bundles arranged nearly as a closed arc are present. These exhibit an evident cambial zone and a sclerenchymatic cap adjoining the phloem (Fig. 3a). Idioblasts with calcium oxalate prisms are found in the collenchyma and ground tissue.

The petiole (Fig. 3d), in transection, reveals a plain-convex shape. The epidermis has the same characteristics of the blade and a continuous strand of annular collenchyma is encountered below it. About two to six collateral vascular bundles resembling a closed arc and presenting a sclerenchymatic cap adjoined to the phloem are embedded in the ground parenchyma. Several prismatic calcium oxalate crystals are found in the petiole.

3.2. Stem morphoanatomy

The stem (Fig. 4a) has a circular transection and the epidermis remains as the dermal system in the incipient secondary growth. It is uniseriate, covered with a thick cuticle and exhibits non-glandular trichomes. A continuous strand of angular collenchyma, few rows of cortical parenchyma that contains amyloplasts, chloroplasts and calcium oxalate prisms, and a starch sheath are found in the cortex (Fig. 4b). The vascular system consists of a cylinder of xylem produced towards the inside and a cylinder of phloem outwards, which is adjoined by perivascular fibre caps. The xylem is wholly lignified and the tracheary

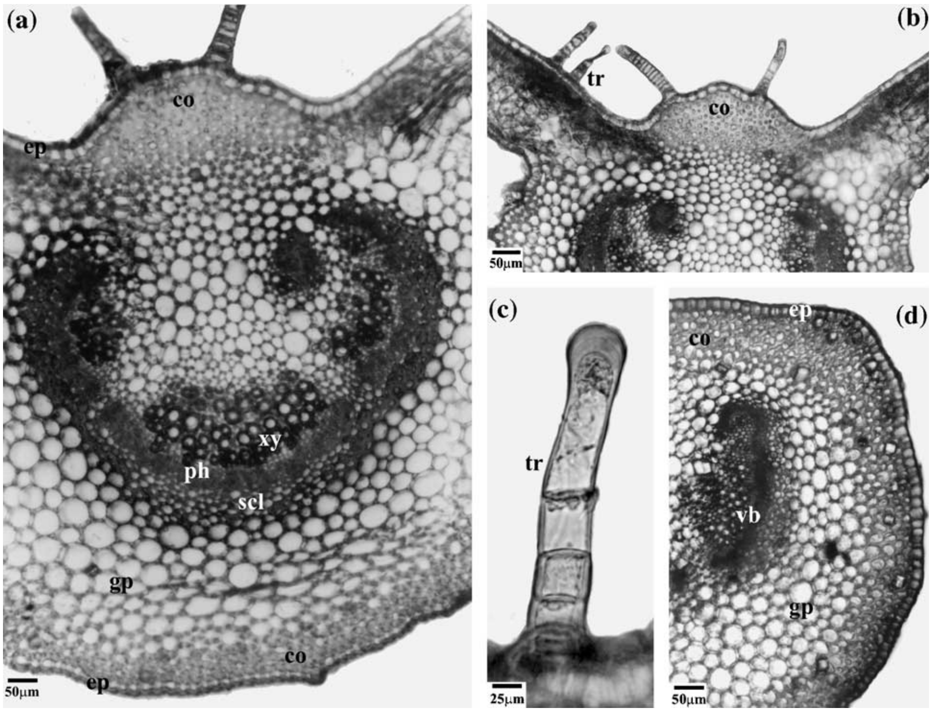

Fig. 3. Midrib and petiole structure of *P. alliacea*. (a) Transection of the midrib, showing collateral vascular bundles arranged as closed arc; (b) trichomes in the midrib; (c) detail of a pluricellular non-glandular trichome with a round thick-walled apex; (d) transverse section of the petiole, revealing a continuous strand of collenchyma below the epidermis and a vascular bundle. co: collenchyma; ep: epidermis; gp: ground parenchyma; ph: phloem; scl: sclerenchymatic cap; tr: trichome, vb: vascular bundle; xy: xylem.

elements are aligned solitary or in small groups (Fig. 4a, c). The pith occupies great part of the stem volume (Fig. 4a) and it consists of parenchymatic cells bearing prismatic crystals of calcium oxalate (Fig. 4d) and several amyloplasts.

4. Discussion

The leaf morphology and phyllotaxy of *P. alliacea* is similar to the description by Martins [33] and Di Stasi, Hiruma-Lima [8]. In addition, according to the latter authors [8] and Carvalho [14], the leaves as well as the roots have a striking aromatic characteristic, due to the garlic-like odour. Regarding the leaf venation, the brochidodromous type observed in *P. alliacea* represents the primitive pattern in Magnoliophyta, as stated by Roth-Nebelsick et al. [34]. Besides being involved with the transport of substances, the venation system is also related to the leaf mechanical stabilization, owing to the presence of the lignified xylem and sclerified elements.

Metcalfe and Chalk [35] have mentioned for the genus *Petiveria* the occurrence of paracytic stomata on both surfaces and very large styloids. In this investigation, *P. alliacea*

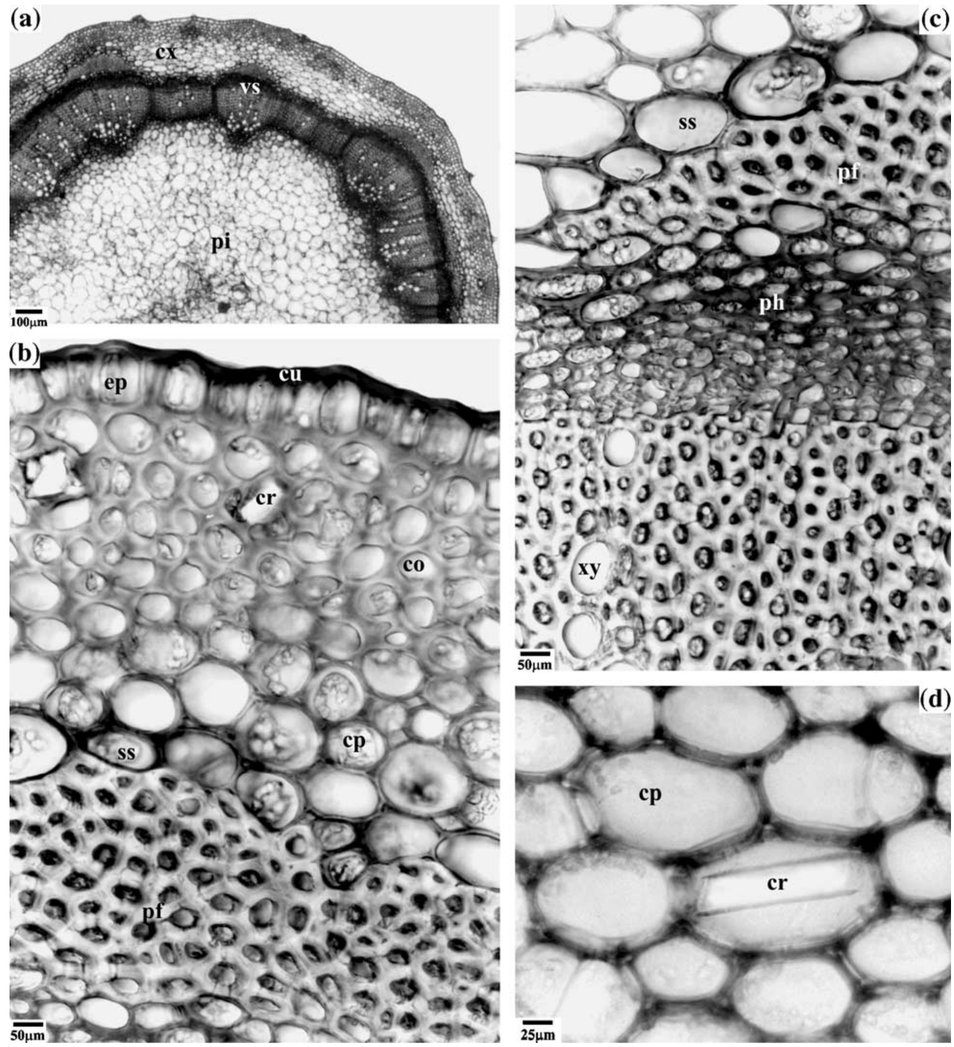

Fig. 4. Stem structure of *P. alliacea*. (a) caulinar organization in incipient secondary growth; (b) stem transection, showing epidermis, prismatic crystals in the cortex, starch sheath and perivascular fibre cap; (c) detail of the vascular system; (d) prismatic crystal in the cortical parenchyma. co: collenchyma; cp: cortical parenchyma; cr: crystal; cu: cuticle; cx: cortex; pf: perivascular fibre cap; ph: phloem; pi: pith; ss: starch sheath; vs: vascular system; xy: xylem.

presents the same type of stomata, although these appendages are confined to the lower epidermis, and the prismatic calcium oxalate crystals may correspond to styloids with greater dimensions. From Woodward's point of view [36], although the presence of stomata increases photosynthetic potential, protects xylem from cavitation favouring water flow and promotes heat dissipation by water loss, herbaceous species maintain low stomatal densities and hydraulic conductances, maximizing the control on loss of water to a dry atmosphere. This control is optimized by a well-developed cuticle, a barrier which contributes to the maintenance of plant water status [37].

Concerning the calcium oxalate crystals, they are by far the most prevalent and widely distributed mineral deposits in higher plants and typically develop within intravacuolar membrane chambers of specialized cells in any organ or tissue [38]. They are formed from environmentally derived calcium and from biologically synthesized oxalate [39]. According to Franceschi and Horner Jr [40], the differentiation of a cell into a crystal idioblast is surely under genetic control and the shape of the crystal formed may also be genetically determined. The functions assigned to calcium oxalate crystals are varied, since they can be related to ionic balance and osmoregulation, storaging form for either calcium or oxalate, mechanical support and protection against foraging animals.

Trichomes are epidermal outgrowths of considerable value for taxonomic purposes. The number of species that are completely devoid of trichomes represent a minority of the Magnoliophyta and environmental conditions influence more the length, size and density than the types of trichomes. These outgrowths play a role in plant defense especially with regard to phytophagous insects, avoiding insect feeding and oviposition responses, and the nutrition of larvae [41]. They may be involved in the regulation of temperature and water-repellency as well [42]. Despite being regarded as infrequent in Phytolaccaceae, when trichomes occur they are unicellular or pluricellular and uniseriate [35,41], as the present observations in *P. alliacea*.

For Metcalfe and Chalk [35], members of the family usually have dorsiventral mesophyll and it includes several layers of palisade parenchyma, contrasting with the two strata found in this investigation. In addition, these authors have reported that the midrib includes six vascular bundles, each supported by fibres and embedded in water-storage tissue in *P. alliacea*. In this work, ground parenchyma not related to typical storage functions is described.

With reference to bundle sheath extensions mentioned in *P. alliacea*, Esau [43] has stated that these are panels of cells which connect the bundle sheaths with the epidermis. The bundle sheath extensions and the minor veins distribute the transpiration stream through the mesophyll and serve as starting points for the uptake of the products of photosynthesis and their translocation out of the leaf.

The stem organization of *P. alliacea* in incipient secondary growth observed in this study corresponds to a typical herbaceous pattern [43]. It differs from the anomalous secondary thickening found in a few genera of Phytolaccaceae, which comprehends successive rings of vascular bundles in the inner parenchymatous portion of the pericycle [35,44,45]. This particular thickening was registered in the root of the genus *Petiveria* [35].

The occurrence of an outer part strongly collenchymatous, broad pith and crystals secreted in the form of large styloids in the family [35] are observed similarly herein. Nevertheless, the medullary bundles reported in Phytolaccaceae [45] are not found in this investigation.

5. Conclusion

The morphoanatomical aspects of *P. alliacea* correspond to the features assigned to the Phytolaccaceae family. Although they have to be evaluated altogether for the species identification, some characters may be pointed out, such as alternate leaf showing non-glandular trichomes with round thick-walled apex, paracytic stomata confined to the lower epidermal side, large prismatic calcium oxalate crystals, dorsiventral mesophyll, minor veins with bundle sheath extensions, collateral vascular bundles in the midrib and petiole, and stem with collateral vascular organization.

Acknowledgments

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