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MICROSCOPIC CHARACTERS OF THE LEAF AND STEM OF *Pachiraaquatica* AUBL. (MALVACEAE)

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ABSTRACT

Pachiraaquatica, commonly known as Malabar chestnut, is a drought-tolerant tree with palmate compound leaves and a swollen stem bottom. It is valued as ornamental, food additive and medicinal. In folk medicine, it is used as antidiabetic and wound healing. This work has studied the leaf and stem microscopic characters of this medicinal plant, in order to contribute to its anatomical characterization for taxonomic and pharmacognostic purposes. Adult leaves and young stems were prepared for light and scanning electron microscopic analyses. Standard microchemical tests were also performed. The leaf is hypostomatic with anomocytic stomata. The epidermis is coated with a striate cuticle and it occurs platelets of epicuticular wax and some glandular trichomes. There are a subepidermal layer, dorsiventral mesophyll and minor vascular bundles with sclerenchymatic bundle extensions. The midrib is biconvex and traversed by collateral vascular bundles in a ring-like arrangement. In the stem, the phellogen has a superficial origin and the phloem is disposed in triangular strands and stratified into fibrous zones. Idioblasts containing druses, phenolic compounds and mucilage are present. These results are novelties for *P. aquatica* and useful for its microscopic characterization.

Keywords: Anatomy, Malabar Chestnut, Medicinal Plant, Mucilage, Pharmacognosy, Phenolic Compounds

INTRODUCTION

The genus *Pachira* Aubl., formerly belonging to Bombacaceae, is currently repositioned in Malvaceae-Bombacoideaeand it includes 24 species **[1, 2]**. Among them, it is encountered *Pachiraaquatica* Aubl., commonly named

Malabar chestnut and native to an area from Southern Mexico to North-Eastern Brazil **[3]**. This species is a drought-tolerant perennial tree with palmate compound leaves and a swollen stem bottom which stores carbohydrates and water. For this unique shape, the tree is valued as ornamental **[4]**.

Besides, *P. aquatica* is used as food additive and also in folk medicine. The leaves and bark stems are considered antidiabetic and wound healing agents **[3, 5-7]**. Phytochemical investigations allied to pharmacological assays have demonstrated that different terpenoids isolated from the species have antimicrobial and antivenomeffects **[4, 8, 9]**. Despite the relevance of *P. aquatica* in different communities, little knowledge is currently available. Therefore, this work has carried out the anatomical study of the leaf and stem of this medicinal plant and potential vegetal drug,using light (LM) and scanning electron microscopic (SEM) techniques, aiming to contribute to the species characterization for taxonomic and pharmacognostic purposes.

MATERIALS AND METHODS

Plant Material

The plant material was collected from specimens grown in open and sunny areas in the city of Caldas Novas, state of Goiás, Brazil (coordinates 17°43'48" S and 48°37'48" W and altitude 686 m), in June 2004. The species was identified at the MuseuBotânico Municipal de Curitiba, Paraná (voucher for reference MBM 270081). Fully-developed leaves from the forth node and below, and stem fragments from 5-20 cm from the shoot were fixed in FAA 70 **[10]**and kept in 70% ethanol (v/v) **[11]**.

Methodology

For light microscopy (LM), samples of the material were freehand sectioned in transverse and longitudinal planes, including paradermal, and stained with astra blue in combination withbasic fuchsine **[12]**. Alternatively, the material was embedded in glycol-methacrylate, sectioned using a rotary microtome and stained with toluidine blue **[13]**. Standard microchemical tests were also carried out to detect cell wall impregnation and cell content, such as ferric chloride for phenolic compounds**[10]**, methylene blue for mucilage**[14]**, Sudan for lipophilic substances**[15]**, hydrochloric phloroglucinfor lignin **[16]** and lugol for starch **[11]**. The chemical nature of the crystals was confirmed with dilute sulphuric acid **[14]**.

For studying the ultrastructure of the leaf surface**[17]**, scanning electron microscopy (SEM) was performedby means of dehydrating in ascending ethanol series and

CO_2 critical point drying, coating with gold and using a high vacuum system.

RESULTS

The leaflet, in face view, has epidermal cells with straight to slightly wavy anticlinal walls **(Figures 1A, 1B)** and anomocytic stomata confined to the abaxial surface **(Figures 1B-D)**. The leaf blade is coated with a striate cuticle **(Figures 1B, 1D)** and upright platelets of epicuticular wax **(Figures2A, 2B)**. Some glandular trichomes occur and possess a short pedicel and an ovate head **(Figures 2A, 2C)**.

In cross-section of the interveinal region, it is encountered a thick cuticle and a single-layered epidermis whose adaxial cells are comparatively larger than the abaxial ones. The stomata are inserted at the same level as the neighboring cells **(Figure 3A)**. A discontinuous subepidermal layer may occur next to the adaxial side and encompasses parenchymatic cells with large vacuoles**(Figures 3A, 3B)**. The mesophyll is dorsiventral and comprises about 4-5 rows of palisade parenchyma and 5-7 strata of spongy parenchyma, the latter forming large intercellular spaces. Traversing the chlorenchyma, there are minor collateral vascular bundles enclosed in a sclerenchymatic sheath which may extend to the epidermis **(Figure 3A)**. The midrib is biconvex **(Figure 3C)** and, below the epidermis, it presents annular collenchyma whose cell walls may be in an initial lignification process determined by the phloroglucin test. Collateral vascular bundles are distributed side by side in a ring-like arrangement and the whole set is encircled by a sclerenchymatic sheath **(Figures 3C, 3D, 4E)**. Several idioblasts identified as cells containing mucilage and phenolic compounds, as well as calcium oxalate druses are present all over the leaflet **(Figures 4A-E)**.

The stem, in cross-section, shows a periderm consisting of a multilayered suber and a phellogen originated peripherally **(Figures 5A, 5B)**. In the cortex, there are some groups of stone cells and cortical parenchyma **(Figures 5A-C)**. The phloem is disposed in triangular strands with portions of medullary rays between them, and it is stratified into fibrous zones **(Figures 6A, 6B)**. The xylem forms a closed cylinder and the pith is parenchymatic **(Figure 6D)**. Scattered idioblasts bearing mucilage (cavities) and phenolic compounds (cells) are often encountered in the cortex and pith **(Figures 5A, 6A, 6E)**. Several cells containing druses of calcium oxalate are present in all the caulinar tissues **(Figures 5A, 6B, 6C)**.

DISCUSSION

At present, there is little information focusing on microscopic attributes of the genus *Pachira* and the anatomical aspects

described in this work for *P. aquatica* are commonly reported for the family species which have tropical arboreal habit. These characters comprise striate and thick cuticle, dorsiventral mesophyll and hypostomatic leaf as well as stem with periderm superficial in origin, stone cells in the cortex, phloem as triangular strands with the apices towards the exterior and stratified by group of fibers, and cells bearing phenolic compounds **[18]**.

Although these characters are shared within the family, there are others that may have taxonomic value at lower levels, such as calcium oxalate crystals, epicuticular wax, subepidermal layers and trichomes **[19, 20]**. Calcium oxalate crystals play different roles in the plant, including protection from herbivory, metal detoxification and tissue calcium regulation **[21]**. Despite the morphology and spatial distribution of calcium oxalate crystals being conserved within specific taxa, assigning diagnostic value to the druses encountered herein in *P. aquatica* is premature because there are scanty data concerning related species.The same consideration applies to the epicuticular wax. Having great ultrastructure diversity and often notable taxonomic significance, waxes are classified in different categories and the most prominent are platelets and tubules **[22]** and the former have been described for *P. aquatica* in this work.

With reference to trichomes, the peltate and stellate types are often observed in the family **[18, 19]**. In partial agreement, *P. aquatica*has shown a few glandular trichomesin this work and they probably correspond to the peltate type mentioned in other related species. On the other hand, regarding the subepidermal layer observed herein, there seems to be some controversy on the origin of this tissue since some surveys on close genera have described it as multiseriate epidermis and hypoderm **[18]**. By definition, these tissues have different meristematic origins, protoderm and ground meristem respectively, and should be distinguished. In this work, in the absence of an ontogenetic approach, this layer has been merely named in accordance with the tissue localization.

Mucilage is common in Malvaceae and this group of metabolites may function as polysaccharide storage and water retention in plants. As expected, it was secreted in cells and cavities of *P. aquatica*. Both mucilage and phenolic compounds, other group of metabolites detected in this investigation, play key roles in the strategies of defense of plants, *e.g.*, protection from injurious UV radiation and deterrence to grazing animals and feeding insects. They provide an evolutionary benefit to plants

and are used to classify them taxonomically **[23]**. Thus, the presence of scattered mucilage and phenolic-storing cells in *P. aquatica* may have a diagnostic value.

CONCLUSIONS

The microscopic characters presented in this work are novelties for *P. aquatica* and some of them, such as platelets of epicuticular wax, glandular trichomes and subepidermal layer in the leaf, as well as druses of calcium oxalate, mucilage and phenolic compounds in the leaf and stem contribute to the speciescharacterization for pharmacognostic purposes. However, further research is needed for comparison among allied species to evaluate their usefulness for taxonomic applicability.

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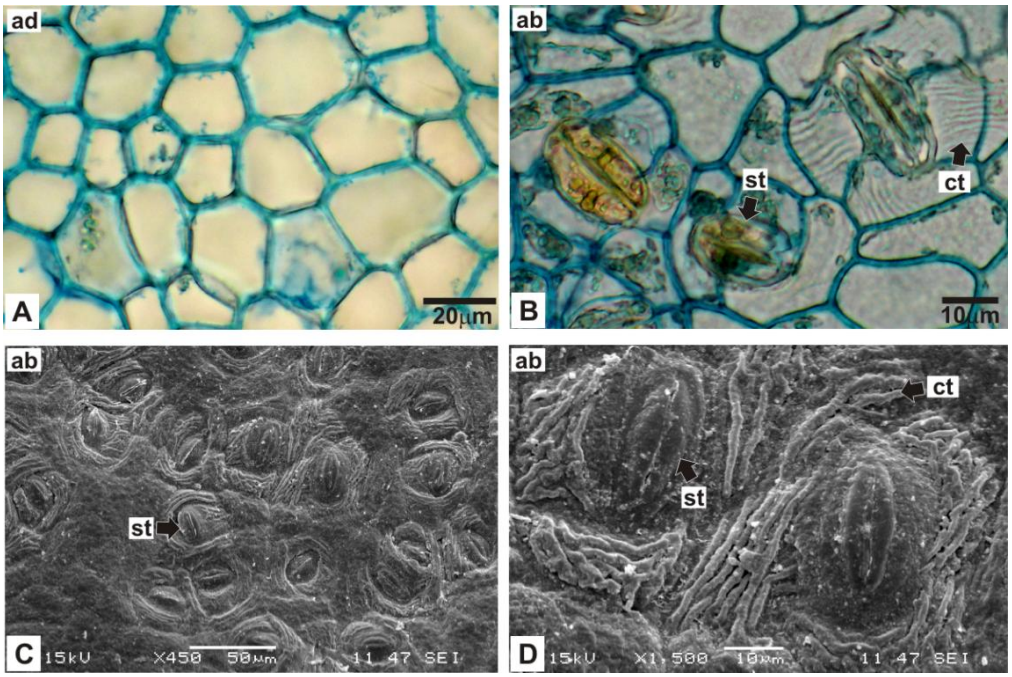

Figure 1: ***Pachiraaquatica*****, face view of the leaf epidermis in LM (A, B) and SEM (C, D); Abbreviations: ab – abaxial surface, ad – adaxial surface, ct – striate cuticle, st – stomatum**

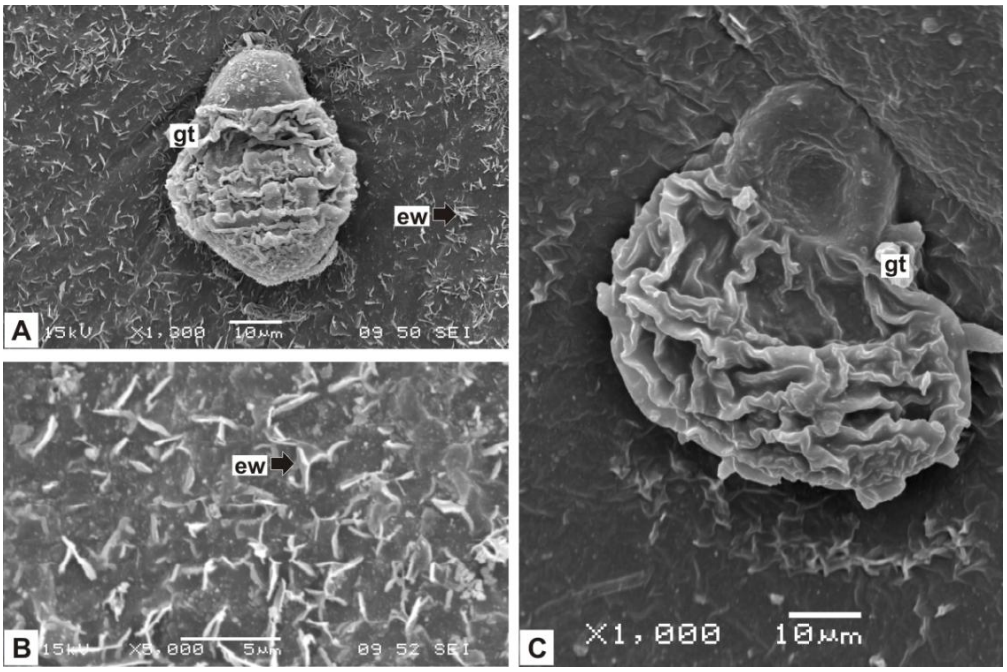

Figure 2: ***Pachiraaquatica,*****SEM examinations of the leaf epidermis (A-C).Abbreviations: gt – glandular trichome, ew – epicuticular wax.**

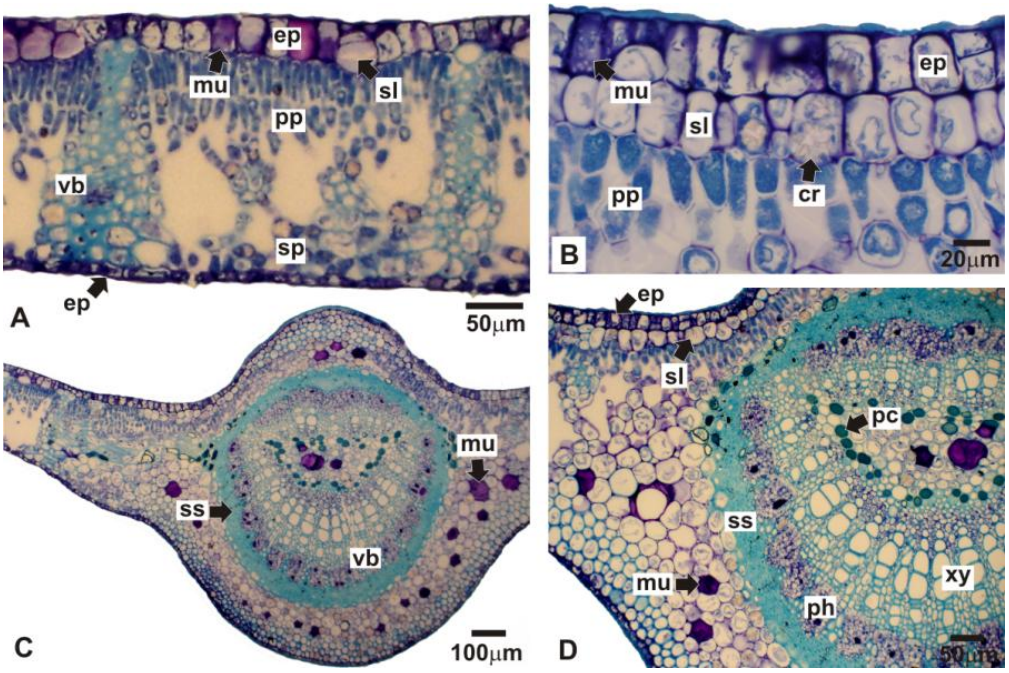

Figure 3: ***Pachiraaquatica,*****LM images of the leaf in cross-section, showing the interveinal region (A, B) and midrib (C, D). Abbreviations: cr – crystal of calcium oxalate, ep – epidermis, mu – mucilage, pc – phenolic compounds, ph – phloem, pp – palisade parenchyma, sl – subepidermal layer, sp – spongy parenchyma, ss – sclerenchymatic sheath, st – stomatum, vb – vascular bundle, xy – xylem**

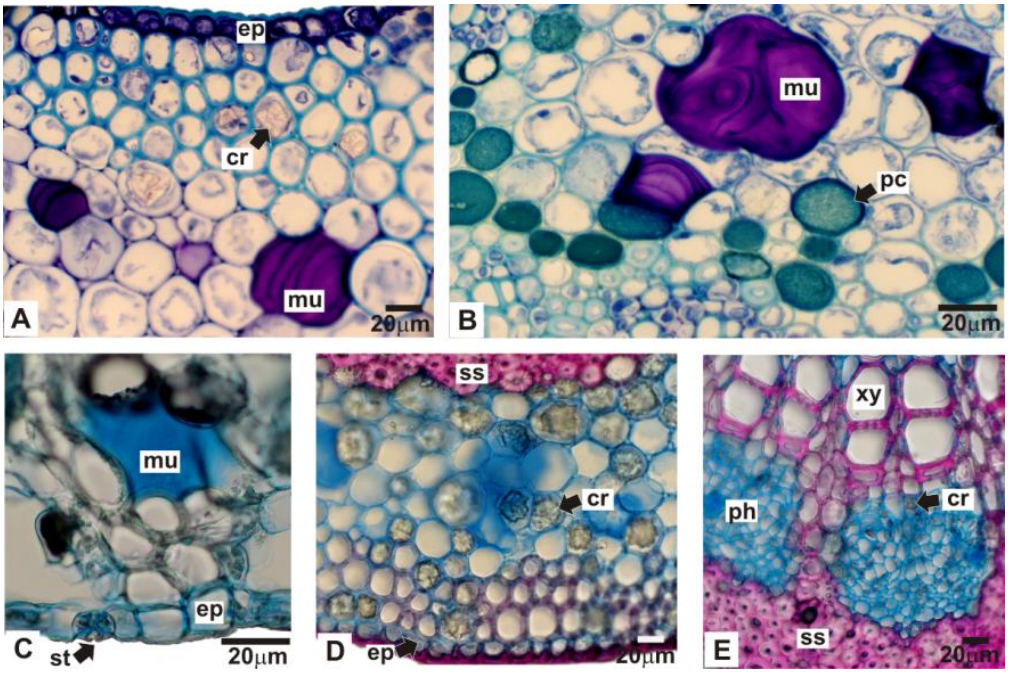

Figure 4: ***Pachiraaquatica,*****LM images of the leaf in cross-section, displaying idioblasts with mucilage, phenolic compounds and crystals (A-D) and detail of the vascular bundle of the midrib (E).Abbreviations: cr – crystal of calcium oxalate, ep – epidermis, pc – phenolic compounds, ph – phloem, mu – mucilage, ss – sclerenchymatic sheath, st – stomatum, xy – xylem.**

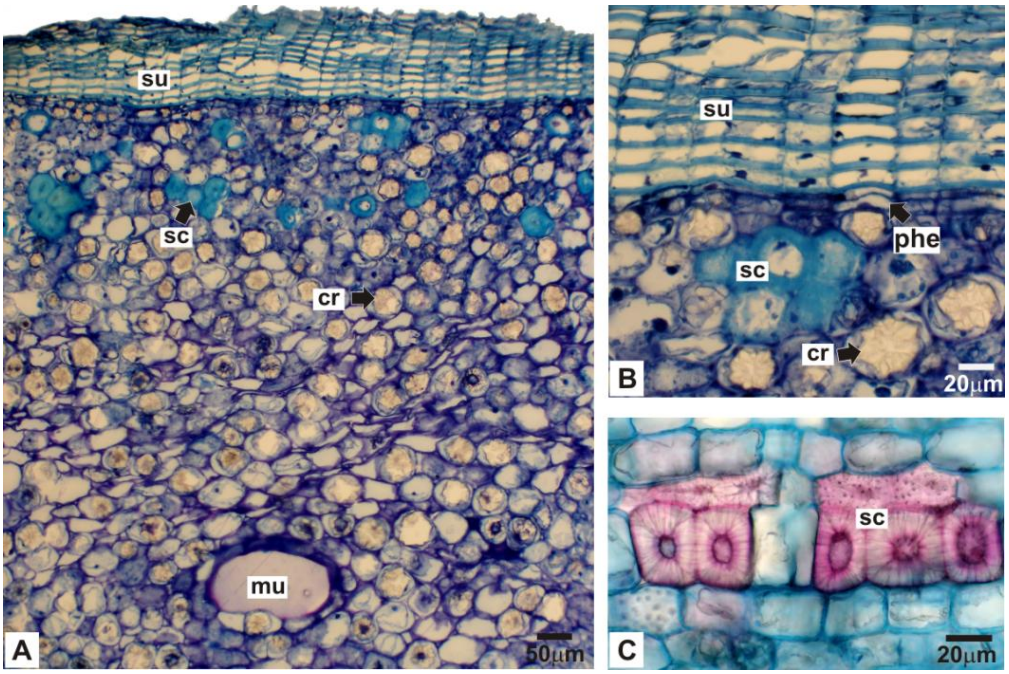

Figure 5: ***Pachiraaquatica*****,LM images of the stem in cross-section, revealing details of the periderm and cortex (A-C).Abbreviations: cr – crystal of calcium oxalate, mu – mucilage, phe – phellogen, sc – stone cell, su – suber.**

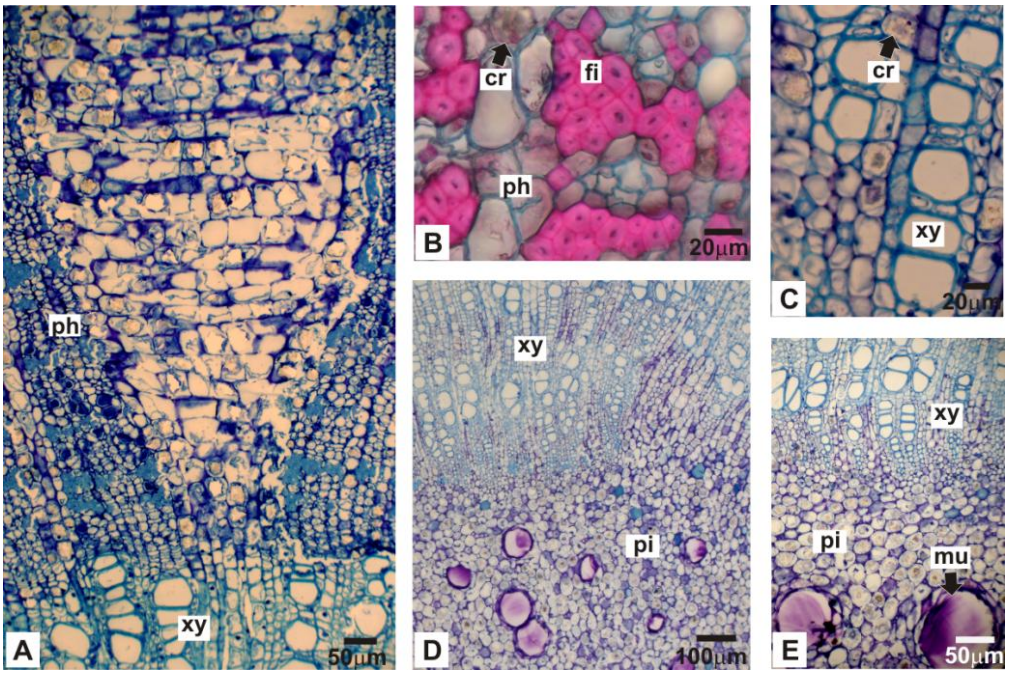

Figure 6: ***Pachiraaquatica,*** **LM images of the stem in cross-section, showing details of the vascular system (A-E).Abbreviations: cr – crystal of calcium oxalate, mu – mucilage, pc – phenolic compounds,ph – phloem, pi – pith, xy – xylem.**