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PHARMACOGNOSTIC CHARACTERIZATION OF THE LEAF AND STEM OF *Xylopia aromatica* (LAM.) MART., ANNONACEAE

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ABSTRACT

Circumscribed to the Annonaceae, there are many tropical species economically important as food and medicinal plants. Among them, it is encountered *Xylopia aromatica* whose leaves and stems are use in folk medicine as carminative, stimulant and antimalarial. Various surveys have demonstrated that the species produces essential oil and has cytotoxic activity against tumor cell lines and antiprotozoal properties. Since a few investigations have dealt with anatomical aspects of *X. aromatica*, this work has studied the microscopic characters of its leaf and stem for pharmacognostic purposes. The botanical material was prepared for light and scanning electron microscopy, including microchemical staining tests. The leaf is hypostomatic, presenting paracytic stomata inserted on the same level as the other epidermal cells. Several non-glandular trichomes, long and uniseriate, are present. The leaf has thick cuticle covering the uniseriate epidermis, subepidermal layer and dorsiventral mesophyll. The midrib is plano-convex and the petiole is circular in cross-section, and both have collateral vascular bundles enclosed in a sclerenchymatic sheath. The stem shows phloem arranged in triangular strands and stratified into fiber zones and xylem with solitary or small groups of tracheary elements. The vascular cylinder is surrounded by a discontinuous sclerenchymatic sheath and the pith is parenchymatic. Small druses of calcium oxalate and round secretory cells occur in the leaf and stem. These microscopic features are a novelty for the species and provide useful information to characterize it in pharmacognostic assays.

Keywords: Medicinal Plant, Plant Anatomy, Secretory Cell, Subepidermal Layer, Trichome, *Xylopia aromatica*

INTRODUCTION

The Annonaceae family includes about 130 genera and 2200 species, largely tropical and economically relevant as a source of food and medicinal plants **[1, 2]**. Amongst them, there is *Xylopia aromatica* (Lam.) Mart., a tree which occurs in South and Central America **[3]**, attains 5-10 m tall and has whitish flowers, scented fruits and spicy seeds **[4, 5]**. In folk medicine, its leaf and stem are used as carminative, stimulant **[6]** and antimalarial **[7]**.

Phytochemical investigations have shown that the species produces essential oil whose main bioactive components are bicyclogermacrene and spathulenol **[4, 8, 9]**. Moreover, pharmacological assays have demonstrated that the leaf and stem extracts have cytotoxic activity against tumor cell lines **[6, 10-12]** and antileishmanial and antitrypanosomal properties**[3, 7, 13]**.

Although these promising results have drawn attention to *X. aromatica*, a few surveys have been carried out to investigate its anatomical aspects. Therefore, this work has studied the microscopic characters of its leaf and stem, which are useful in the characterization of this medicinal plant and potential vegetal drug for pharmacognostic purposes.

MATERIALS AND METHODS

Plant Material

Samples of *Xylopia aromatica* (Lam.) Mart. (Annonaceae) were collected in a sunny field located in the city of Caldas Novas, Goias, in the center of Brazil (coordinates approximately $17^{o}43'$ S and $48^{o}37'$ W and altitude of 670 m), in June 2004. The species identification was confirmed in the herbarium at the Museu Botânico Municipal de Curitiba (voucher MBM 287624).

Methodology

Fresh adult leaves from the forth node and below and young stems obtained 5-30 cm from the shoot were chemically fixed with FAA 70 **[14]** and stored in 70% ethanol ethanol **[15]**. For light microscopic observations, the material was freehand sectioned in transverse and longitudinal planes, stained with astra blue-basic fuchsine **[16]** and mounted with 50% glycerin. For long term storage of slides, leaf and stem fragments were embedded in synthetic resin, sectioned using a rotary microtome, stained with toluidine blue **[17]** and prepared with a synthetic mounting medium.

Additionally, microchemical staining tests were performed to detect some metabolites in the cell as well as the nature of the cell wall impregnation: Sudan for lipophilic substances**[18]**, lugol for starch**[15]**, acid phloroglucin for lignin **[19]**, ferric chloride for phenolic compounds **[14]**, methylene

blue for mucilage and dilute sulfuric acid for calcium oxalate crystals **[20]**.

To investigate the epidermal relief, fixed leaf fragments were dehydrated in an ascending ethanol series and by the CO_2 critical point, coated with gold and examined in a scanning electron microscope using high vacuum **[21]**.

RESULTS

The leaf, in face view, has epidermal cells with polygonal anticlinal walls on both surfaces **(Figures 1A, 1B)** and paracytic stomata restricted to the abaxial side following a typical hypostomatic pattern **(Figure 1B)**. In cross-section, it is observed that the guard-cells are inserted on the same level as the surrounding ones **(Figure 1C)**.

There are several non-glandular trichomes **(Figures 1D, 1E)**, long, multicellular, uniseriate, with an acute apex and thick cell walls. The interveinal region, in cross-section, shows a uniseriate epidermis coated with a thick cuticle **(Figures 2A-D)**. Below the adaxial side, it is encountered a subepidermal layer, occasionally 2- or 3-seriate, consisting of parenchymatic cells with large vacuoles **(Figures 2A-D)**. The mesophyll is dorsiventral, encompassing about five rows of palisade parenchyma and seven strata of spongy parenchyma **(Figures 2A, 2B)**. Traversing the chlorenchyma, there are minor vascular bundles, collateral and encircled by a sclerenchymatic sheath which may extend to the epidermis forming bundle sheath extensions.

The midrib is plano-convex, in cross-section **(Figure 2A)**. A thick cuticle covers a uniseriate epidermis which has convex external periclinal walls **(Figures 3A, 3C)**. Collateral vascular bundles are present and arranged in open arc **(Figures 2A, 3B, 3D)**, and the whole set is enclosed in a conspicuous sclerenchymatic sheath **(Figures 2A, 3A, 3B)**. The petiole is approximately circular and is traversed by three collateral vascular bundles, disposed side by side and embedded in a sclerenchymatic sheath **(Figures 4A, 4B)**. Large groups of sclerenchymatic cells are found in the ground parenchyma near the vascular system **(Figure 4A)**.

Some small calcium oxalate crystals, usually as druses, and various scattered secretory cells, often round, with moderately thick walls and lipophilic content, are found in the interveinal region **(Figures 2B-D)**, midrib **(Figures 3A, 3C)** and petiole **(Figures 4A, 4C)**.

The stem, in incipient secondary development, shows a periderm and few-layered cortex **(Figure 5A)**. In the vascular cylinder, it is encountered a discontinuous sclerenchymatic sheath outside the phloem **(Figure 5A)**. This conducting system is arranged in triangular strands separated by medullary rays and stratified into fiber

zones **(Figures 5A, 5B)**. The xylem is completely lignified and traversed by narrow rays. The tracheary elements are solitary or disposed in small groups. The pith is parenchymatic **(Figure 5B)**. Few secretory cells bearing lipophilic substances **(Figure 5A)** and small druses of calcium oxalate are present in the stem.

DISCUSSION

Based on the results of this survey, *X. aromatica* has shown microscopic characters in accordance with Annonaceae, a family considered rather uniform in structure, particularly in wood features. The leaf characteristics observed in this work and commonly mentioned for the taxon are hypostomatic leaf with paracytic stomata, guard-cells usually on the level of the surface, thick cuticle covering an epidermis with polygonal cells in outline, and dorsiventral mesophyll **[22, 23]**.

Some other aspects are considered of important diagnostic value, such as the multilayered epidermis in certain species, usually referred as hypodermis **[22]**. This tissue is often translucent when parenchymatous and much larger than the epidermis **[23]**. In this study, it was observed a subepidermal layer, uni- to 3-seriate subjacent to the adaxial surface which is possibly equivalent to the hypodermis. However, in the absence of an ontogenetic approach in this analysis, this layer was merely named adopting a topographic designation to avoid inaccuracy.

With regard to the vascular system, minor vascular bundles with sclerenchymatic extensions into the mesophyll, often vertically transcurrent are reported for some species of Annonaceae **[22, 23]**, in agreement with the present findings. For the midrib, Metcalfe **[22]** has stated that the vascular system is usually in the shape of a crescentiform strand in the family and it can be interrupted by and supported on its abaxial side by sclerenchyma. In that author's opinion, the vascular organization of the petiole has taxonomic relevance and for the genus *Xylopia* the bundles are in number of three and they remain clearly distinguishable and surrounded by sclerenchymatous fibers, in the same manner described herein for *X. aromatica*.

In *Xylopia*, the occurrence of secretory cells, mostly referred to as oil cells, although mucilage or resinous substances may be frequent, can contribute to distinguish species according to their reaction stains, size and distribution. Having similar importance, calcium oxalate crystals are very common in the family, especially in the epidermis, mesophyll or surrounding the veins and are described as small druses in *Xylopia* **[22]**. These characteristics are confirmed in this work, exhibiting *X.*

aromatica round secretory cells with lipophilic substances and small druses distributed in the leaf.

In comparison with related species, Justo *et al*. **[24]** have described for*X. Brasiliensis* Spreng. leaf with uniseriate non-glandular trichomes, one-layered epidermis with sinuous anticlinal cell walls, paracytic stomata confined to the abaxial surface, dorsiventral mesophyll, druses and idioblasts containing lipophilic substances. For Santos *et al*. **[25]**, these idioblasts are secretory cavities in *X. brasiliensis* and they possibly have lysigenous origin. Although *X. brasiliensis* and *X. aromatic* share many anatomical features, they can be distinguished for the latter displaying epidermis with polygonal anticlinal cell walls and an evident subepidermal layer, uni- to 3-seriate.

Although *X. benthamii* R.E.Fr. and *X. nitida* Dunal investigated by Almeida *et al*. **[26]** show microscopic features similar to the present description of *X. aromatica*, they can be differentiated from each other. The species *X. benthamii* possesses epidermal cells with wavy contour in face view, secretory cavities and absence of subepidermal layer. On the other hand, *X. nitida* displays various secretory cavities in the leaf, in contrast with the secretory cells of *X. aromatica*.

Nevertheless, distinguishing *X. aromatica* and *X. frutescens* Aubl., based on the work by Silva and Grotta **[27]**, authors who published a brief anatomical study of the latter species, is a difficult task. Both species have many structures in common, including the presence of subepidermal layer next to the adaxial surface, druses and secretory cells bearing essential oil droplets distributed in different regions of the leaf. However, those authors have not mentioned trichomes in *X. Frutescent s*and, if the lack of these epidermal appendages is confirmed, it can be useful for identifying both species.

With respect to the stem in Annonaceae, often the periderm is superficial, strands of pericyclic fibers occur, the phloem is cone-shaped and stratified tangentially into fibrous and non-fibrous zones, the vascular cylinder is traversed by a combination of narrow and broad rays, and secretory cells and crystals are present **[22, 23]**. According to Luchi *et al*. **[28]**, who have analyzed the wood anatomy of *X. aromatica*, the vessels are mostly solitary or in radial multiples, and secretory idioblasts are found. In general, these characters have been confirmed in this study for *X. aromatica*.

CONCLUSION

The majority of these features are a novelty for *X. aromatica* and, as expected, the species has exhibited general anatomical characters of the leaf in agreement with

Annonaceae and shared by allied species, with emphasis on paracytic stomata on the abaxial epidermal surface, dorsiventral mesophyll, collateral vascular bundles and small druses of calcium oxalate. For distinguishing *Xylopia* species, useful diagnostic characters include the presence of non-glandular trichomes, subepidermal layer and secretory cells. The stem features are predicted to be rather uniform in the family and assigning distinctive significance for some characters requires further investigations on the genus.

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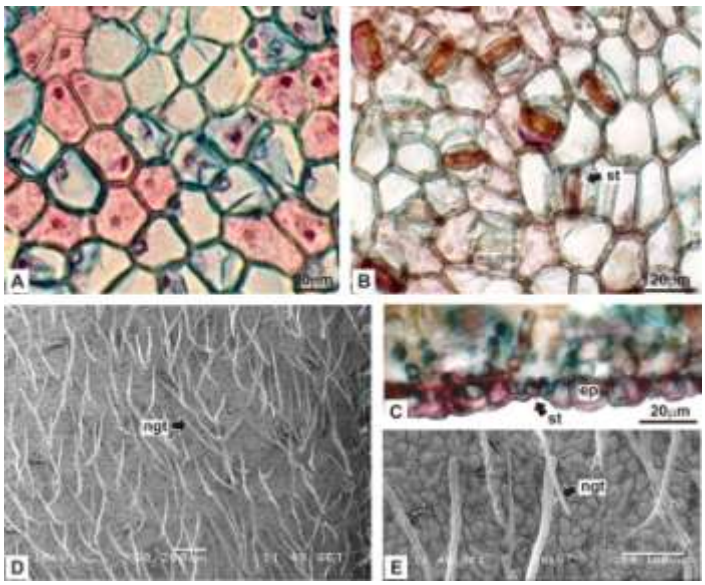

Figure 1: *Xylopia aromatica* leaf: A, B. Face view of the epidermis, adaxial and abaxial surfaces, respectively; C. Cross-section of the abaxial surface of the epidermis, showing the stomatum position; D, E. Scanning electron micrographs of the abaxial surface of the epidermis, displaying numerous non-glandular trichomes. Abbreviations: ep - epidermis, ngt – non-glandular trichome, st – stomatum

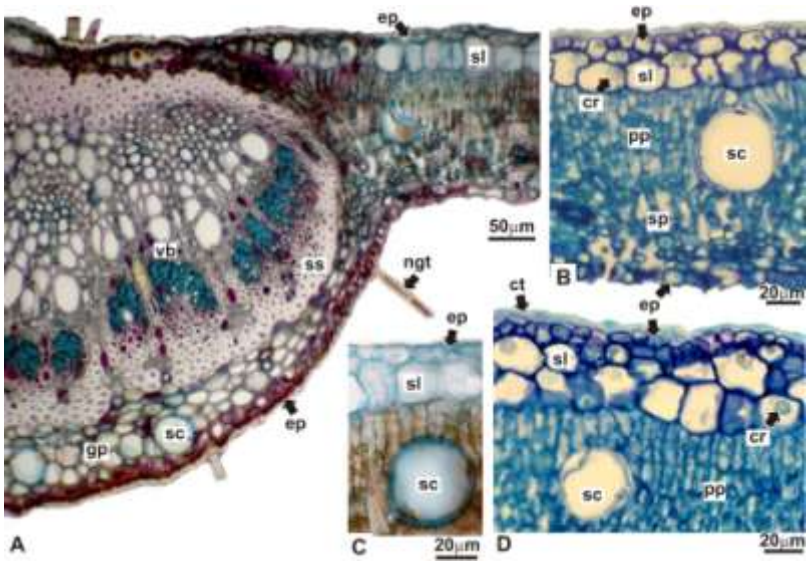

Figure 2: ***Xylopia aromatica*** **leaf, in cross-section: A. Interveinal region and midrib; B-D. Details of the interveinal region. Abbreviations: cr – crystal, ct – cuticle, ep – epidermis, ngt – non-glandular trichome, pp – palisade parenchyma, sc – secretory cell, sl - subepidermal layer, sp – spongy parenchyma, ss – sclerenchymatic sheath, vb – vascular bundle**

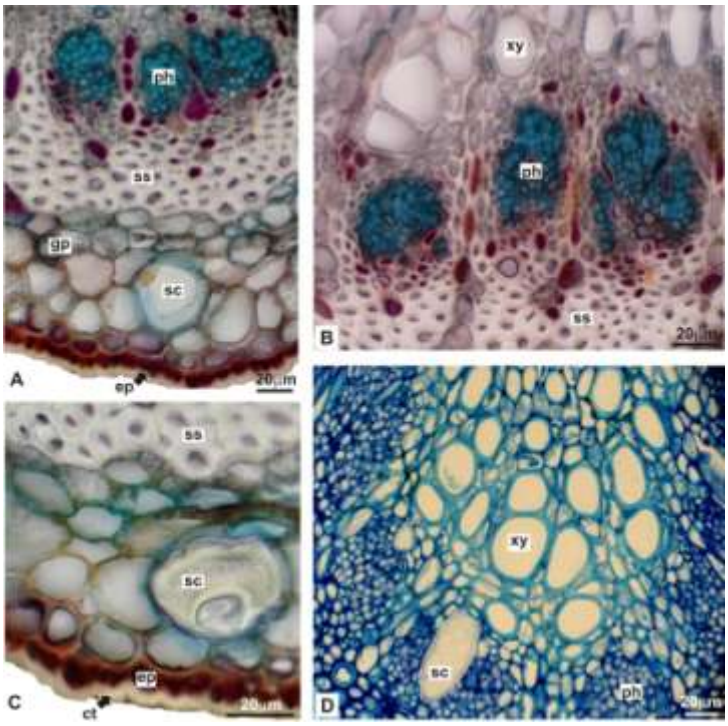

Figure 3. ***Xylopia aromatica*** **midrib, in cross-section: A, B, D. Vascular system; C. Detail of a secretory cell. Abbreviations: ct – cuticle, ep – epidermis, gp – ground parenchyma, ph – phloem, sc – secretory cell, ss – sclerenchymatic sheath, xy - xylem**

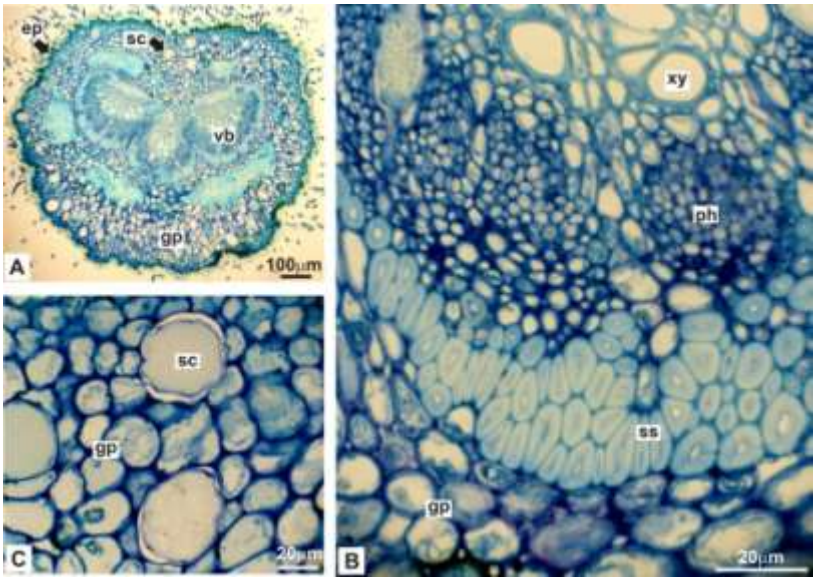

Figure 4: ***Xylopia aromatica*** **petiole, in cross-section: A. Overall aspect; B. Detail of the vascular system; C. Secretory cells in the ground parenchyma. Abbreviations: ep – epidermis, gp – ground parenchyma, ph – phloem, sc – secretory cell, ss – sclerenchymatic sheath, vb – vascular bundle, xy – xylem.**

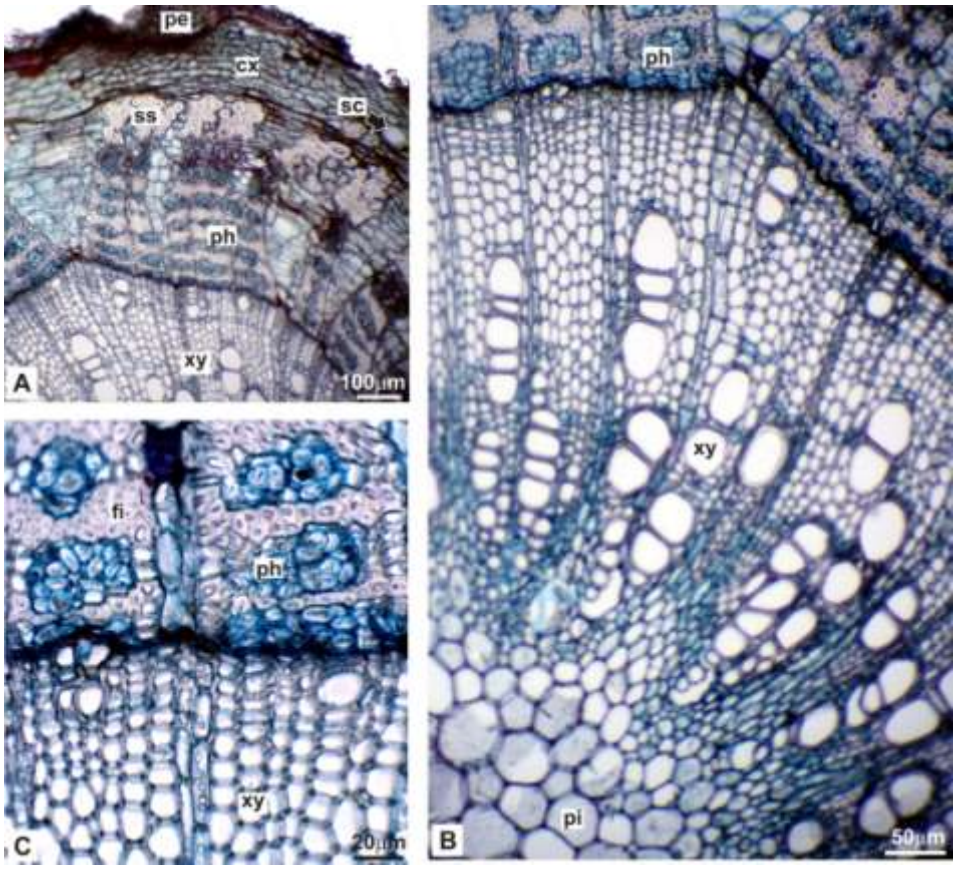

Figure 5: ***Xylopia aromatica*** **stem, in cross-section: A. General feature; B, C. Details of the vascular cylinder. Abbreviations: cx – cortex, fi – fiber, pe – periderm, ph – phloem, pi – pith, sc – secretory cell, ss – sclerenchymatic sheath, xy – xyle**m.