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Original Article

Anatomical and Histochemical Characterization of Leaves of *Luffa cylindrica* (L.) M. Roem

Laís Emanuelle Bernardo Vieira, Rafaela Damasceno Sá, Karina Perrelli Randau*

ABSTRACT

Background: *Luffa cylindrica* (L.) M. Roem. (Cucurbitaceae) is an herbaceous plant used for food as compose salads and do sweets and also used in traditional medicine as treat parasitic infections and intestinal diseases. Although this information, there are not many relates about anatomic characters for use in your quality control. **Objective:** The aim was investigating the anatomical characters of petiole and leaf blade and characterizing the metabolites in the leaf blade of *L. cylindrica*. **Materials and Methods:** Semipermanent histological slides were prepared for analysis of petiole and leaf blade in optical microscopy. Histochemical tests were also performed in the leaf blade. **Results:** The anatomical study revealed information about the type of trichomes, cuticle, vascular bundles and arrangement of the tissues that determine the botanical identity of this species. It was also identifying, for the first time, the presence of two types of trichomes in both of leaf blades faces. The histochemistry allowed determining which metabolites are in the leaf blade and also their location. **Conclusion:** The study described new characters for *L. cylindrica* and the results provide support to quality control of the species.

Key words: Anatomy, Cucurbitaceae, Histochemistry, *Luffa cylindrica*, Microscopy.

INTRODUCTION

The Cucurbitaceae family have 134 genera and encompass over 965 species. The principal genera about to the family are *Bryonopsis, Citrullus, Corallocarpus, Cucumis, Cucurbita, Lagenaria, Luffa, Momordica* and *Trichosanthes* and they can be cultivated in the tropics, subtropics, arid and temperate regions.[1-3]

The cucurbits are herbaceous. The most species having large leaves, the foliage is whole or in deep lobes and they can be climbing or creeping plants. The flowers are having differences sexes and a large inferior ovary. After fertilization, the ovary develops into a fruit processing hard exocarp, fleshy mesocarp and endocarp.[4-5] Many cucurbits' fruits have been an ingredient for the culinary and are eaten when immature or mature by salads, sweets, desserts or pickled.[6-7]

Besides being food, some species are often used in popular medicine throughout the world, such as in America,[8-9] Africa[10-11] and Asia.[12-13] The principal parts of the plant used to treat icterus, gastric diseases, diabetes, sinusitis, lung diseases, fever, cancer, inflammation, skin infections, pain, among others, are roots, leaves, fruits and seeds.[8-13] Many plants of the Cucurbitaceae family contain cucurbitanes, which is a type of triterpene.[14]

Brazil has some cucurbit genera. Around 28 genera and 160 species occur in all states in the country. Among them, *Luffa* is a genus it has had two species: *Luffa cylindrica* (L.) M. Roem. moreover, *Luffa operculata* (L.) Cogn.[15] *Luffa cylindrica* is popularly known as "Bucha" and its root, leaves, fruits and seeds has been used to treat infections and gastric disease.[16-17] The leaves are used in Guinea Bissau to intestinal diseases, fever and malaria,[18] in Bolivia to anuria,[19] in Cuba to parasitic infections,[20] in India to indigestion[21] and in Pakistan as laxative.[22]

Although the medicinal use of Cucurbitaceae family has been much reported in the literature, the quality control about many members of its family is not too reported. Therefore, this research aimed to perform the anatomic and histochemistry characterization of leaves of *L. cylindrica*, in order to expend the pharmacobotanical information about species of Cucurbitaceae family.

MATERIALS AND METHODS

Plant material

Adult leaves of specimens of *Luffa cylindrica* (L.) M. Roem., Cucurbitaceae, were collected in the city of Aliança, Pernambuco, Brazil. A voucher specimen was deposited in the Herbarium Dárdano de Andrade Lima of the Instituto Agronômico de Pernambuco (IPA), under collection number 92082.

Anatomical characterization

Cross-sections were obtained by hand, using a common razor blade, at the middle region of petiole

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and leaf blade fixed in FAA 50.[23] Leaf blade paradermal sections were also performed on the adaxial and abaxial surfaces. All sections were clarified with sodium hypochlorite solution (50%),[24] followed by washing with distilled water. Lastly, cross-sections were stained with safranin and astra blue[25] and paradermal sections were stained with methylene blue (1%).[26] Semipermanent histological slides were prepared to contain the sections of botanical material, following common plant anatomy procedures.[23,27] The analysis of the slides was conducted using a polarized microscope (Leica DM750M) coupled with a digital camera (Leica ICC50 W), through images processed in software (LAS EZ).

Histochemical characterization

Histochemical tests were made on cross-sections of fresh leaf blades obtained by hand, using a common razor blade.[23] The specific reagents used were: potassium dichromate (10%) for phenolic compounds,[28] Dragendorff's reagent for detecting alkaloids,[29] vanillin-chloridric acid for tannins,[30] antimony trichloride for triterpenes and steroids,[31] Nadi reagent for essential oils,[32] Sudan III for lipophilic substances,[27] phloroglucinol for lignin,[23] Lugol's iodine reagent for starch.[23] Controls were performed in parallel with the tests and semipermanent histological slides were prepared containing the cross-sections,[23,27] which were analyzed under an optical microscope (Alltion).

RESULTS

In cross-section, the petiole of *L. cylindrica* has rounded outline, with two ribs in the adaxial face (Figure 1A, B). It shows an epidermis with a single stratum of cells, covered with a thin cuticle (Figure 1A). This species present two types of trichomes: the non-glandular trichomes and the glandular trichomes. The non-glandular trichomes are pluricellular uniseriate and the glandular trichomes have peduncle and apex both of them multicellular (Figure 1C, D). The collenchyma has an angular form and its constitution is around four to five cell layers (Figure 1A). The vascular system is composed of eleven bicollateral vascular bundles, being two bundles in two ribs on the adaxial face (Figure 1A) and nine bundles are in the central region of the petiole, forming a ring (Figure 1B).

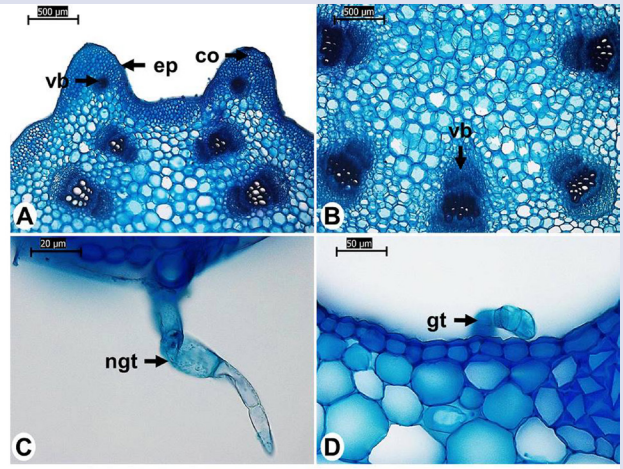

Figure 1: Cross-Sections of the Petiole of *Luffa cylindrica* (L.) M. Roem. A, B. General view. C. Non-glandular trichome. D. Glandular trichome. co: Collenchyma, ep: Epidermis, gt: Glandular trichome, ngt: Non-glandular trichome, vb: Vascular bundle.

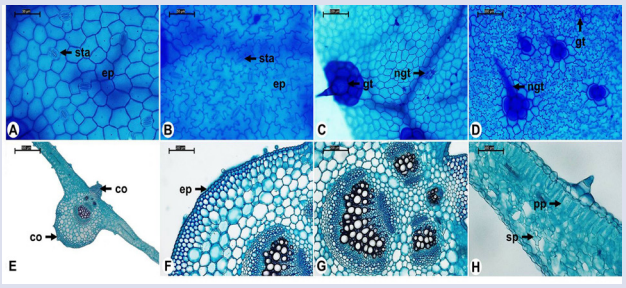

Figure 2: Frontal view and cross-sections of the leaf blade of *Luffa cylindrica* (L.) M. Roem. A, C. Adaxial surface. B, D. Abaxial surface. E, F, G. Midrib. H. Mesophyll. co: Collenchyma, ep: Epidermis, gt: Glandular trichome, ngt: Non-glandular trichome, pp: Palisade parenchyma, sp: Spongy parenchyma, sta: Stomata.

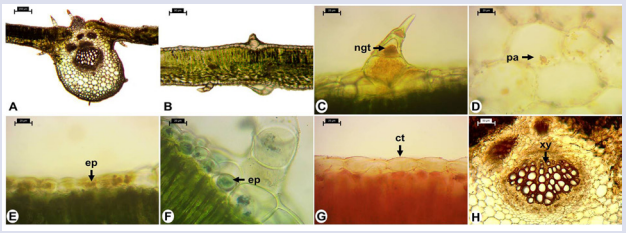

Figure 3: Histochemistry of the leaf blade of *Luffa cylindrica* (L.) M. Roem. A, B. Control. C. Potassium dichromate (10%). D. Dragendorff's reagent. E. Antimony trichloride. F. Nadi reagent. G. Sudan III. H. Phloroglucinol. ct: Cuticle, ep: Epidermis, ngt: Non-glandular trichome, pa: Parenchyma, xy: Xylem.

The leaf blade, in frontal view, is amphistomatic with anomocytic stomata (Figure 2A, B). The epidermis has cell walls with the straight or slightly sinuous contour on the adaxial face (Figure 2A), although in the abaxial face the epidermis has cell walls with a strongly sinuous contour (Figure 2B). Both of them leaf blade faces have the same types of trichomes seen at petiole (Figure 2C, D).

In cross-section, the midrib has a biconvex outline (Figure 2E). Its epidermis is uniseriate with thin cuticle (Figure 2F). The collenchyma is angular composed of eight to ten cells layer in the adaxial face and two to three cells layer in the abaxial face (Figure 2E). The vascular system is made with bundles arranged in ring form as the petiole, however in midrib are just four bundles (Figure 2G). About the bundle distribution, three of them are bicollateral and one comes back to adaxial face and it is collateral. The mesophyll is dorsiventral with one layer of palisade parenchyma and its spongy parenchyma has braciform cells (Figure 2H).

The Figure 3A and 3B show the cross-section of leaf blade without the addition of reagent. After the addition of reagents to identify secondary metabolites were obtained these results: phenolic compounds were found in non-glandular trichomes (Figure 3C); alkaloids were seen in parenchyma of the midrib (Figure 3D); triterpenes, steroids and essentials oils were evidenced in epidermal cells (Figure 3E, F); Sudan III revealed lipophilic compounds in cuticle (Figure 3G) and the phloro-

glucinol showed the existence of lignin in xylem (Figure 3H). Negative results were identified for tannins and starch.

DISCUSSION/ CONCLUSION

The petiole outline found in *L. cylindrica* is different from other species of Cucurbitaceae family. Rus *et al.*[33] found a semicircular outline, laterally compressed in *Bryonia alba* L. In *Momordica charantia* L., Sá *et al.*[34] observed a convex outline with two ribs on the adaxial surface and a triangular outline on the abaxial surface. *M. tuberosa* Cogn. revealed a convex outline on the adaxial surface and triangular outline on the abaxial surface.[35] About the presence of collenchyma in the petiole, this tissue has also been observed in *B. alba* and some species of *Momordica*.[36] In the latter case, Aguoru and Okoli have verified that the number of collenchyma layers can be a character to differentiate them.[36] In the same work with species of *Momordica*, Aguoru and Okoli described that the number of bicollateral vascular bundles in the petiole ranged from six to eighteen.[36] Săvulescu and Hoza[37] found seven bicollateral bundles in the central region of the petiole and two bicollateral bundles in the ribs of *M. charantia*, while Sá *et al.*[34] visualized five bicollateral bundles in the central region of the petiole and two bicollateral bundles in the ribs of *M. charantia*. Rus *et al.*[33] found nine bicollateral bundles in *B. alba,* being two situated near the compression.

The number of vascular bundles also varies in the leaf blade of the Cucurbitaceae species. Ajuru and Okoli[38] identified eight bicollateral bundles in *Cucumis melo* L., four in *Cucurbita moschata* Duchesne and two in *Cucumeropsis mannii* Naudin. Mohammed and Guma[39] reported the presence of seven bicollateral bundles in species of *Citrullus* and *Cucurbita*, three in *Cucumis* and four in *Luffa*. Although the presence of bicollateral bundles in the family is common,[40] in this research was identified one collateral vascular bundle in the leaf blade of *L. cylindrica*. Collateral vascular bundles have also been identified in *B. alba*[33] and *M. charantia*.[41]

Non-glandular and glandular trichomes are common in the family Cucurbitaceae and, in this study, they were found in the petiole and leaf blade of *L. cylindrica*. Some researches relative to leaf blade of *Luffa* species mentioned only the presence of non-glandular trichomes as in *L. acutangular* (L.) Roxb., *L. echinate* Roxb., *L. cylindrica*[38] and *L. aegyptiaca*.[39] Alves *et al.*[42] described non-glandular and glandular trichomes in *L. aegyptiaca* and Rus *et al.*[33] in *B. alba*. Thus, in this work is possible to note the first description about the presence of non-glandular and glandular trichomes in *L. cylindrica*.

Alves *et al.*[42] showed that *L. aegyptiaca* also has an amphistomatic leaf blade, with anomocytic stomata. However, a useful character to distinguish it from *L. cylindrica* is that *L. aegyptiaca* presents collenchyma only on the adaxial face of the midrib, while *L. cylindrica* showed collenchyma in both faces.[39] In relation to mesophyll, in *C. melo*, *C. moschata* and *C. mannii* the palisade parenchyma is constituted of two to three layers of cells.[38] Phytochemical investigations demonstrated the presence of phenolic compounds, alkaloids, terpenes and steroids in leaves of *L. cylindrica*,[43-45] corroborating the results found through histochemistry tests in this study. The presence of tannins in the leaves of the species is divergent, as it was not evidenced in the present study nor the investigation of Aboh *et al.*[44] but Partap *et al.*[43] evidenced tannins. Flavonoids, tannins, alkaloids and terpenes have been identified in *L. acutalanga* and *L. aegyptiaca*.[46-48]

The findings of this study can be used as a tool for identification of *L. cylindrica* and assists in its pharmacobotanical standardization.

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CONFLICT OF INTEREST

There are no conflicts of interest.

ABBREVIATIONS

CO: Collenchyma; **CT:** Cuticle; EP: Epidermis; **GT:** Glandular Trichome; **NGT:** Non-Glandular Trichome; **PA:** Parenchyma; **PP:** Palisade Parenchyma; **SP:** Spongy Parenchyma; **STO:** Stomata; **VB:** Vascular Bundle; **XY:** Xylem..

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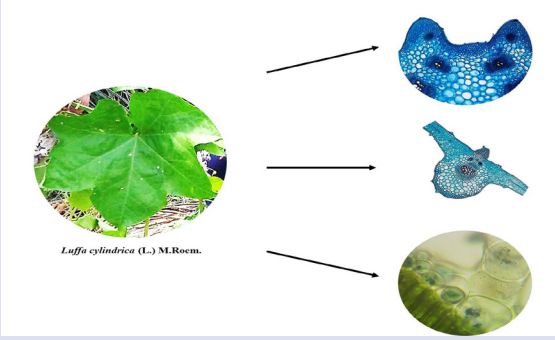
GRAPHICAL ABSTRACT



SUMMARY

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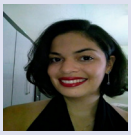

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