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Original Article

Anatomical and histochemical characterization of stem and leaves of *Bauhinia pulchella* Benth. (Fabaceae)

Caracterização anatômica e histoquímica de caule e folhas de *Bauhinia pulchella* Benth. (Fabaceae)

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Abstract

Bauhinia pulchella Benth. (Fabaceae), is native to Brazil and popularly known as pata-de-bode. In folk medicine, it is used to treat diabetes. Pharmacological studies have demonstrated different properties, such as cytotoxic and antioxidant, and different chemical constituents, such as essential oil, triterpenoids, steroids, among others. In order to highlight the morphological differences of the species *B. pulchella* from the others of the genus, and its pharmacological potential, the present study aimed to carry out the anatomical and histochemical characterization of the stem and leaves of *B. pulchella*. Usual methods in plant anatomy were used in the preparation of semi-permanent slides containing cross sections of the stem, petiole and leaf blade and paradermal sections of the leaf blade for analysis in light microscopy and polarized light. Histochemical tests were also performed to localize the metabolites in the stem and leaf blade. The stem of *B. pulchella* has a cylindrical outline, the bark is composed of 7-8 layers of cells and discontinuously distributed sclerenchyma; petiole presents flat convex contour, prominences in the adaxial region, concentric amphicrivral vascular bundle, surrounding a small concentric anfivasal intramedullary bundle, adaxially two accessory bundles and tector trichomes; leaf blade with anisocytic and tetracytic stomata on the adaxial surface and anisocytic, tetracytic and anomocytic on the abaxial surface; and midrib with plain-convex outline, 1-2 layers of collenchyma and covering trichomes. Characteristics that differ from other species of the genus *Bauhinia*. Alkaloids, phenolic compounds, lipophilic compounds, lignin, triterpenes, steroids and tannins were observed in the species. The results are fundamental for the pharmacobotanical standardization of the studied species.

Keywords: *Bauhinia pulchella*, histochemical analysis, pharmacobotany.

Resumo

Bauhinia pulchella Benth. (Fabaceae), é nativa do Brasil e popularmente mais conhecida como pata-de-bode. Na medicina popular, é utilizada no tratamento de diabetes. Estudos farmacológicos demonstraram diferentes propriedades, como por exemplo, citotóxica e antioxidante, e diferentes constituintes químicos, como óleo essencial, triterpenoides, esteroides, entre outros. A fim de evidenciar as diferenças morfológicas da espécie *B. pulchella* das demais do gênero, e seu potencial farmacológico, o presente estudo teve como objetivo realizar a caracterização anatômica e histoquímica do caule e das folhas de *B. pulchella*. Métodos usuais em anatomia vegetal foram utilizados na preparação de lâminas semipermanentes contendo seções transversais do caule, pecíolo e lâmina foliar e seções paradérmicas da lâmina foliar para análises em microscopia óptica de luz e luz polarizada. Testes histoquímicos também foram realizados para localizar os metabólitos no caule e lâmina foliar. O caule de *B. pulchella* apresenta contorno cilíndrico, casca composta de 7-8 camadas de células e esclerênquima distribuído descontinuamente; pecíolo apresenta contorno plano convexo, proeminências na região adaxial, feixe vascular concêntrico anficrivral, circundando pequeno feixe concêntrico anfivasal intramedular, adaxialmente dois feixes acessórios e tricomas tectores; lâmina foliar com estômatos anisocíticos e tetracíticos na face adaxial e anisocíticos, tetracíticos e anomocíticos na face abaxial; e nervura central com contorno plano-convexo, 1-2 camadas de colênquima e tricomas tetores. Características que difere de outras espécies do gênero *Bauhinia*. Alcaloides, compostos fenólicos, compostos lipofílicos, lignina, triterpenos, esteroides e taninos foram observados na espécie. Os resultados são fundamentais para a padronização farmacobotânica da espécie estudada.

Palavras-chave: *Bauhinia pulchella*, análise histoquímica, farmacobotânica.

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1. Introduction

The Fabaceae (Lindl.) family corresponds to the largest family in the number of species in Brazil with a cosmopolitan distribution, with 253 genera and about 3031 species in the country (JBRJ, 2023). Due to the ecological plasticity of this family, it presents centers of biodiversity in different habitats with varied climates, soils, and topography (Wojciechowski, 2003; Wojciechowski et al., 2004). For many years, the family has been studied in taxonomic (Bentham, 1865; Cronquist, 1981; Ribeiro, 1998; Lima, 2000; Gomes et al., 2017), economic, ornamental and medicinal research (Miotto et al., 2008; Souza and Lorenzi, 2008; Martins, 2009; Souza and Souza, 2011; Pereira et al., 2019).

The genus *Bauhinia* L. is included in the Fabaceae family and belongs to the Cercidoideae subfamily, which is considered a monophyletic subfamily according to phylogenetic studies (Vaz and Tozzi, 2005). In Brazil, several representatives of the genus are used in traditional medicine as hypoglycemic agents. The leaves have antidiabetic, diuretic, and hypocholesteremia properties, popularly used against cystitis, intestinal parasites, and elephantiasis (Mors et al., 2000). In addition, antifungal, antibacterial, antimicrobial, antioxidant, and antidiabetic properties were attributed to the genus (Silva and Cechinel-Filho, 2002; Rashed and Butnariu, 2014; Prabhu et al., 2021). The medicinal potential of the genus is quite high, with three representatives on the National Relation of Medicinal Plants of Interest to the Unified Health System (RENISUS) list: *B. affinis* Vogel, *B. forficata* Link and *B. variegata* L. (Brasil, 2022).

Among the species of the genus, *B. pulchella* is native to Brazil and occurs in the phytogeographic regions of the Amazon, Caatinga, and Cerrado (Santos et al., 2019), where it is popularly known as catingueira, miroro, mororo, mororo-de-bode, pata-de-cabra, pata-de-bode, and pata-de-vaca (Queiroz, 2009; Aguiar and Barros, 2012). As some popular names suggest, it has a split leaf in the middle, forming two lobes or leaflets, which resembles a bovine or goat foot, with morphological characteristics that are similar to representatives of the genus *Bauhinia* (Lorenzi and Matos, 2008; Lusa and Bona, 2009), confusing its identification and, in some cases, leading to intoxication, since in the genus may contain some toxic species (Rivera et al., 1994; Nogueira and Sabino, 2012).

In folk medicine, the leaves and stem bark of *B. pulchella* are used as a tea to treat diabetes (Aguiar and Barros, 2012). Pharmacological studies have shown that the plant has larvicidal activity against the *Aedes aegypti* and cytotoxic, anthelmintic, and antioxidant properties (Sousa et al., 2016; Lopes et al., 2016; Carvalho et al., 2018). Reports from the literature emphasize that the vegetable extracts used to treat diabetes also have a significant antioxidant effect (Khalil et al., 2008; Cunha et al., 2010). Moreover, chemical studies have shown flavonol glycosides (Sousa et al., 2016; Carvalho et al., 2018), triterpenoids, steroids, phenolic compounds and essential oil (Monteiro et al., 2022).

Highlighting the morphological similarity of *Bauhinia* species, the pharmacological potential presented by *B. pulchella*, and the scarcity of chemical studies of the species, it is evident the need for more studies, which can significantly contribute to the diagnosis of the characteristics that help to differentiate the species of the genus and elaborate new drugs. For this purpose, this study aims to anatomically characterize the aerial vegetative organs of *B. pulchella*, in addition to identifying the points of accumulation of metabolites present in the species through histochemistry to contribute with information on the species differentiation for greater quality control. In addition, the description of native plants contributes to the recognition of Brazilian biodiversity.

2. Material and Methods

According to standard herbarium techniques (Bridson and Forman, 2010), adult shrub specimens of *B. pulchella* were collected in the city of São Benedito - Ceará, on the state road CE-321, km 59, geographical coordinate 4°4'54" S, 40°50'21' W. Exsiccate No. 29.463 was deposited in the Herbarium Jaime Coelho de Moraes of the Federal University of Paraíba in the Campus de Areia and identified by Professor Dr. Leonardo Pessoa Felix.

For anatomical characterization, the material was fixed in FAA50 (formaldehyde Vetec®, acetic acid Vetec®, and 50% ethyl alcohol Vetec®, 1:1:18 v/v) (Johansen, 1940). Cross-sections of stem in secondary structure, petiole, and leaf blade were obtained freehand, using steel blades and pith of the *embaúba* petiole (*Cecropia* sp.) as support material. For the leaf blade, paradermal sections were performed on the adaxial and abaxial surfaces. Then, the sections were subjected to a sodium hypochlorite (Vetec®) solution (50%) for decolorization (Kraus and Arduin, 1997). After washing in distilled water, the cross-sections were stained according to the technique described by Bukatsch (1972), with Safranin (Ranylab®) and Astra blue (Ranylab®); paradermal sections were stained with methylene blue (Ranylab®) (Krauter, 1985). Subsequently, all sections were mounted on semi-permanent slides, following usual procedures in plant anatomy (Johansen, 1940; Sass, 1958).

Histochemical tests were performed on cross-sections of stem and fresh leaf blades obtained by the same method used for anatomical characterization. The following reagents were used to indicate the presence of metabolites: potassium dichromate (Interlab®, 10%) for phenolic compounds (Johansen, 1940), Dragendorff (Synth®) for alkaloids (Yoder and Mahlberg, 1976), hydrochloric vanillin (Synth®) for tannins (Mace and Howell, 1974), Sudan III (Interlab®) for lipophilic compounds (Sass, 1958), antimony trichloride (Dinâmica®) for triterpenes and steroids (Mace et al., 1974), Lugol (Interlab®) for starch (Johansen, 1940), phloroglucinol (Interlab®) for lignin (Johansen, 1940), and hydrochloric acid (Vetec®, 10%) to establish the nature of crystals (Jensen, 1962). Controls without reagents were performed in parallel to the histochemical tests, and semi-permanent slides were prepared containing cross-sections (Johansen, 1940; Sass, 1958).

The analysis was conducted on images using an optical light microscope (Leica DM750M) coupled with a digital camera (Leica ICC50W) and processed in software (LAS EZ).

3. Results and Discussion

In the cross-section of the stem of *B. pulchella* in secondary growth, a cylindrical contour with a bark composed of 7-8 layers of flattened oblong cells is observed, followed by 5-6 layers cortical parenchyma (Figure 1a and 1b). Sclerenchyma is observed discontinuously distributed throughout the stem surrounding the vascular cylinder (Figure 1b). The vascular cylinder is a closed collateral (Figure 1a and 1b). In the central region, medullary parenchyma is observed (Figure 1a). Duarte and Debur (2003) described the stem of *B. microstachya* (Raddi) J. F. Macbr. with a flattened shape which is slightly enlarged in the central portion, with periderm with tabular cells constituting the bark. Adjacent to it, parenchyma cells and a sclerenchymatic sheath involving the vascular system were described. In the central region, the pith formed four poles resembling a cross with thick-walled parenchymatic cells where idioblasts containing crystals were observed, often prismatic and, rarely assuming the drusen form. In *B. pulchella*, no crystals were observed.

In the cross-section, the convex plane contour of the petiole can be observed with its prominences in the adaxial region (Figure 2a). The epidermis is uniseriate (Figure 2a and 2b) and covered by a thick cuticle (Figure 2b). Adjacent to the epidermis is the fundamental parenchyma composed of 5-6 cell layers (Figure 2a and 2b). Sclerenchymatic tissue is observed surrounding the entire vascular bundle (Figure 2c). It is observed a large amphicribral concentric vascular bundle, surrounding a small intramedullary amphivasal concentric bundle (Figure 2c), and being flanked adaxially by two small collateral accessory bundles (Figure 2a and 2d). Tector trichomes can be observed throughout the petiole epidermis (Figure 2e). Duarte and Debur (2003) described the petiole of *B. microstachya* with a semicircular contour and slightly flattened on the adaxial surface, presenting a unistratified epidermis. Then, 4-6 layers of fundamental parenchyma were described, and in the central region, an anficrival vascular bundle with sclerenchymatic sheath involving the entire vascular system. The authors did not describe accessory bundles for *B. microstachya*, a feature that differs from the present study. Ferreira et al. (2003), analyzing the characters of *B. blakeana* Dunn., described the petiole with a flat-convex contour with two protrusions, unistratified epidermis covered by a striated cuticle, followed by 3-4 layers of collenchyma with prism and druse crystals. The fundamental parenchyma is formed by 6-7 layers of cells, and discontinuous sclerenchymatic tissue surrounds the vascular region. The authors also described 8-10 vascular bundles for *B. blakeana*; of these, one to two collaterals in each salience and the other bilaterals located in the central region. In addition, unicellular and multicellular tector trichomes were observed, and rare glandular trichomes were also noted. Lusa and Bona (2009) described the *B. forficata* petiole with an elliptical shape presenting two lateral projections. On the adaxial surface, the uniseriate epidermis is covered by a thin cuticle. Internally to the epidermis, there is a discontinuous band of collenchyma, followed by cortical parenchyma and an extensive sheath of fibers, which surrounds the vascular cylinder with a collateral vascular bundle. Elbanna et al. (2016) described the *B. vahlii* Wight and Arnott. petiole with circular contour, epidermis covered by smooth cuticle, followed by 7-9 layers of collenchyma, with an open collateral vascular bundle and observed the presence of non-glandular trichomes and prism-like crystals detected in all layers of the petiole and the pith may have agglomerated.

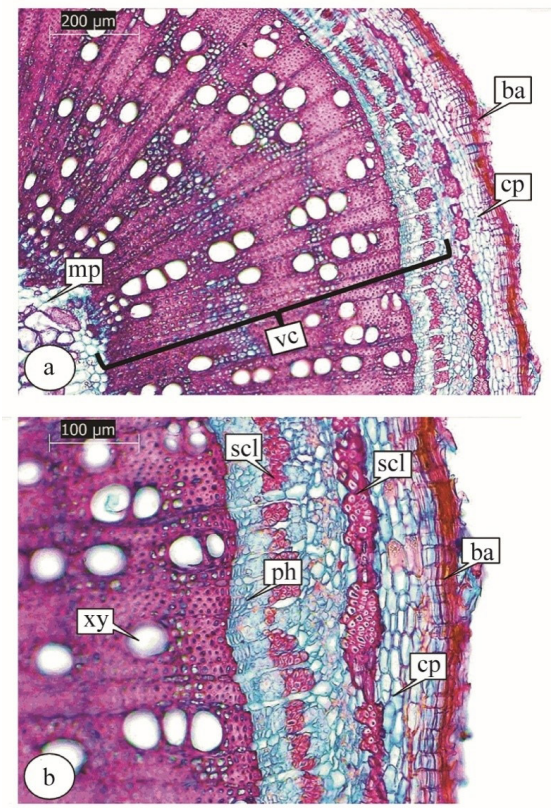

Figure 1. Cross-section of the stem of *B. pulchella*. (a) Stem in secondary growth; (b) Details of sclerenchyma, phloem, cortical parenchyma, bark, and xylem. vc: vascular cylinder; scl: sclerenchyma; ph: phloem; cp: cortical parenchyma; mp: medullary parenchyma; ba: bark; xy: xylem.

In the paradermal sections, the leaf blade of *B. pulchella* presents epidermal cells with slightly curved walls on both adaxial (Figure 3a) and abaxial (Figure 3b-d) surfaces. The leaf blade is classified as amphistomatic with anisocytic and tetracytic stomata on the adaxial surface (Figure 3a) and anisocytic, tetracytic, and anomocytic stomata on the abaxial surface (Figure 3b-d). Lusa and Bona (2009) observed in *B. forficata* an adaxial surface with slightly curved walls and an abaxial surface with straight anticline walls. Anomocytic stomata were observed on both surfaces. Pereira et al. (2018) described the anatomical characters of *B. cheilantha* (Bong.) Steud., *B. pentandra* (Bong.) Steud., and *B. ungulata* L., observed straight to slightly curved epidermal walls in *B. cheilantha*, *B. pentandra*, and *B. ungulata* on the adaxial surface. On the abaxial surface of *B. cheilantha* and *B. pentandra* they observed curved walls, and in *B. ungulata*, curved and papillose

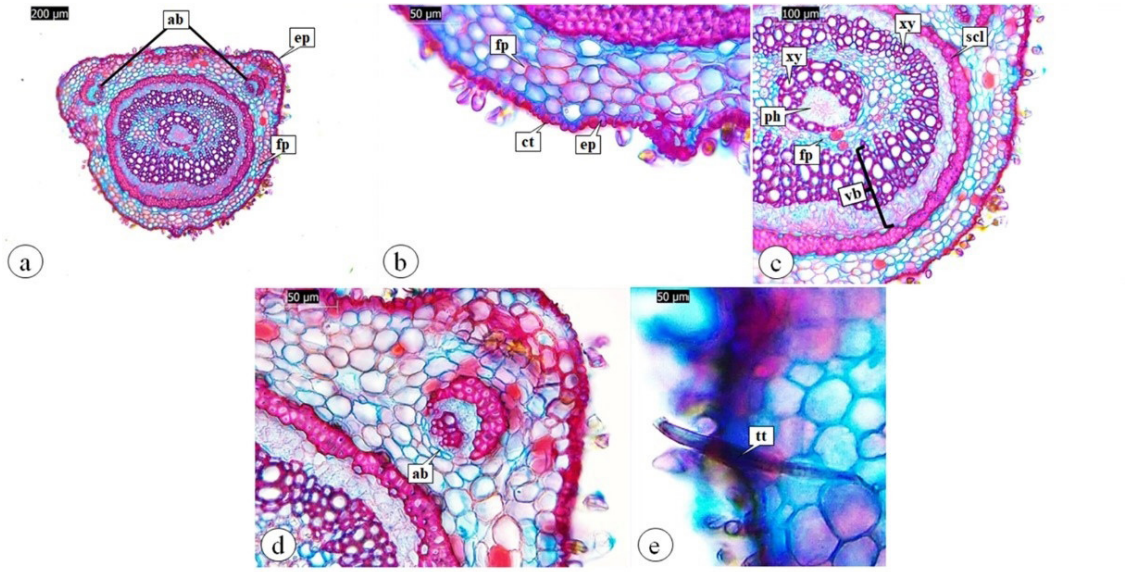

Figure 2. Cross-section of the *B. pulchella* petiole. (a) General aspect of the petiole; (b) Details of the epidermis, cuticle, and fundamental parenchyma; (c) Detail of the vascular bundle and sclerenchyma; (d) Detail of the accessory vascular bundle; (e) Detail of the tector trichome. ct: cuticle; ep: epidermis; scl: sclerenchyma; ab: accessory beam; ph: phloem; vb: vascular bundle; fp: fundamental parenchyma; tt: tector trichome; xy: xylem.

walls. Furthermore, the authors described *Bauhinia* species with amphistomatic leaves with anomocytic and anisocytic stomata occurring on both surfaces. Paracytic-type stomata were also observed on both surfaces of *B. pentandra* and *B. ungulata*. Antunes et al. (2021) described the species *B. monandra* Kurz with sinuous to straight cell walls on the adaxial surface and straight on the abaxial surface, classifying the leaf blade as amphistomatic with paracytic and anomocytic stomata on the abaxial surface. The stomata are scarce on the adaxial surface.

The midrib exhibits a flat-convex contour (Figure 3e). The epidermis is uniseriate (Figure 3e and 3f) and covered by a thin cuticle (Figure 3f). The midrib comprises 1-2 layers of collenchyma on the adaxial surface (Figure 3e and 3g), and the fundamental parenchyma fills the entire vein (Figure 3e and 3f). The vascular bundle is the collateral type (Figure 3g). Around the vascular bundle, sclerenchymatic tissues are observed (Figure 3f and 3g). Tector trichomes are distributed on the abaxial face (Figure 3f). Duarte and Debur (2003) described the leaf blade of *B. microstachya* as a biconvex contour with prominence on the abaxial surface; the epidermis is uniseriate with collenchyma interrupting, being replaced by the fundamental parenchyma where a collateral vascular bundle surrounded by a sclerenchyma sheath is immersed. The species *B. cheilantha*, *B. pentandra*, and *B. ungulata* presented uniseriate and papillose epidermis, with a thick cuticle in *B. cheilantha* and *B. ungulata* but a thin cuticle in *B. pentandra*. The midrib of the species is flat-convex, with collateral vascular bundles delineated by two sclerenchyma ribbons, with a crystalline sheath and collenchymatous cortex with sparse drusiferous idioblasts; The median portion of the midrib of *B. cheilantha* has an arch-shaped central vascular bundle, "V" shaped in *B. pentandra* and "U" shaped in *B. ungulata* (Pereira et al., 2018). Antunes et al. (2021) described the *B. monandra* uniseriate epidermis adjacent to the abaxial epidermis, with two layers of collenchyma, 5-6 collateral vascular bundles interconnected by a band of parenchyma and surrounded by a thick layer adjacent to the phloem, constituted by septate fibers, in addition to describing the species with drusen-like crystals in the parenchyma.

The mesophyll is dorsiventral, composed of 2-3 layers of palisade parenchyma and 1-3 layers of spongy parenchyma (Figure 3h). Ferreira et al. (2003) described *B. blakeana* as a dorsiventral mesophyll with 3-4 layers of palisade parenchyma and 4-5 layers of spongy parenchyma with large intercellular spaces. The mesophyll of *B. ungulata* is dorsiventral in the cross-section, with a layer of palisade parenchyma; in *B. cheilantha* and *B. pentandra*, two layers of the same parenchyma are observed with drusiferous idioblasts; The spongy parenchyma showed 2–4 layers in *B. cheilantha* and *B. pentandra*, with small intercellular spaces; In *B. ungulata*, the spongy parenchyma has 4-5 layers, with large intercellular spaces; Prismatic crystal idioblasts were observed in the vascular systems of secondary bundles, mainly in *B. pentandra* (Pereira et al., 2018). Antunes et al. (2021) described the species *B. monandra* as a dorsiventral mesophyll with two layers of palisade parenchyma and about two layers of spongy parenchyma. Also, the authors observed the presence of drusen-like crystals in the mesophyll.

Figure 4 shows the stem in cross-sections subjected to histochemical characterization. Figure 4a shows the stem without any reagent. Alkaloids (Figure 4b), starch (Figure 4c), and phenolic compounds (Figure 4d) were evidenced in the cortical parenchyma. In the suber and parenchyma, lipophilic compounds were observed (Figure 4e). Figure 4e shows lignin observed

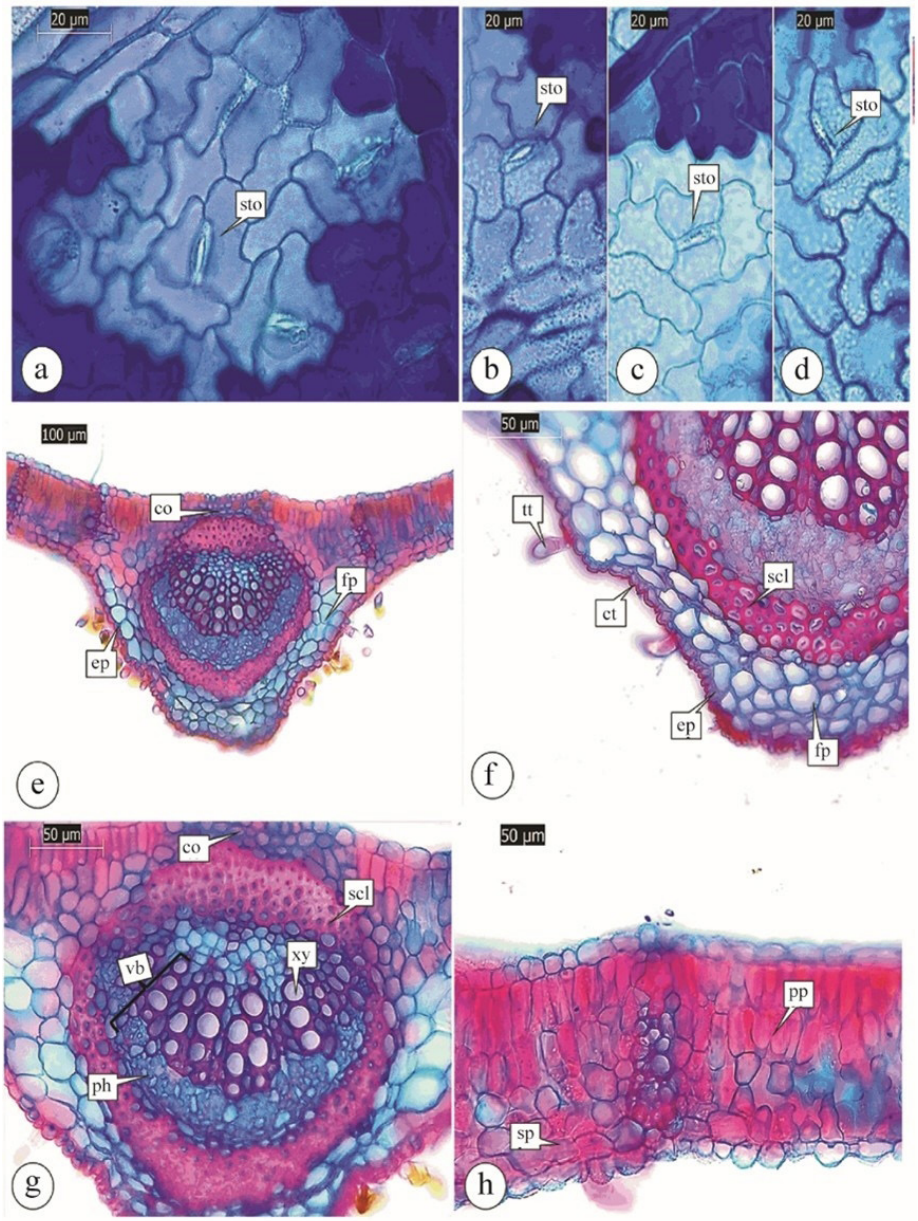

Figure 3. Paradermal and transverse sections of the *B. pulchella* leaf blade. (a) Adaxial surface; (b-d) Abaxial surface; (e-h) General appearance and details of the leaf blade; (f) General appearance of the mesophyll; (h) Details of the mesophyll. co: collenchyma; ct: cuticle; ep: epidermis; scl: sclerenchyma; sto: stoma; ph: phloem; vb: vascular bundle; sp: spongy parenchyma; fp: fundamental parenchyma; pp: palisade parenchyma; tt: tector trichome; xy: xylem.

in the sclerenchyma and in the xylem. Tests for tannins, triterpenes and steroids were negative. Monteiro et al. (2022), using Sephadex LH-20 column chromatography and semi-preparative HPLC, identified triterpenoids, steroids, flavonoids, and phenolic compounds in the stem of the species *B. pulchella*, corroborating, in part, with the findings in the present study. Tests for starch were negative. Monteiro et al. (2022), using Sephadex LH-20 column chromatography and semi-preparative HPLC, identified triterpenoids, steroids, flavonoids, and phenolic compounds in the stem of the species *B. pulchella*, corroborating, in part, with the findings in the present study.

Figure 5 shows the leaf blade in cross-sections subjected to histochemical characterization. Figure 5a shows the leaf blade without any reagent. Alkaloids were evidenced in the fundamental parenchyma (Figure 5b), and phenolic compounds in the sclerenchyma and the fundamental parenchyma (Figure 5c). In the cuticle, lipophilic compounds were observed (Figure 5d). Figure 5e shows lignin observed in the sclerenchyma and the vascular bundle

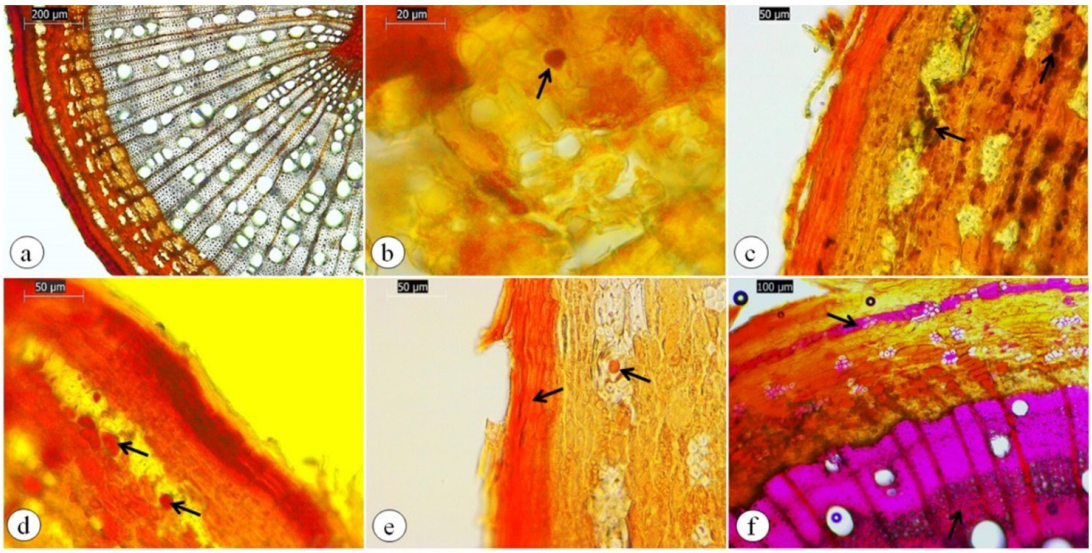

Figure 4. Histochemistry of the stem of *B. pulchella*. (a) control; (b) Dragendorff; (c) Iodine solution; (d) Potassium dichromate; (e) Sudan III; (f) Phloroglucinol.

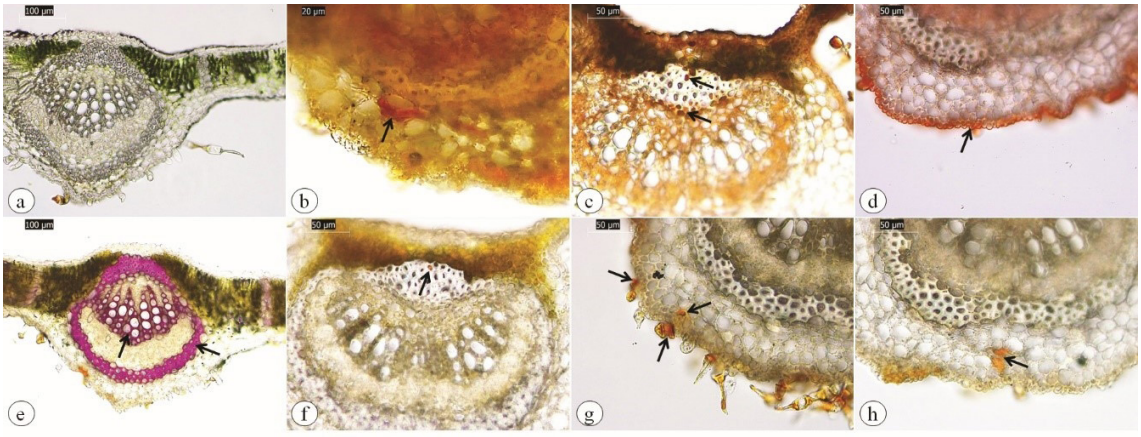

Figure 5. Histochemistry of the leaf blade of *B. pulchella*. (a) control; (b) Dragendorff; (c) Potassium dichromate; (d) Sudan III; (e) Phloroglucinol; (f-g) Antimony trichloride; (h) Hydrochloric vanillin.

of the midrib, highlighted in the xylem. Triterpenes and steroids were evidenced in the sclerenchyma (Figure 5f), the fundamental parenchyma, and the trichome (Figure 5g). In the fundamental parenchyma, clusters of tannins were also observed (Figure 5h). Tests for starch were negative.

4. Conclusion

The present study enabled the identification of anatomical and histochemical parameters for the diagnosis of the species *B. pulchella*, since different representatives of the genus *Bauhinia* are recommended in the RENISUS list for their medicinal potential. In the light microscopy evaluation, it was possible to identify and anatomically characterize the stem, petiole, and leaf blade, showing differentiating characteristics such as stem shape, absence of collenchyma and organization of vascular bundles in the petiole, stomata typification, presence and absence of glandular and non-glandular trichomes in the leaf blade, absence of prismatic crystals or druses in the stem, the petiole and leaf blade. Therefore, these findings are essential to corroborate the data in the literature on the species studied. Through the histochemical analysis, alkaloids, phenolic compounds, lipophilic compounds, lignin, triterpenes, steroids and tannins were observed in the species. With this, this study contributed to the pharmacobotanical standardization of the *B. pulchella* species, to evidence and corroborate with findings already described for the *Bauhinia* genus in the scientific literature and to assist in the differentiation of species through

anatomy since external morphological characteristics are similar, such as the leaf blade, for instance.

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