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ARTICLE

Anatomical and histochemical study of the stem and leaves of *Jasminum azoricum* L.

Hanna Karolyne de Melo Pereira[1], Cledson dos Santos Magalhães[2] & Karina Perrelli Randau[3]

1 Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco, 50740521, Recife, PE, Brasil

2 Programa de Pós-Graduação em Inovação Terapêutica, Laboratório de Farmacognosia, Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco, 50670901, Recife, PE, Brasil.

3 Programa de Pós-Graduação em Inovação Terapêutica, Programa de Pós-Graduação em Ciências farmacêuticas, Laboratório de Farmacognosia, Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco, 50740521, Recife, PE, Brasil

Correspondence
K.P. Randau
E-mail: karina.prandau@ufpe.br
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Resumen

Estudio anatómico e histoquímico del tallo y las hojas de Jasminum azoricum *L.*

El presente estudio tuvo como objetivo caracterizar la anatomía e histoquímica del tallo y las hojas de *Jasminum azoricum* L. Para ello, se realizaron preparaciones histológicas semipermanentes que contenían cortes transversales de tallo y hoja, además de cortes paradérmicos de la lámina foliar, analizados por microscopía óptica. También se realizaron pruebas histoquímicas para verificar la ubicación de acumulación de constituyentes químicos en secciones transversales de la hoja. Las caracterizaciones anatómicas aportaron importantes datos para distinguir las especies y el estudio histoquímico reveló los sitios de síntesis y/o almacenamiento de los metabolitos que poseen importantes propiedades medicinales.

Palabras clave: Oleaceae; Jazmín; Taxonomía; Farmacobotánica; Microscopía.

Abstract

The present study aimed to characterize the anatomy and histochemistry of the stem and leaves of *Jasminum azoricum* L. Thus, semi-permanent slides were prepared containing transverse sections of the stem and leaf, in addition to paradermal sections of the leaf blade, analyzed by optical microscopy. Histochemical tests were also carried out in order to verify the site of accumulation of chemical constituents in cross-sections of the leaf blade. The anatomical characterizations allowed the identification of important characteristics to distinguish the species and the histochemistry revealed the sites of synthesis and/or storage of the metabolites. The results found help to identify the species studied and point out possible metabolites that have considerable medicinal properties.

Key words: Oleaceae; Jasmim; Taxonomy; Pharmacobotany; Microscopy.

Introduction

Jasminum azoricum L., popularly known as white jasmim, jasmim-dos-açores and jasmim limão (Cordeiro 2020) is a species belonging to the Oleaceae family and can be found in the Middle East, India, China and Africa (El-Hawary *et al.* 2020). In the late 17th and early 18th centuries, this same species caught the attention of many European collectors who began to cultivate it in botanical gardens in Holland and England, having majority of its use as an ornamental plant, and soon after being brought to the Americas (Cordeiro 2020).

Brazil has a very favorable climate for the propagation and development of the species, even if it is not endemic, with some records focusing on coastal regions, with confirmed occurrences in the northeast, southeast and south, as well as the phytogeographic domains of the caatinga. and the Atlantic Forest (Sampaio 2020).

J. azoricum presents itself as a climbing shrub, with opposite phyllotaxis, perennial life cycle, with leathery consistency, trifoliate leaves, glabrous texture and shiny appearance, composed of oval to ovate-lanceolate leaflets. Its flowering period lasts almost the entire year in tropical climates. Its dazzling white flowers and quite fragrance are gathered in terminal racemes, having a different scent from other jasmins for having a more citrus touch. The flowers of this species are very attractive to pollinators such as butterflies and other insects, which are considered mainly responsible for the propagation of the species. Its fruits are berry-like, darkish color and small size. (El-Sayyad *et al.* 1983, Kumar & Sabeena 2013, Akhtar *et al.* 2021).

However, even though its use is mainly ornamental, in the western side of the globe, the species has a long history of use in traditional oriental medicine. Its medicinal properties are seen from religious rites to treatments against some comorbidities. According to studies by Hari & Nair (2018), *J. azoricum* in traditional Indian medicine it is used to treat pain, itching and inflammation, even in traditional Chinese medicine infusions of its dried flowers are used to cure gallstones and in cooking it is used in the preparation of sweets, teas and puddings.

Properties of *J. azoricum* were investigated, such as its antioxidant and antimicrobial role (Thiruvengadam *et al.* 2018), a great cytotoxic power of its extract, with potential for anticancer action (Ali 2019, El-Hawary *et al.* 2020). With current science, many of these properties have been evidenced and attributed to the presence of phytochemicals, such as flavonoids, tannins and oleacein (Thiruvengadam *et al.* 2018). However, more research on this species is needed to describe their properties and the best way to extract compounds and thus see its phytochemical and pharmaceutical applications (Hari & Nair 2018).

It is noteworthy that factors such as the wide variety of species within the genus *Jasminum* L. added to the scarcity of information on the species *J. azoricum* and the difficulty in accessing the data, caused a series of mistakes in the identification and registration of the species in some countries, in which *J. azoricum* was described and identified as a new species, that is, a species that had not been previously identified, leading to duplication of records (Green 1969).

Thus, given the scarcity of data on the anatomical and histochemical characterization of *J. azoricum*, which contribute to the identification and differentiation of *J. azoricum* from other species of the genus, the present study aimed to characterize the anatomy and carry out the histochemical study of *J. azoricum* species to aid in its identification and quality control of the species.

Materials and methods

Anatomical characterization

Adult specimens of *Jasminum azoricum* were collected in the city of Camaragibe located in the state of Pernambuco, Brazil. Exsiccate nº 93891 was deposited in the Herbarium Dárdano de Andrade Lima, of the Agronomic Institute of Pernambuco (IPA) for botanical identification.

For anatomical characterization, the material was fixed in FAA50 (formaldehyde, acetic acid and 50% ethyl alcohol, 1:1: 18 v/v) (Johansen 1940). Transversal and longitudinal sections of the stem, as well as transverse sections of the petiole and leaf blade were obtained freehand, using steel blades and pith from the petiole of embaúba (*Cecropia* sp.) as support material. For the leaf blade, paradermal sections were also made, on the adaxial and abaxial faces. Then, the sections were submitted to a sodium hypochlorite solution

(50%) for classification for 3 minutes (Kraus & Arduin 1997). After washing in distilled water, cross -sections were stained according to the technique described by Bukatsch (1972) with safranin and Astra blue and the paradermal sections were stained with methylene blue (Krauter 1985). Subsequently, all sections were mounted on semi-permanent slides, following usual procedures in plant anatomy (Johansen 1940, Sass 1951). For the analysis of the slides, an optical light and polarization microscope (Leica DM750M) was used, coupled with a digital camera (Leica ICC50W), through which images processed in software (LAS EZ) were obtained.

Histochemical characterization

Histochemical tests were performed on cross-sections of 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 (10%) for phenolic compounds (Johansen 1940), Dragendorff for alkaloids (Yoder & Mahlberg 1976), hydrochloric vanillin for tannins (Mace & Howell 1974), Sudan III for lipophilic compounds (Sass 1951), antimony trichloride for triterpenes and steroids (Mace *et al.* 1974), Lugol for starch (Johansen 1940) and phloroglucinol to lignin (Johansen 1940). Controls without the addition of reagents were performed in parallel to the histochemical tests and semi-permanent slides were prepared containing cross-sections (Johansen 1940, Sass 1951). 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).

Results and discussion

Anatomical characterization

Stem

In cross-section, the stem has a circular shape (Fig. 1A) with a thick cuticle covering the uniseriate epidermis with isodiametric cells (Figs. 1A, 1B). Adjacent to the epidermis, angular collenchyma composed of 1-2 layers of cells is observed (Fig. 1B), followed by the cortical parenchyma (Figs. 1A,1B). Sclerenchymatous tissue is observed surrounding the entire vascular cylinder (Fig. 1B). The vascular cylinder is of the bicollateral type (Fig. 1C). In the central region of the stem, medullary parenchyma is observed (Fig. 1A). Stomata were observed above the height of the epidermis (Figs. 1D,1E) of the paracytic, anisocytic and tetracytic types, an uncommon feature in the species of the genus.

El-Sayad *et al.* (1983), in a previous study, described the stem of *J. azoricum* as circular in shape, the epidermis formed by a single strip of cells covered by a thick cuticle, followed by the parenchymal cortex, with the pericycle being represented by an uninterrupted band of parenchyma and sclerenchyma, bicollateral vascular bundle and in the central region parenchymal medulla can be seen. In the present study, no trichomes were observed, however, Deng *et al.* (2012), described trichomes on the stem *Jasminum sambac* (L.) Aiton both in primary and secondary growth, being rare, and possible of the tector or glandular type.

Petiole

In cross-section, the biconvex contour of the petiole can be observed with two prominences on the adaxial surface (Fig. 2A). In a study analyzing different species of *Jasminum*, it was observed that the presence of prominences on the petiole is not a common characteristic of this genus (Gonzales & Solís 2016), however, in the present study it was a striking feature to distinguish the species. Thick cuticle covers the uniseriate epidermis (Figs. 2A, 2B). Adjacent to the epidermis is the angular collenchyma arranged in 1-2 layers (Fig. 2B), followed by fundamental parenchyma as well as in the central region of the petiole (Figs. 2A, 2B). Close to the vascular bundle, sclerenchyma can be seen distributed in small portions throughout the petiole (Fig. 2B). In *J. sambac* and *Jasminun polyanthum* Franch., the sclerenchyma is continuously distributed (Gonzales & Solís 2016). There are 3 collateral vascular bundles, one in the central region of the petiole (Fig. 2C) and two accessories located at the ends of the adaxial surface (Figs, 2A-2D). Stomata (Fig. 2E) and glandular trichomes are observed throughout the petiole epidermis (Fig. 2F).

Leaf

In paradermal sections, the leaf blade of *J. azoricum* presents epidermal cells with straight walls on both the adaxial and abaxial sides (Figs. 3A, 3B). The leaf blade is classified as amphistomatic with tetracytic and anomocytic stomata on both sides of the leaf blade (Figs. 3A, 3B). The

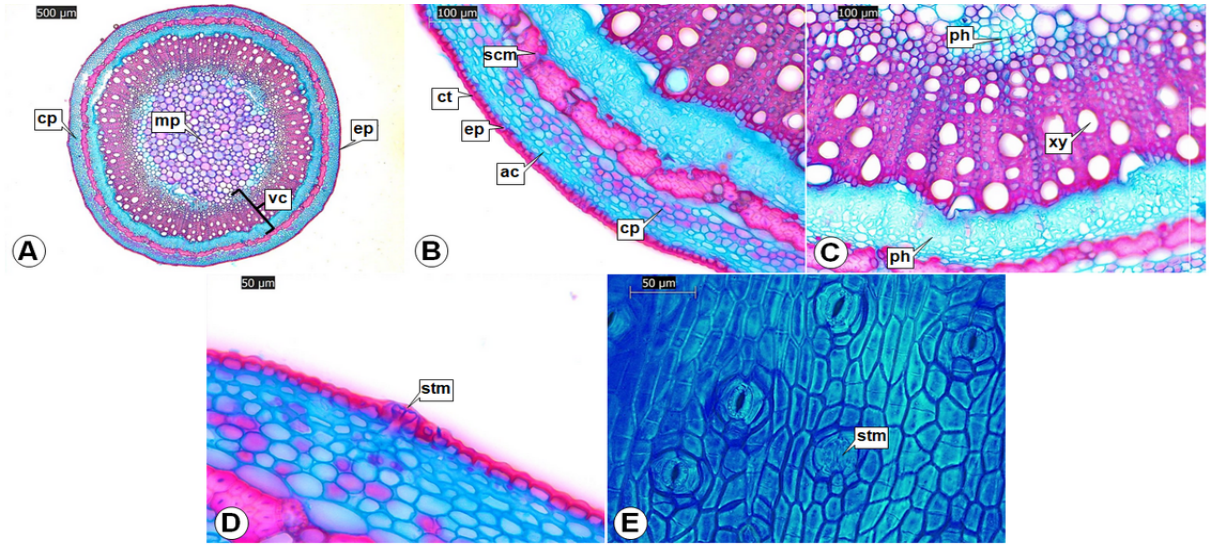

Figura 1. Sección transversal del tallo de *Jasminum azoricum* L. **A:** Aspecto general del tallo; **B-D:** Detalles del tallo; **E:** Detalle de los estomas de tipo paracítico, anisocítico y tetracítico. ac: colénquima angular; ct: cutícula; vc: cilindro vascular; ep: epidermis; scm: esclerénquima; stm: estoma; ph: floema; cp: parénquima cortical; mp: parénquima medular; xy: xilema.

Figure 1. Cross section of the stem of *Jasminum azoricum* L. A: General appearance of the stem; **B-D:** Stem details; **E:** Detail of the stomatas paracytic, anisocytic and tetracytic types. ac: angular collenchyma; ct: cuticle; vc: vascular cylinder; ep: epidermis; scm: sclerenchyma; stm: stoma; ph: phloem; cp: cortical parenchyma; mp: medullary parenchyma; xy: xylem.

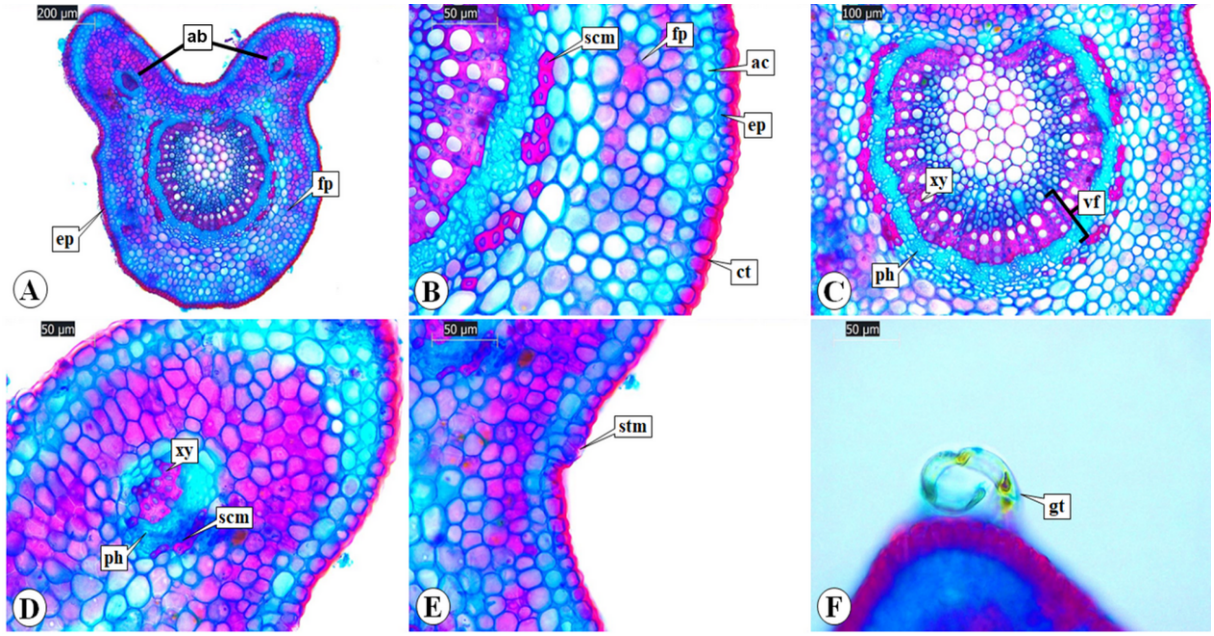

Figura 2. Sección transversal del pecíolo de *Jasminum azoricum* L. **A:** Aspecto general del pecíolo; **B-F:** Detalles del pecíolo. ab: haz accesorio co: colénquima; ct: cutícula; ep: epidermis; fp: parénquima fundamental; scm: esclerénquima; stm: estoma; ph: floema; vb: haz vascular; gt: tricoma glandular; xy: xilema.

Figure 2. Cross section of the petiole of *Jasminum azoricum* L. **A:** General appearance of the petiole; **B-F:** Details of the petiole. ab: accessory bundle co: collenchyma; ct: cuticle; ep: epidermis; fp: fundamental parenchyma; scm: sclerenchyma; stm: stoma; ph: phloem; vb: vascular bundle; gt: glandular trichome; xy: xylem.

presence of stomata on both sides becomes a differentiator when compared to other jasmim species, such as *J. polyanthum* and *J. sambac* that have stomata only on the abaxial surface (El-Sayyad *et al.* 1983; Gonzales & Solís 2016). Metcalfe & Chalk (1950) described anomocytic stomata for the genus.

The midrib exhibits a biconvex outline (Fig. 3C). A thick cuticle is observed covering the uniseriate epidermis (Figs. 3C, 3D). Then, 1-2 layers of angular collenchyma are observed (Fig. 3D) and after that, fundamental parenchyma is observed (Figs. 3C, 3D). The vascular bundle is of the collateral type (Fig. 3E). Gonzales & Solís (2016)

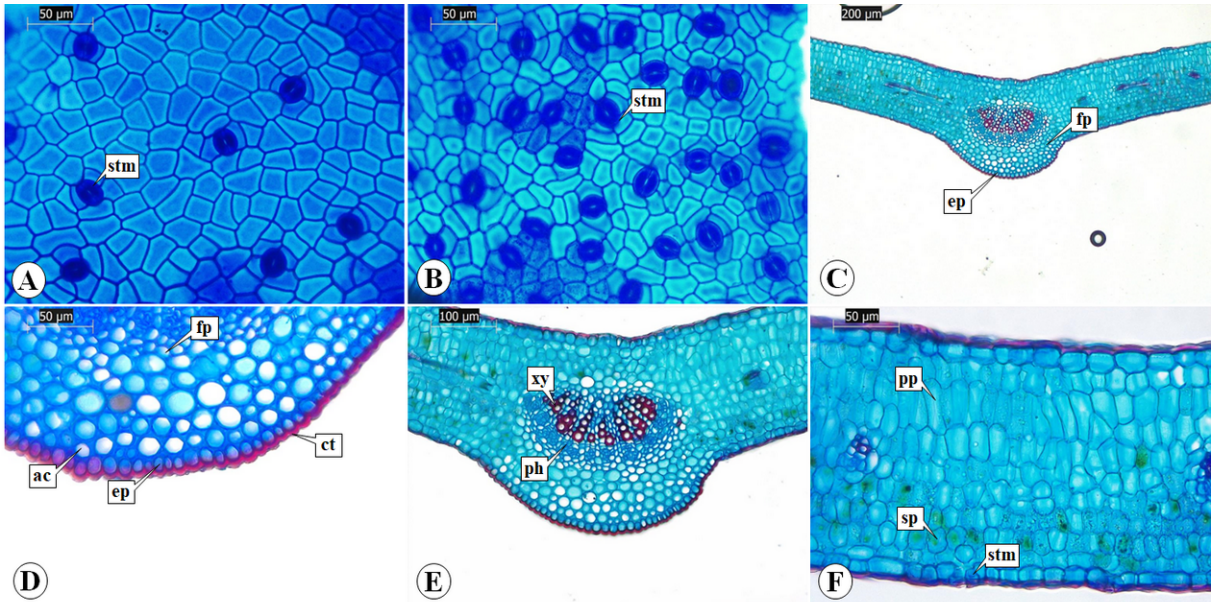

Figura 3. Secciones paradérmicas y transversales del limbo de *Jasminum azoricum* L. **A:** Cara adaxial; **B:** Cara abaxial; **C-F:** Detalles del limbo. ag: colénquima angular; ct: cutícula; ep: epidermis; stm: estomas tetracíticos y anomocíticos; ph: floema; pp: parénquima en empalizada; sp: parénquima esponjoso; fp: parénquima fundamental; xy: xilema.

Figure 3. Paradermal and transverse sections of the leaf blade of *Jasminum azoricum* L. **A:** Adaxial face; **B:** Abaxial face; **C-F:** Leaf blade details. ag: angular collenchyma; ct: cuticle; ep: epidermis; stm: tetracytic and anomocytic stomata; ph: phloem; pp: palisade parenchyma; sp: spongy parenchyma; fp: fundamental parenchyma; xy: xylem.

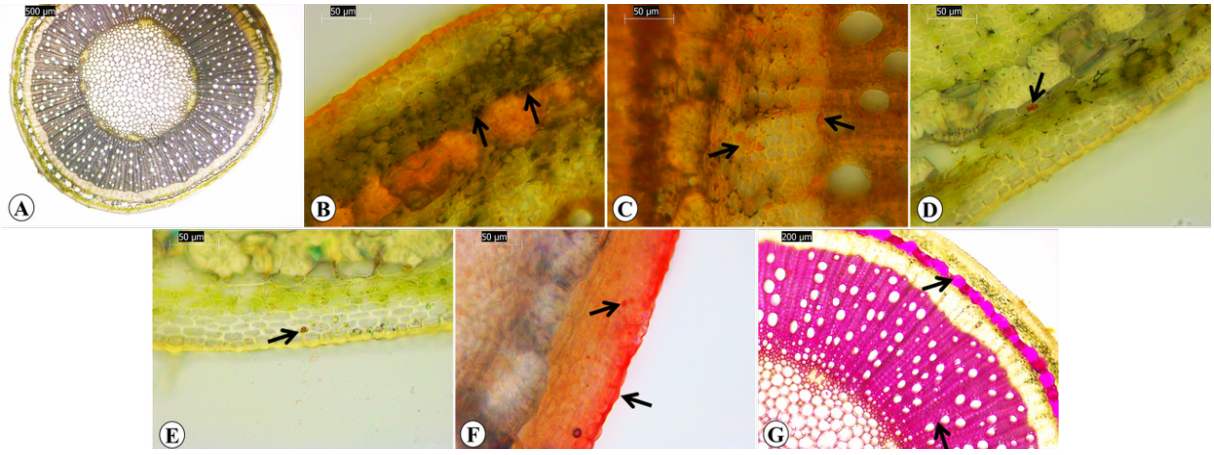

Figura 4. Caracterización histoquímica del tallo de *Jasminum azoricum* L. **A:** Control; **B:** Lugol; **C:** Dragendorff; **D-E:** dicromato potásico; **F:** Sudán III. **G:** cloroglucinol.

Figure 4. Histochemical characterization of the stem of *Jasminum azoricum* L. **A:** Control; **B:** Lugol; **C:** Dragendorff; **D-E:** potassium dichromate; **F:** Sudan III; **G:** phloroglucinol.

characterize the epidermis of *J. azoricum* as uniseriate, formed by small and polygonal cells covered by a thick cuticle, characteristics also described for the species *J. polyanthum* and *J. sambac*. In addition, the authors observed trichomes for these species, diverging from what was found in this study.

The mesophyll is dorsiventral type, composed of 3-4 layers of palisade parenchyma and 5-7 layers of spongy parenchyma (Fig. 3F). In the mesophyll epidermis, the presence of epidermal attachments, the stomata, is observed (Fig. 3F). Deng *et al.* (2012) described the presence of these two tissue types in the leaf anatomy of the genus Jasminum. Studies describe the presence of crystals in chlorophyll parenchyma cells in *Jasminum* species, being described as micro druses (Deng *et al.* 2012, Ali & Sosa 2016), however, no crystals were observed in the present work.

Histochemical characterization

Stem

Figure 4 shows the stem of *J. azoricum* in sections submitted to histochemical characterization. Fi-

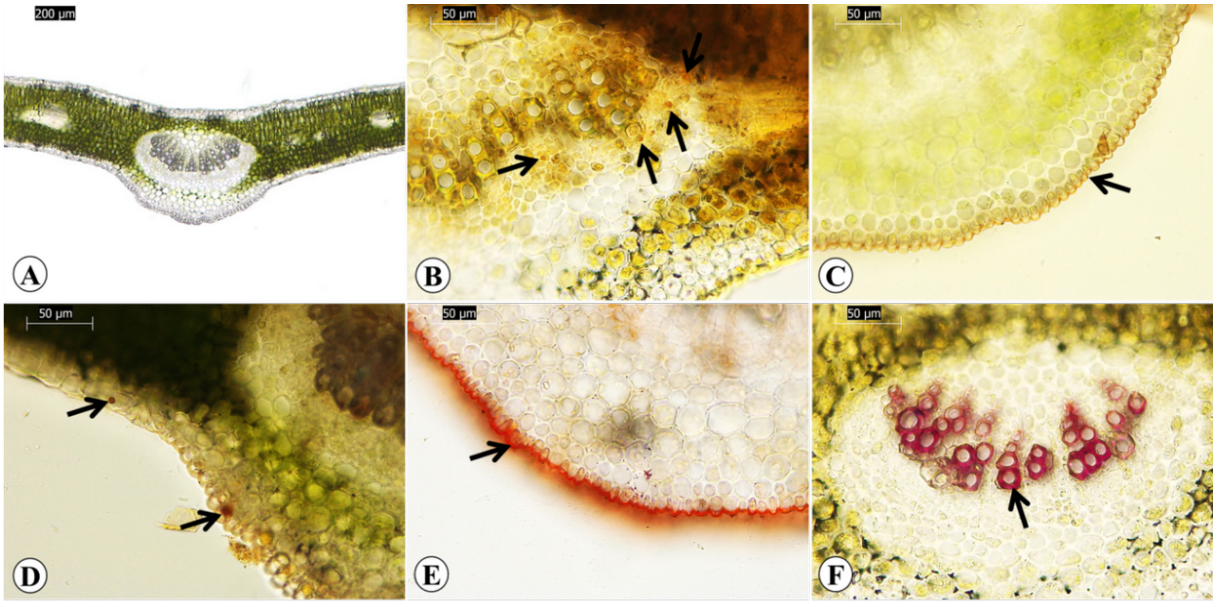

Figura 5. Caracterización histoquímica del limbo foliar de *Jasminum azoricum* L. **A:** Control; **B:** Dragendorff; **C-D:** dicromato potásico. **E:** Sudán III; **F:** cloroglucinol.

Figure 5. Histochemical characterization of the leaf blade of Jasminum azoricum L. **A:** Control. **B:** Dragendorff; **C-D:** potassium dichromate; **E:** Sudan III; **F:** phloroglucinol.

gure 4A demonstrates the stem without the addition of reagent. Starch was observed in the parenchyma (Fig. 4B) Alkaloid concentrations were observed in the phloem (Fig. 4C). Phenolic compounds were evidenced in the fundamental parenchyma (Fig. 4D) and in the collenchyma (Fig. 4E). In the cuticle and collenchyma, lipophilic compounds were observed (Fig. 4F). Lignin was evidenced in the sclerenchyma and xylem (Fig. 4G). Tests for tannins and triterpenes and steroids were negative.

Leaf

Figure 5 shows the leaf blade of *J. azoricum* in cross-sections submitted to histochemical characterization. Figure 5A shows the leaf blade without the addition of any reagent. Concentrations of alkaloids were observed in the phloem (Fig. 5B). Balkrishna (1989) and Thiruvengadam (2018) identified alkaloids in the leaves of the species in phytochemical research. Phenolic compounds were evidenced in the cuticle and epidermis of the species (Figs. 5C, 5D). The presence of lipophilic compounds was also observed in the cuticle (Fig. 5E). Figure 5F shows the presence of lignin observed in the vascular bundle of the midrib, highlighted in the xylem. Tests for starch, tannins and triterpenes and steroids were negative. A result that differs from that reported in studies Balkrishna (1989), Rastogi *et al.* (2001) and Thiruvengadam (2018), which confirm the presence of these three compounds in the species.

Conclusion

Based on the microscopic analysis of the present study, it was possible to determine important anatomical characters, which can be applied to differentiate the species of the genus *Jasminum*, as well as for the quality control of the species *Jasminum azoricum*, such as presence of stomata and absence of trichome on the stem, presence of prominence and trichome on the petiole, and amphistomatic leaf blade. Through histochemical tests of leaf blades, the presence of alkaloids, phenolic compounds, lipophilic substances and lignins.

The findings of this work suggest in *J. azoricum* the presence of components that have therapeutic potential, helping with data for future pharmacological research, as well as the anatomy for taxonomy and diagnosis of useful characters for distinguishing the species of the genus.

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