

## Morphoanatomical and histochemical study of *Ipomoea hederifolia* L. (Convolvulaceae)

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### Resumen

*Estudio morfoanatómico e histoquímico de Ipomoea hederifolia* L. (Convolvulaceae)

*Ipomoea hederifolia* L. es una planta herbácea, nativa de la región tropical en América con importancia medicinal. Se realizó un estudio farmacobotánico de las hojas y tallos, efectuando macro y microscópicos morfodiagnósticos y pruebas histoquímicas. *I. hederifolia* presentó características anatómicas típicas de la familia Convolvulaceae, sin embargo, la epidermis y sus apéndices (p. ej. cutícula estriada y tricomas pelta), así mismo, el peciolo y la anatomía del tallo, presentaron características relevantes en el reconocimiento taxonómico de la especie. La prueba histoquímica mostró la presencia de lignina y cutina, demostrando reacciones positivas para el almidón, compuestos fenólicos y proteínas. La anatomía y las pruebas histoquímicas muestran un conjunto de características relevantes para la caracterización farmacobotánica de *I. hederifolia*, Aumentando el conocimiento de la especie y proporcionando subsidios para el control de calidad de sus productos vegetales.

**Palabras clave:** *Ipomoea hederifolia*, Histoquímica; Farmacobotánica; Microscopía; Anatomía de tallos y hojas.

### Abstract

*Ipomoea hederifolia* L. is a herbaceous vine native to the tropical Americas with important medicinal properties. Was realized a pharmacobotanical study of the leaves and stems of this species, performing macroscopic and microscopic morphodiagnoses and histochemical tests. Anatomical characteristics typical of the family Convolvulaceae were found. However, the epidermis and its appendages (e.g. striated cuticle and peltate trichomes) and the anatomy of the petiole and the stem presented relevant characters for the taxonomic recognition of the species. Histochemical tests evidenced the presence of lignin and cutin and positive reactions for starch, phenolic compounds, and proteins. The anatomy and the histochemical tests indicated a set of characteristics relevant to the pharmacobotanical characterization of *I. hederifolia*, expanding our knowledge of the species and providing subsidies for the quality control of its vegetal products.

**Key words:** *Ipomoea hederifolia*; Histochemistry; Pharmacobotany; Microscopy; Leaf and stem anatomy.

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## Introduction

The family Convolvulaceae comprises 59 genera and approximately 1.880 species with cosmopolitan distribution, mainly found in tropical and subtropical regions (Stevens 2017). Four hundred and twenty-one species subordinated to 25 genera are known to occur in all biomes of Brazil, and 193 species are considered endemic (Flora do Brasil 2020).

*Ipomoea* L. is considered the most numerous genus of Convolvulaceae, with approximately 750 widely distributed species (Stevens 2017). The genus is represented by 159 species in Brazil, of which 62 are endemic. Eighty-eight species have been recorded in the Northeastern region of the country, 48 of them in the Atlantic Forest (Flora e Funga do Brasil 2020).

Many representatives of *Ipomoea* have economic importance. Some species are sources of human food resources, such as *I. batatas* (L.) Lam. (sweet potato) and *I. aquatica* Forssk. (water spinach); others have nutritional (*I. alba* L., *I. albivenia* Sweet, *I. pileata* Roxb.) (Meira *et al.* 2012) and medicinal value (*I. nil* (L.) Roth, *I. pes-caprae* (L.) R. Br., *I. purga* (Wender.) Hayne), and ornamental uses (*I. horsfalliaek* Hook., *I. purpurea* (L.) Roth, *I. alba*). Some species are considered invasive (*I. cairica* (L.) Sweet, *I. tricolor* Cav., *I. triloba* L., *I. purpurea*) or weeds (*I. asarifolia* (Desr.) Roem. & Schult., *I. cairica*, *I. carnea* Jacq., *I. tiliacea* (Willd.) Choisy, *I. indica* (Burm.) Merr., *I. nil*) (Garcia-Blanco 1972, Lorenzi 2000). The main chemical constituents of *Ipomoea* are ergoline alkaloids, indolizidine alkaloids, nortropanic alkaloids, phenolic compounds, coumarins, norisoprenoids, diterpenes, isocoumarins, benzenoids, flavonoids, anthocyanosides, glycolipids, lignans, and triterpenes (Meira *et al.* 2012).

*Ipomoea hederifolia* L., popularly known as “jitirana”, is an herbaceous vine native to Brazil that occurs in all regions and phytogeographic domains of the country (Flora e Funga do Brasil 2020). It was introduced as an ornamental plant and naturalized in the paleotropics. Its wide geographic distribution is a result its usefulness in phytoremediation, its resistance to herbicides, longer growth cycles, and morphophysiological characteristics such as heat tolerance (Moshobane *et al.* 2022).

*I. hederifolia* is an annual species, reproducing

mainly by seeds and grows well in fertile soils with good levels of humidity (Azania *et al.* 2011). It presents best development during the austral summer, autumn, and beginning of winter (Kissmann & Groth 1999). According to Brandão & Gavilanes (1997), the species is invasive and frequently occurs along road sides, in anthropic fields, pastures, abandoned areas, as well is in annually or perennially cultivated areas such as potato (Moreira & Bragança 2011) or sugarcane plantations, where it is considered a weed and detrimental to productivity (Silva *et al.* 2009, Azania *et al.* 2011).

*I. hederifolia* has economic importance for its ornamental value (Pulido-Salas 1993, Lorenzi 2000). It is used in hedges and in the horticulture industry in general (Srivastava & Rauniyar 2020).

This species is also used in popular medicine (Agra *et al.* 2008). The leaves, stems, roots, and fruits are the most used parts, consumed in the form of infusion or decoction to treat dermatitis, rheumatism (Agra *et al.* 2008), nervous system disorders, tumors, stomachaches, intestinal parasites, and as a purgative drug (Pandurangan & Rana 2015). The seeds can also have anti-inflammatory, cathartic, diuretic, and expectorant application, being used for constipation, edema, and parasitosis, and the roots are used as sternutatory (Srivastava & Rauniyar 2020). Given its importance to popular medicine of the leaves and stems, these organs were used in this study according to hypothesis of these organs can be accumulation sites of the big amount of the chemical substances useful to pharmacobotanical.

Chemically, the plant produces ipanguline A/ isoipanguline A, pyrrolizidine alkaloids (Jenett-Siems *et al.* 1993), nortropanic alkaloids (Meira *et al.* 2012), platinecine derivatives (Jenett-Siems *et al.* 1993), and organic acids (phenylacetic acid, decanoic acid, octadecanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid) (Osorio *et al.* 2018) with oxytocic, anticancer, antipsychotic, anti-inflammatory, antioxidant, and antibacterial properties (Pandurangan & Rana 2015).

Studies of *I. hederifolia* in Brazil, more specifically in the Northeastern region, have principally focused on taxonomic (Conceição *et al.* 2014, Delgado Junior *et al.* 2014, Bandeira *et al.* 2019, Lima & Melo 2019) and floral biology aspects (Kiill & Ranga 2003), or on its control as an invasive species in cultivated areas (Silva 2013). Research on the anatomy of the species includes

the classic studies of Lowell & Lucansky (1986) and Monquero *et al.* (2005) who examined the leaf epidermis from a physiological perspective. Plant anatomy has proven to be useful, together with taxonomy, to the quality control of plant species considered medicinal, such as *Solanum* L. (Nurit-Silva & Agra 2011, Nurit-Silva *et al.* 2012), *Ficus* L. (Araújo *et al.* 2013), *Cissampelos* L. (Porto *et al.* 2016), *Dalbergia* L.F. (Neves *et al.* 2016), *Bauhinia* L., and *Schnella* (Raddi.) Wund. (Pereira *et al.* 2018).

Although some studies have examined the medicinal potential of *I. hederifolia*, no information was performed on the exact localization of the source of its chemical compounds. In addition, anatomical studies that report characters of taxonomic value for *Ipomoea* and Convolvulaceae are scarce and therefore necessary. The correct identification of *I. hederifolia* is fundamental for its safe use for medicinal purposes. Thus, this study aimed to provide a detailed and inedited macroscopic and microscopic description of the stems and leaves of *I. hederifolia* to identify the main sites of synthesis and accumulation of secondary metabolites through histochemical tests.

## Materials and methods

*Ipomoea hederifolia* was collected in August/2016 in an Atlantic Forest area near the Federal University of Paraíba (Campus I), at João Pessoa, Paraíba State, Brazil (07°06'54"S, 34°51'47"W). The material collected was used for the botanical identification of the species and for morphological, anatomical, and histochemical studies.

Morphological analyses were undertaken for the macroscopic morphodiagnosis of the species, using fresh material and/or material conserved in 70% alcohol. Leaves and stems were analyzed and described using a Zeiss binocular stereomicroscope.

The microscopic morphodiagnoses involved anatomical analyses of 10 samples of stems and mature fresh leaves as well as specimens that had been fixed in 50% FAA (Formaldehyde, Acetic Acid and Alcohol) for 24 hours and subsequently conserved in 70% alcohol (Johansen 1940). Free-hand paradermal (leaf blade) and transverse (petiole, leaf blade, and stem) sections were made at the median region of the organs to prepare semi-permanent slides. The sections were cleared using 20% sodium hypochlorite (NaClO), neutralized

with an acetic acid solution (1:00), washed in distilled water, stained with a mixture of safranin and astra blue (transverse sections) or safranin (paradermal sections), and mounted with coverslips in 50% glycerin (Kraus & Arduin 1997).

The histochemical tests were performed with freehand transverse sections of fresh leaves (leaf blades and petioles) and stem fragments (not cleared) treated with the following reagents: lugol to detect starch grains (Berlyn & Miksche 1976); Sudan III to detect lipophilic substances (Jensen 1962); a solution of acidic phloroglucinol for identifying lignin (Sass 1951); ferric chloride for phenolic compounds (Johansen 1940); and xylydine Ponceau for total proteins (Vidal 1970). Controls were run parallel to these tests.

Morphological descriptions follow the terminology proposed by Harris & Harris (2001); the anatomical terminology followed Metcalfe & Chalk (1950). Plant structures were analyzed using a Leica ES2 optical microscope and photographed using a DV150F Samsung Digital Camera. Anatomical analyses were undertaken in the Botany Teaching Laboratory in the Education and Health Center at the Federal University of Campina Grande, Cuité, Paraíba State, Brazil.

## Results

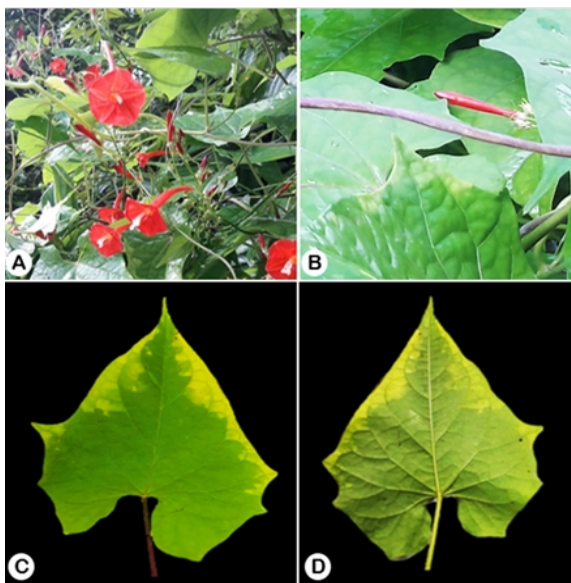
### Macroscopic morphodiagnosis

*Ipomoea hederifolia* is a herbaceous vine with a bright red corolla (Fig. 1A), simple, alternate, membranaceous, cordiform or 3-lobed leaves (1.7–12 × 1.2–10 cm) with cordate, subcordate or hastate base, cuspidate to mucronate apex, entire to slightly undulate margins, with small acute lobes (Figs. 1C-D) and glabrescent indumenta composed of glandular trichomes on both epidermal faces. The petiole is semicircular, up to 7.0 cm long, glabrescent. The stem is climbing, striated, green to vinaceous (Fig. 1B).

### Microscopic morphodiagnosis

#### Leaf

The paradermal section of the epidermis of leaf blade in frontal view shows cells with sinuous anticlinal walls on both faces, but more accentuated on the abaxial face (Figs. 1A & 1C). Indument glabrescent, composed of peltate and sessile glandular trichomes (Figs. 2B & 2D) sparsely distributed on both faces of the leaf blade. The stomata



**Figura 1.** *Ipomoea hederifolia*. **A:** Hábito; **B:** Detalle del tallo; **C-D:** Hoja simple, cordiforme; **C:** Cara adaxial; **D:** Cara abaxial.

**Figure 1.** *Ipomoea hederifolia*. **A:** Habit; **B:** Detail of the stem; **C-D:** Simple, cordiform leaf; **C:** Adaxial face; **D:** Abaxial face.

are of the paracytic type and have amphistomatic distribution (Figs. 2A and 2C).

The leaf blade, in transverse section, showed a heterogeneous, asymmetrical, dorsiventral mesophyll (Fig. 2D) with 1-2 seriate palisade parenchyma and 2-3 seriate spongy parenchyma. The leaf edge is rounded and slightly flexed abaxially (Fig. 2D), with small-diameter vascular bundles near the edge, where palisade parenchyma cells contact the spongy parenchyma.

The midrib, in transverse section, is biconvex, abaxially more prominent and adaxially sharp (Fig. 2F). The salience of the adaxial face is composed of 4 to 5 layers of vertically organized collenchymatous tissue (Fig. 2G). The abaxial face has a subepidermal layer with 1 to 2 layers of collenchyma (Fig. 2H) and, more internally, a fundamental parenchyma with idioblasts containing druses (Fig. 2H). The vascular system is arc shape and composed of a single central bicollateral bundle (Fig. 2F).

The petiole, in transverse section, is concave on the adaxial face and convex on the abaxial face, and slightly lobate (Fig. 2I). The epidermis is uniseriate, composed of tabular cells, and covered by a moderately thick cuticle with glandular trichomes below the level of the epidermis (Fig. 2L). Internally, the epidermis has a layer of hypodermic cells (Fig. 2J) followed by 4 to 5 layers of collenchymatous tissue of the angular type (Fig. 2L), and then parenchymatic tissue containing

secretory canals that are probably laticiferous. The vascular system is composed of four bicollateral bundles, with the two central bundles in an arc with two dorsal accessory bundles. Starch grains and idioblasts containing druses were present in the phloem cells (Fig. 2K).

### Stem

The stem has a circular outline in transverse section with sharp lateral protuberances (Fig. 3A). The epidermis is composed of a single layer of quadrangular cells covered by a thin and striated cuticle. The cortex presented 3-4 layers of angular collenchyma and, more internally, 2-3 layers of parenchyma with occasional secretory canals, probably lactiferous (Fig. 3B). The vascular system is bicollateral and, as the stem demonstrates secondary growth, it is possible to identify a vascular cylinder resulting from vascular cambium activity (Fig. 3B) initiating growth-ring formation. Discontinuous sclerenchyma bundles were external to the phloem (Fig. 3B); an endodermis composed of irregular cells was observed subjacent to the fiber bundles. Young and completely formed vessel elements were situated between the cambium and the xylem (Fig. 3D).

The medullar region is composed of medullar parenchyma with isodiametric cells, with largest diameter in the central region and smaller cells along the periphery (Fig. 3A). Secretory canals are located near the internal phloem (Fig. 3C), as are numerous starch grains (Figs 3E and 3F) and idioblasts with druses (Fig. 3E).

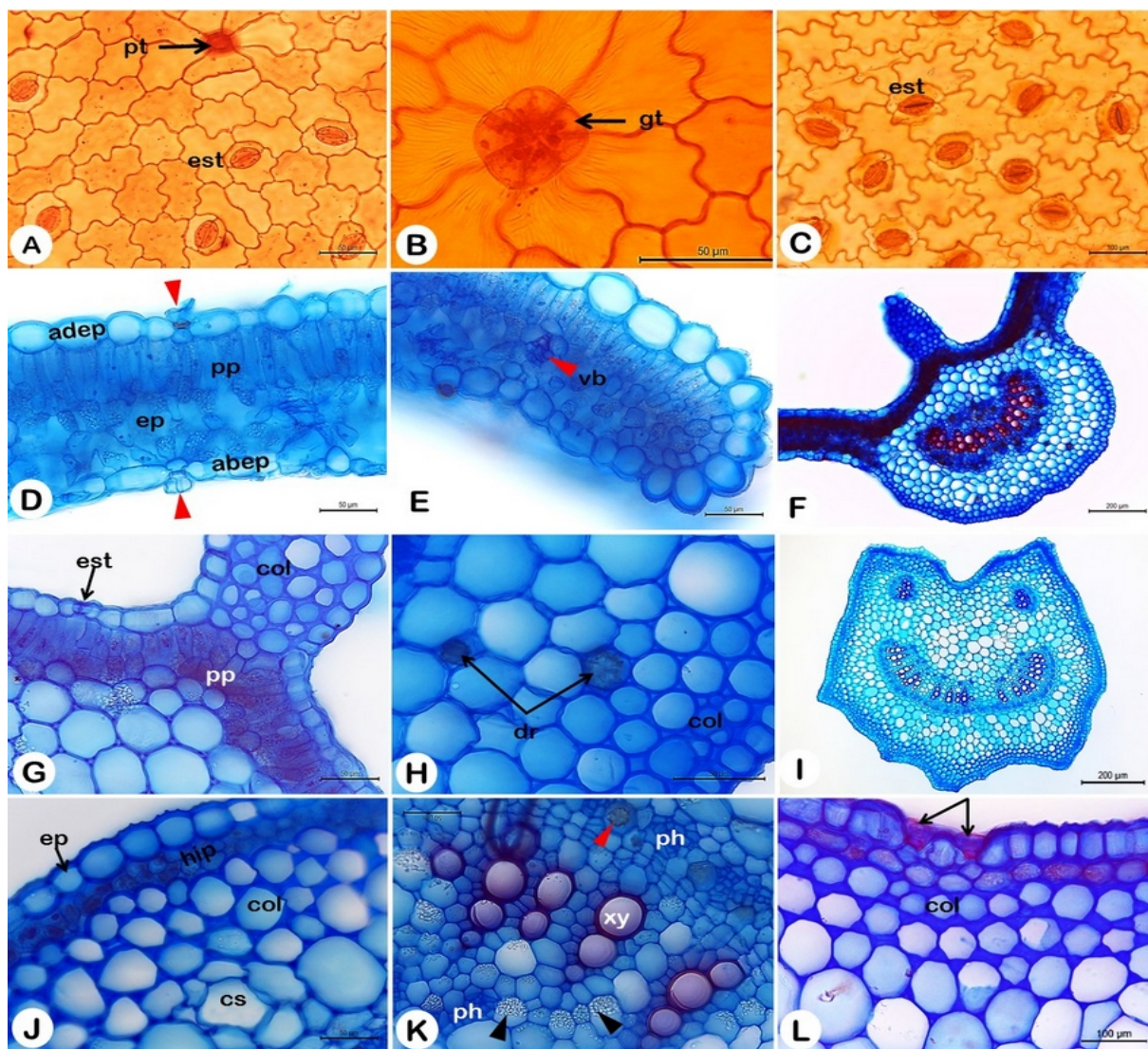
### Histochemical tests

The reagent acidic phloroglucinol applied to transverse sections of the midrib, petiole and stem (Fig. 4B) reacted with tissues such as the xylem and sclerenchyma. The ferric chloride reagent reacted only with the palisade parenchyma of the mesophyll (Fig. 4C), evidencing the presence of phenolic compounds in that leaf region. The presence of starch grains in the mesophyll cells, in the petiole (Fig. 4D), and in the cortical and medullar parenchyma of the stem were observed in transverse sections treated with lugol. Proteins were evidenced by the xylidine Ponceau test in the midrib and petiole, and in the cortical and medullar parenchyma of the stem (Fig. 4E). The Sudam III reagent indicated the presence of lipidic substances in the cutinized walls of the epidermal regions of the petiole, midrib (Fig. 4F), and stem (Table 1).

## Discussion

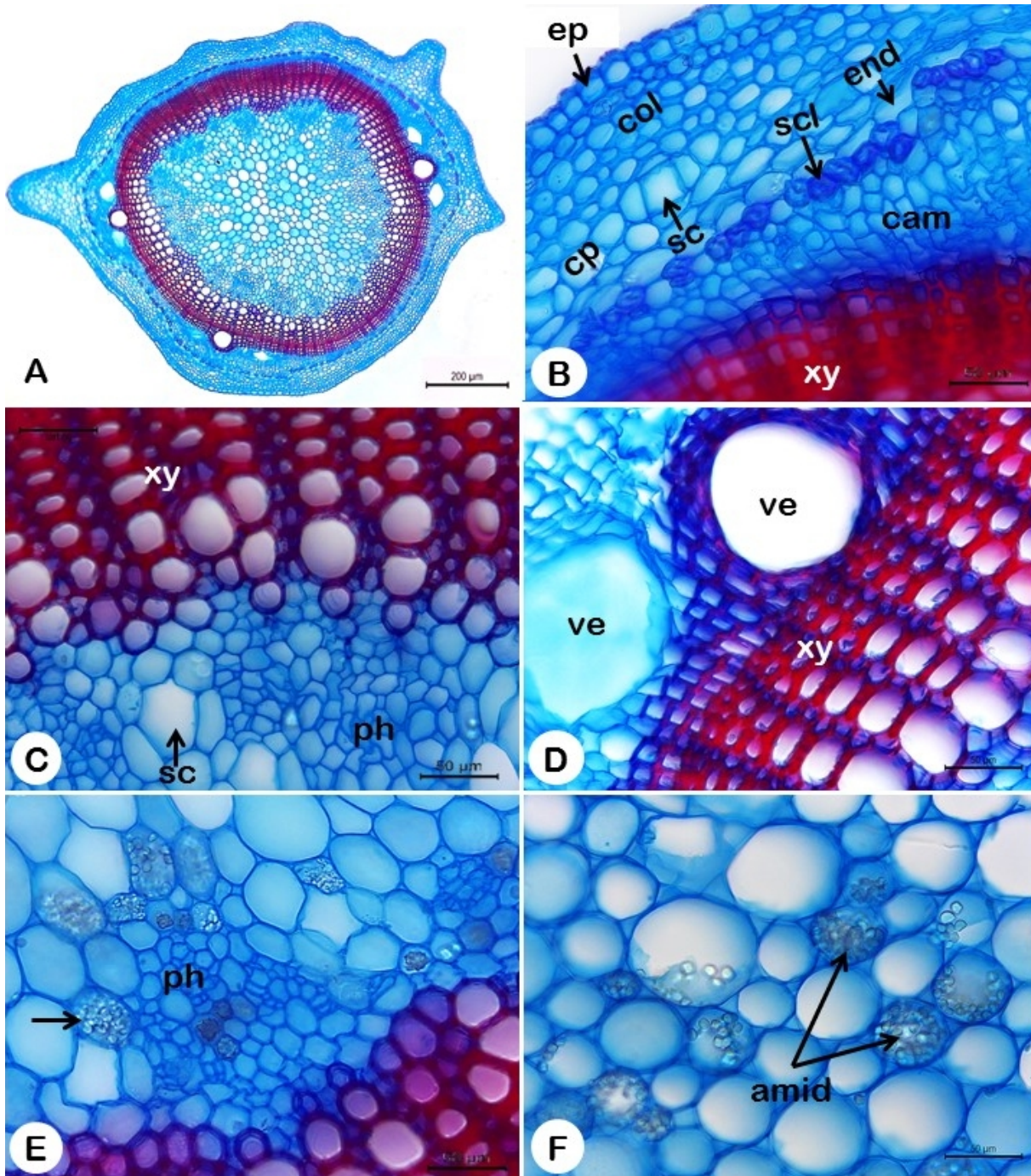
The morphological characters of *I. hederifolia* corresponded to those described for the species by Bandeira *et al.* (2019) and Delgado Junior *et al.* (2014). The anatomical characters of the leaves corresponded in many aspects to the pattern described

for Convolvulaceae by Metcalfe & Chalk (1950). The morphological characters of the anticlinal walls likewise followed patterns shared in common among *Ipomoea* species such as *I. purpurea*, *I. tricolor*, *I. cairica*, *I. batatas*, *I. eriocarpa* R.Br., *I. pes-tigridis* L., and *I. quamoclit* L. (Ashfaq *et al.* 2019). Environmental factors may influence the structure of the



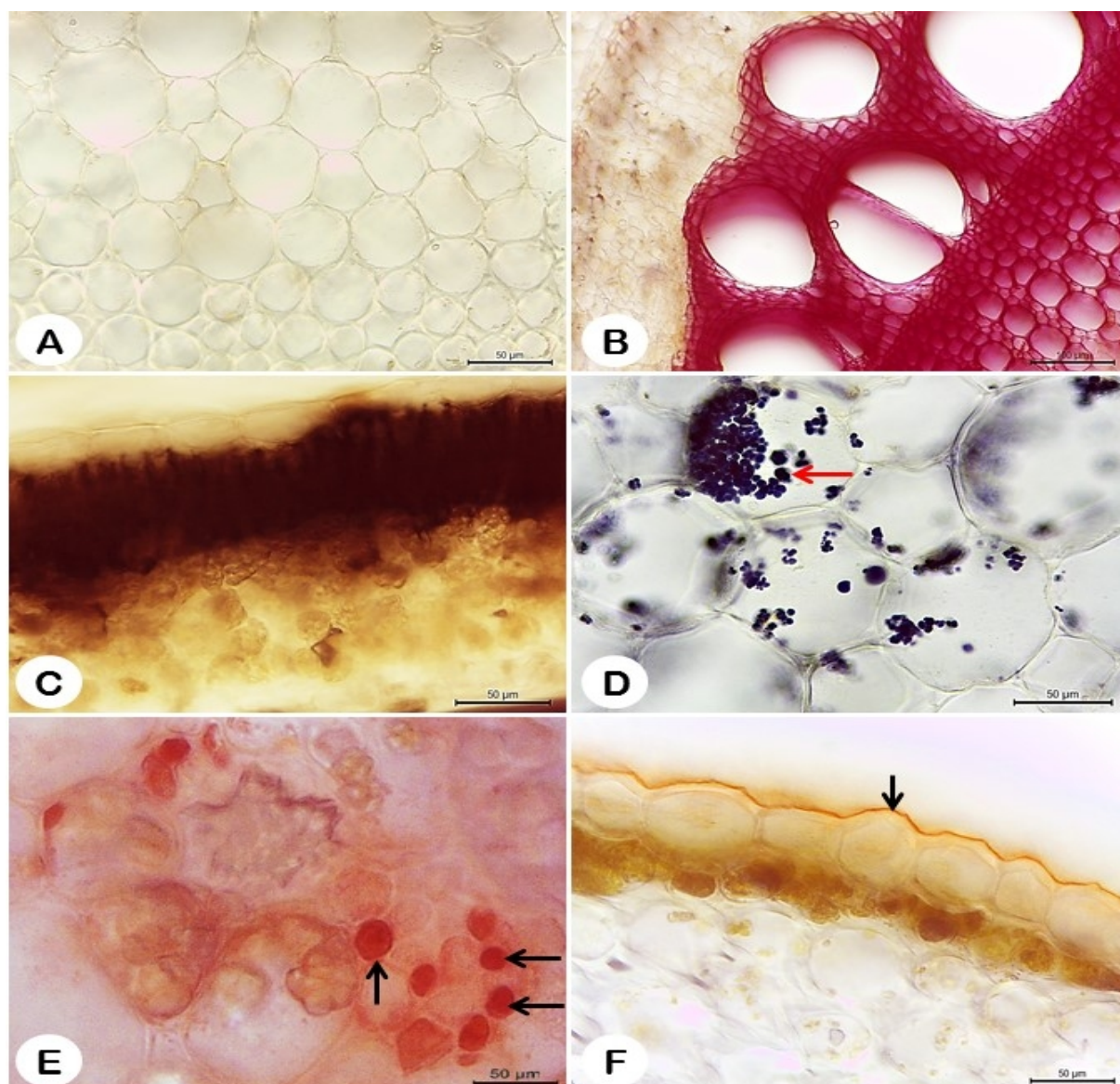
**Figura 2.** Hoja. Epidermis en vista frontal: **A**: Cara adaxial con estomas paracíticos (est) y tricomas peltados (pt); **B**: Detalle de la cara adaxial, con tricomas glandulares (gt); **C**: Cara abaxial; Limbo foliar en sección transversal: **D**: Mesófilo dorsiventral, con parénquima en empalizada (pp), parénquima esponjoso (ep) y tricomas glandulares (flecha) en la cara adaxial (ad ep) y la cara abaxial (ab ep) de la epidermis; **E**: Borde redondeado; Nervadura central: **F**: Descripción general; **G**: Detalle, evidenciando estomas (est), parénquima en empalizada (pp) y colénquima (col); **H**: Detalle, evidenciando colénquima (col) e idioblastos con drusas (dr); Peciolo: **I**: Descripción general; **J**: Detalle del hipodermis (hip) adyacente a la epidermis y un canal secretor (SC) en el parénquima cortical; **K**: Detalle del sistema vascular con énfasis en: xilema (xi), floema externo e interno (fl), granos de almidón (flecha roja) y drusas en el floema (flecha negra); **L**: Detalle de los tricomas glandulares de la epidermis (flecha). Colorante: safranina y azul Astra (D, E, F, G, H, I, J, K y L), safranina (A, B y C). Microscopio óptico y DV150F Samsung Camera Digital.

**Figure 2.** Leaf. Epidermis in frontal view: **A**: Adaxial face with paracytic stomata (est) and peltate trichomes (pt); **B**: Detail of the adaxial face showing glandular trichomes (gt); **C**: Abaxial face; Leaf blade in transverse section: **D**: Dorsiventral mesophyll with palisade parenchyma (pp), spongy parenchyma (ep), and glandular trichomes (arrow) on the adaxial face (ad ep) and abaxial face (ab ep) of the epidermis; **E**: Rounded edge; Midrib: **F**: Overview; **G**: Detail showing stomata (est), palisade parenchyma (pp) and collenchyma (col); **H**: Detail showing collenchyma (col) and idioblasts with druses (dr); Petiole: **I**: Overview; **J**: Detail of the hypodermis (hip) adjacent to the epidermis and a secretory canal (sc) in the cortical parenchyma; **K**: Detail of the vascular system showing: xylem (xi), external and internal phloem (fl), starch grains (red arrow), and druses in the phloem (black arrow); **L**: Detail of glandular trichomes on the epidermis (arrow). Stain: safranin and Astra blue (D, E, F, G, H, I, J, K and L), safranin (A, B and C). Optical microscopy and DV150F Samsung Digital Camera.



**Figura 3.** Tallo, en sección transversal que muestra crecimiento secundario. **A:** Resumen; **B:** Detalles del colénquima (col), parénquima cortical (cp), esclerénquima (scl), endodermo (extremo) y cambium multiseriado (cam); **C-D:** Detalles del sistema vascular: canal secretor (sc), floema interno (ph), xilema (xy) y elementos vasculares (ve); **E:** Detalle del floema interno (ph), que contiene idioblastos con drusas y células parenquimatosas con granos de almidón; **F:** Detalle del parénquima medular, que contiene numerosos granos de almidón (medio). Colorante: safranina y azul Astra (A, B, C, D, E y F). Microscopio óptico y DV150F Samsung Camera Digital.

**Figure 3.** Stem, in cross-section, showing secondary growth- **A:** Overview; **B:** Detail of the collenchyma (col), cortical parenchyma (cp), sclerenchyma (scl), endoderm (end), and multiseriate cambium (cam); **C-D:** Details of the vascular system: secretory canal (sc), internal phloem (ph), xylem (xy), and vessel elements (ve); **E:** Detail of the internal phloem (ph) containing idioblasts with druses and parenchymatous cells with starch grains; **F:** Detail of the medullary parenchyma, containing numerous starch grains (amid). Stain: safranin and Astra blue (A, B, C, D, E and F). Optical microscopy and DV150F Samsung Digital Camera.



**Figura 4.** Pruebas histoquímicas de secciones transversales de las hojas y el tallo **A:** Blanco (control); **B:** Floroglucinol ácido: xilema lignificado en el tallo; **C:** Cloruro férrico: compuestos fenólicos en el parénquima en empalizada del mesófilo; **D:** Lugol: granos de almidón en el parénquima fundamental del pecíolo; **E:** Xilidina Ponceau: células del parénquima medular del tallo indicadoras de proteínas teñidas con xilidina; **F:** Sudam III: compuestos lipídicos en las paredes cutinizadas de la epidermis de la nervadura central. Microscopio óptico y DV150F Samsung Camera Digital.

**Figure 4.** Histochemical tests of cross-sections of leaf and stem. **A:** White (control); **B:** Acidic phloroglucinol – lignified xylem in the stem; **C:** Ferric chloride: phenolic compounds in the palisade parenchyma of the mesophyll; **D:** Lugol: starch grains in the fundamental parenchyma of the petiole; **E:** Xylidine Ponceau: stained cells of the medullary parenchyma of the stem indicating proteins; **F:** Sudam III: lipidic compounds in the cutinized walls of the epidermis of the midrib. Optical microscopy and DV150F Samsung Digital Camera.

anticlinal walls of epidermal cells: plants growing in the shade show sinuous cells while those exposed to direct sunlight have straight cell walls (Wilkinson 1979). Leaf macro- and micro-morphological characters have shown to be very plastic in different environments according to anatomical studies with ecological approach (Esau 1977, Fahn 1990, Cutter 1986). Lemos *et al.* (2019) observed that the anticlinal epidermal walls in the leaves of *Eugenia puniceifolia* (Kunth) DC growing in shaded environments demonstrated greater sinuosity than those in well-

illuminated sites. Nonetheless, this character is essentially genetically determined in some species, not influenced by environmental conditions, so that cell wall characteristics can be of taxonomic value (Wilkinson 1979).

The striated nature of the cuticle in *I. hederifolia*, as previously reported for this species (Monquero *et al.* 2005, Bolarinwa *et al.* 2018), is also frequently seen in the genus *Ipomoea*, such as in *I. asarifolia*, *I. mauritiana* Jacq. (Folorunso 2013) and *I. longerosa* Choisy (Santos & Nurit-Silva 2018). From an

	Tests /Metabolites				
	Acidic phloroglucinol Lignin	Ferric chloride Phenolic compounds	Lugol Carbohydrates	Xylidine ponceau Total proteins	Sudan III Lipids
	Mesophyll				
Ep	-	-	-	-	+
Sp	-	+	+	-	-
Pp	-	+	+	-	-
Midrib					
Ep	-	-	-	-	+
Cp	-	-	+	-	-
Vs	+	-	+	+	-
Petiole					
Ep	-	-	+	-	+
Cp	-	-	+	-	-
Vs	+	-	+	+	-
Stem					
Ep	-	-	-	-	+
Cp	+	-	+	-	-
Vs	+	-	+	+	+

**Tabla 1.** Ensayos histoquímicos realizados en hojas y tallos de *Ipomoea hederifolia* L. Epidermis (Ep), Parénquima cortical (Cp), Parénquima esponjoso (Sp), Parénquima en empalizada (Pp), Sistema vascular (Vs).

**Table 1.** Histochemical tests performed on the leaves and stems of *Ipomoea hederifolia* L. Epidermis (Ep), Cortical parenchyma (Cp), Spongy parenchyma (Sp), Palisade parenchyma (Pp), Vascular system (Vs).

ecological point of view, just as the sinuous nature of the anticlinal epidermal cell walls is modified by environmental factors, the cuticle seems to be plastic, with striations commonly observed in well-illuminated habitats and usually absent in shaded plants (Pereira *et al.* 2003). Pegorini *et al.* (2008) reported that xerophytic plants generally have wrinkled cuticles, which contribute to prevent water loss to the environment.

The wrinkled cuticle observed in individuals of *I. hederifolia* is most likely associated with sunlight incidence, especially in individuals collected in the forest edges. According to Esau (1977), cuticles have a number of functional roles, including mechanical protection and the restriction of leaf transpiration and aeration.

Glandular and peltate trichomes were observed in *I. hederifolia*. Monquero *et al.* (2005) reported simple, unicellular, and glandular trichomes in *I. hederifolia*, while Bolarinwa *et al.* (2018) observed only simple trichomes, differently from the present study. The glandular trichomes observed in our sample correspond to those identified in other species of Convolvulaceae, such as *I. asarifolia* (Martins *et al.* 2012), *I. pes-caprae*, *I. imperati* (Vahl) Griseb (Kuster *et al.* 2016), *I. sepiaria* Koenig ex Roxb. (Prasanth *et al.* 2018), and in species of *Stictocardia* (Olaranont *et al.* 2018). According to Ashfaq *et al.* (2019), glandular and peltate trichomes are common among Convolvulaceae species. Trichomes, like the cuticle, exercise different functions in different plants. High concentrations of simple trichomes can act as barriers against high sunlight incidence and water losses (Werker 2000, Zini *et al.* 2016). Some

researchers have suggested the production of chemical compounds such as volatile terpenes that serve as defensive substances against herbivory as one possible function of glandular and peltate trichomes (Peiffer *et al.* 2009).

The amphistomatic leaves seen in *I. hederifolia* have been observed in other species of the genus, such as *I. cairica* (Mandal *et al.* 2015), *I. eriocarpa* (Khalifa *et al.* 2017), *I. pes-tigridis* (Babu *et al.* 2018), *I. longeramosa* Choisy (Santos & Nurit-Silva 2018), and *I. bahiensis* Willd. ex Roem. & Schult. (Silva & Lemos 2020), although hypostomatic patterns have also been reported in the genus, such as in *I. learii* Knight ex J. Paxton (Porwal *et al.* 2015) and *I. pes-caprae* (Nilam *et al.* 2018). Paracytic stomata are common in Convolvulaceae species (Metcalf & Chalk 1950), as observed in *I. eriocarpa* (Khalifa *et al.* 2017), *I. pes-caprae* (Nilam *et al.* 2018), *I. longeramosa* (Santos & Nurit-Silva 2018), and *I. bahiensis* (Silva & Lemos 2020). Stomatal types observed in *Ipomoea* also include cyclocytic (e.g., *I. carnea*, Abba *et al.* 2018), staurocytic (e.g., *I. purpurea*, Bolarinwa *et al.* 2018), anisocytic and anomocytic (e.g. *I. cairica*, Mandal *et al.* 2015), and diacytic (e.g., *I. learii*, Porwal *et al.* 2015) stomata. In *I. hederifolia*, paracytic stomata predominate (Monquero *et al.* 2015).

Stomatal types represent taxonomically important characters and are relevant in the distinction of species with otherwise strong vegetative similarities (Lopes-Silva *et al.* 2021). Ten stomatal types, together with other epidermal characters, give strong support to the taxonomy of the tribe Bignoniaceae (Bignoniaceae), and the ecological role of stomata is



extremely relevant to our understanding of physiological processes in plants (Xiong & Flexas 2020). Their distribution on the leaf surface has been associated to environmental conditions. For example, plants with amphistomatal distribution (such as *I. hederifolia*) generally occupy environments with high incident sunlight, so that the entry of carbon dioxide into the leaves of those plants is greater when compared to plants growing in the shade (Muir 2019). *Ipomoea hederifolia* rows preferentially along forest edges, where luminosity is higher. As such, stomata distribution in this species is intrinsically related to environmental conditions.

The dorsiventral mesophyll of *I. hederifolia*, with its uniseriate palisade parenchyma, is similar to that of *I. pes-tigridis* (Babu *et al.* 2018), and *I. sepiaria* (Prasanth *et al.* 2018), and different from *I. burchellii* Meisn. (Santos *et al.* 2020), *I. imperati*, and *I. pes-caprae*, which have an isobilateral mesophyll (Arruda *et al.* 2009). The leaf edge of *I. hederifolia* is similar to that described in species of other genera of Convolvulaceae, such as *Argyreia* spp. (Traiperm *et al.* 2017) and *Stictocardia beraviensis* (Vatke) Hallier f. (Olaranont *et al.* 2018).

The biconvex shape of the midrib and the presence of a vascular system composed of a single vascular bundle organized in an arc observed in *I. hederifolia* coincides with the descriptions of *I. triloba*, *I. longeramosa* (Santos & Nurit-Silva 2015 2018), and *I. eriocarpa* (Khalifa *et al.* 2017). Other vascular bundle shapes have been described in Convolvulaceae, such as planar-convex and concave-convex (Tayade & Patil 2012). The the vertically organized angular collenchyma and the adaxial salience seen in *I. hederifolia* represent differential characters of this species.

The concave-convex shape of the petiole of *I. hederifolia* is a common character in the genus and is seen, for example, in *I. asarifolia* (Martins *et al.* 2012), *I. triloba* (Santos & Nurit-Silva 2015), and *I. pes-tigridis* (Babu *et al.* 2018). The presence of a hypoderm followed by collenchyma in the petiole of *I. hederifolia* is similar to that described in *I. eriocarpa* (Khalifa *et al.* 2017). The probable lactiferous canals observed in the petiolar parenchyma is a diagnostic character for Convolvulaceae (Metcalfe & Chalk 1950) and has been recorded in many species of *Ipomoea* (Martins *et al.* 2012, Kuster *et al.* 2016, Santos & Nurit-Silva 2018), as well as in other genera, as in *Distimake tuberosus* (L.) AR Simões & Staples (Tamaio *et al.* 2021).

The anatomical features of the stem of *I. hederifolia* are similar to those observed in other *Ipomoea* species, such as *I. cairica* (Dan-Sheng *et al.* 2007),

*I. quamoclit* (Rajendran *et al.* 2007), and *I. pes-tigridis* (Babu *et al.* 2018), and characterize the genus. The organization of the vascular system with intraxylemic phloem in the periphery of the medulla is a universal characteristic of Convolvulaceae according to Carlquist & Hanson (1991). In their study of the development of the intraxylemic phloem and internal cambium in *I. hederifolia*, Patil *et al.* (2009) reported that the internal phloem originates from procambial activity after the formation of the protoxylem.

The fibers in the stems of *I. hederifolia*, described as sclerenchymal bundles, are very similar to those observed in *I. nil* (L.) Roth, although the latter have been described as gelatinous. Gelatinous fibers are common among vines and tendrils and allow them to wrap around other plants (Bowling & Vaughn 2009). More precise studies of the cortical and vascular regions of the stem are necessary to determine whether the fibers in *I. hederifolia* are gelatinous or not.

Idioblasts containing druses in the petiole parenchyma and cortical and medullar regions of the stem are a common characteristic in other representatives of Convolvulaceae and have been reported in *I. eriocarpa* (Khalifa *et al.* 2017), *I. sepiaria* (Prasanth *et al.* 2018), and *Stictocardia beraviensis* (Olaranont *et al.* 2018). Druses presumably have diverse and ecologically important functions in plants, including defense against herbivores and prevention of water loss in situations of water stress, aiding in the closure of the stomata (Nakata 2012, Tooulakou *et al.* 2016).

In terms of the histochemical tests, the substances identified here have been reported in other *Ipomoea* species. For example, phenolic compounds have been reported in *I. asarifolia* (Martins *et al.* 2012) and lignin in the xylem of *I. obscura* (L.) Ker Gawl. (Meena & Santhi 2018). Although many of the proteins visualized in the parenchymatous and vascular tissues of *I. hederifolia* may be macromolecules fundamental to cellular structure and functioning, similar tests for proteins in *I. asarifolia* and *I. fistulosa* Mart. ex Choisy gave negative results (Martins *et al.* 2012, Mukherjee *et al.* 2011).

Histochemical studies of Convolvulaceae are rare. Various chemical substances were detected in the glandular trichomes of *I. asarifolia* by Martins *et al.* (2012), *Bonamia ferruginea* (Choisy) Hallier f. by Paes & Mendonça (2008), *I. cairica* by Mandal *et al.* (2015), *I. pes-caprae* and *I. imperati* by Kuster *et al.* (2016), and *S. beraviensis*, *Stictocardia tiliifolia* (Desr.) Hallier f. and *I. purpurea* by Olaranont *et al.* (2018). Further research is most likely to

reveal reveal valuable information on the biological and ecological aspects of chemical substances in the group. Corroborating the hypothesis of this study, stem and leaves in *I. hederifolia* were important sites of the accumulation of chemical compounds which may be related to defense against herbivory and pathogens, attraction of pollinators, and with the therapeutic potential of the species. This is a relevant contribution of this study since it can help to locate regions of the plant with the greatest amount of chemical compounds.

Distinct species that are widely used in popular medicine are known by the same common name and commercialized as substitutes in folk medicine. The structural and chemical characterization of plants is, therefore, fundamental for the quality control of their therapeutic drugs and to avoid adulteration.

So, the study of *I. hederifolia* brought new information about morphoanatomical characters useful for the correct identification and recognition of the species, essential to its medicinal use. In addition, the morphoanatomical characterization provided here represents a relevant contribution to the taxonomy of the genus *Ipomoea* and the family Convolvulaceae. The histochemical analyses showed new information about the diversity, origin and accumulation of secondary metabolites, which is important for the quality control of plant remedies. The chemical compounds encountered are most likely closely related to the therapeutic potential and wide use of the species in folk medicine. Therefore, the data obtained here may be used further and improve the study of *I. hederifolia* contributing to understand its therapeutic potential described in the literature through understanding the role and quantities of the secondary metabolites of the species.

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