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ANATOMY AND HISTOCHEMISTRY OF THE SOUTH AMERICAN NATIVE PLANT Allophylus semidentatus (SAPINDACEAE)

ANATOMIA E HISTOQUÍMICA DA PLANTA NATIVA SUL-AMERICANA *Allophylus semidentatus* (SAPINDACEAE)

ANATOMÍA E HISTOQUÍMICA DE LA PLANTA NATIVA SUDAMERICANA Allophylus semidentatus (SAPINDACEAE)

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ABSTRACT
To date, no anatomical or histochemical studies have been reported for Allophylus semidentatus. This study represents the first anatomical and histochemical investigation of the species. It aimed to explore the vegetative anatomy of A. semidentatus using light and scanning electron microscopy. Histochemical and energy-dispersive X-ray spectroscopy (EDS) analyses were performed to determine the chemical nature of cellular constituents and the elemental composition of the crystals. The main anatomical features observed include the presence of three types of trichomes (peltate and capitate glandular, and conical non-glandular trichomes), hypostomatic leaves, a biconvex midrib with a prominent adaxial ridge, and a biconvex petiole and petiolule with two adaxial wings. Druses and prismatic crystals of calcium oxalate were also identified. The anatomical and histochemical characteristics described here fill an important knowledge gap for the species and provide diagnostic features that support species identification and contribute to the taxonomy of the genus Allophylus.

KEYWORDS: Allophylus semidentatus. Microchemistry. Micromorphology. Microscopy. Sapindaceae.

RESUMO
Até o momento não foram relatados estudos anatômicos ou histoquímicos para *Allophylus semidentatus*. Este estudo representa a primeira investigação anatômica e histoquímica da espécie. Teve como objetivo explorar a anatomia vegetativa de *A. semidentatus* por meio de microscopia óptica e eletrônica de varredura. Análises histoquímicas e de espectroscopia de raios X por dispersão de energia (EDS) foram realizadas para determinar a natureza química dos constituintes celulares e a composição elementar dos cristais. As principais características anatômicas observadas incluem a presença de três tipos de tricomas (tricomas glandulares peltados e capitados, e tricomas cônicos não glandulares), folhas hipostomáticas, nervura central biconvexa com uma crista adaxial proeminente e pecíolo e peciólulo biconvexos com duas asas adaxiais. Drusas e cristais prismáticos de oxalato de cálcio também foram identificados. As características anatômicas

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e histoquímicas descritas neste trabalho preenchem uma importante lacuna de conhecimento sobre a espécie e fornecem características diagnósticas que auxiliam na identificação da espécie e contribuem para a taxonomia do gênero *Allophylus*.

PALAVRAS-CHAVE: *Allophylus semidentatus*. Micromorfologia. Microquímica. Microscopia. Sapindaceae.

RESUMEN
Hasta el momento no se han reportado estudios anatómicos ni histoquímicos sobre *Allophylus semidentatus*. Este estudio representa la primera investigación anatómica e histoquímica de la especie. Tuvo como objetivo explorar la anatomía vegetativa de *A. semidentatus* mediante microscopía óptica y microscopía electrónica de barrido. Se realizaron análisis histoquímicos y de espectroscopía de rayos X por dispersión de energía (EDS) para determinar la naturaleza química de los constituyentes celulares y la composición elemental de los cristales. Las principales características anatómicas observadas incluyen la presencia de tres tipos de tricomas (tricomas glandulares peltados y capitados, y tricomas cónicos no glandulares), hojas hipostomáticas, nervadura central biconvexa con una cresta adaxial prominente, y pecíolo y peciólulo biconvexos con dos alas adaxiales. También se identificaron drusas y cristales prismáticos de oxalato de calcio. Las características anatómicas e histoquímicas descritas en este trabajo llenan una importante laguna de conocimiento sobre la especie y proporcionan características diagnósticas que ayudan en la identificación de la especie y contribuyen a la taxonomía del género *Allophylus*.

PALABRAS CLAVE: *Allophylus semidentatus*. Micromorfología. Microquímica. Microscopía. Sapindaceae.

INTRODUCTION

Sapindaceae Juss. is a large family comprising about 1900 species grouped into 144 genera distributed mainly in the tropical and temperate regions of the world (Buerki *et al*., 2021). In Brazil, it is represented by about 436 species and 32 genera distributed throughout the country, with a greater presence in the Amazon region (Flora do Brasil, 2020). *Allophylus* L. is the sixth-largest genus in the family, with 28 species, including trees, shrubs, and lianas, distributed throughout all Brazilian regions, predominantly in the North (Coelho, 2020a).

Several biological activities have been reported for *Allophylus* species, including anti-inflammatory (Santos *et al*., 2021), antioxidant (Trevizan *et al*., 2016), antimicrobial (Islam *et al*., 2012), antiulcerogenic (Dharmani *et al*., 2005), and antidiabetic (Calero *et al*., 2014). These biological activities of the species are credited to their vast chemical components, for example, essential oils, flavonoids, and tannins in *Allophylus edulis* (A. St.-Hil. *et al*.) Hieron. ex Niederl. (Trevizan *et al*., 2016; Santos *et al*., 2021; Sobottka *et al*., 2021), flavonoids in *Allophylus cominia* Sw. (Semaan *et al*., 2017) and *Allophylus africanus* P. Beauv. (Ferreres *et al*., 2018), among others as coumarins, triterpenoids, and alkaloids (Ibrahim *et al*., 2018).

Allophylus semidentatus Radlk. is a native tree found in Bolivia, Brazil, and Peru (Plants of the World, 2023). In Brazil, it occurs in the Amazon, Cerrado, and Atlantic Forest biomes (Coelho, 2020b). Despite the pharmacological relevance reported for the genus, no studies describing the anatomy and histochemistry of *A. semidentatus* have been published to date. The absence of structural data for the species limits the availability of diagnostic anatomical characters that are traditionally employed in taxonomic studies (Metcalfe; Chalk, 1950). Anatomical and histochemical features, such as trichome types, stomatal distribution, vascular organization, and crystal occurrence, constitute important sources of diagnostic information for species characterization and botanical identification (Rodrigues *et al*., 2009; Rodrigues *et al*., 2025).

Histochemical staining, to allow for *in situ* analysis of bioactive compounds while maintaining three-dimensional cellular and tissue spatial information, has become a widely applied technique in the field of plant sciences (Yadav *et al*., 2021). The identification and histolocalization of secondary metabolites are performed through histochemical tests, in fresh or previously fixed plant material (Ventrella *et al*., 2013), which are based on the application of specific or nonspecific reagents capable of reacting selectively with certain classes of phytoconstituents (Rodrigues *et al*., 2009; Yadav *et al*., 2021). The results allow for the rational selection of the most suitable plant organ for phytotherapeutic applications, contributing to the sustainable use of species and promoting a more effective application of plant material (Rodrigues *et al*., 2025).

Among the various secondary metabolites present in plant species, phenolic compounds, such as flavonoids, anthocyanins, and tannins, stand out. These substances are involved in physiological processes *in planta*, but act *ex planta*, mainly due to their antioxidant capacity, reducing the production of free radicals and mitigating oxidative damage (Yadav *et al*., 2021). Flavonoids, generally stored in superficial tissues in the form of heterosides, when accumulated, form yellowish masses detectable histochemically (Rodrigues *et al*., 2009). Other phytoconstituents, such as lignins, alkaloids, lipids, proteins, and starch, can also be characterized by means of histochemical assays (Yadav *et al*., 2021; Rodrigues *et al*., 2025).

Thus, the integration of anatomical and histochemical analyses represents a robust and complementary approach for plant characterization, providing essential support for botanical identification and taxonomic delimitation, particularly in morphologically similar taxa. In this context, the present study aimed to investigate the microscopic and histochemical characteristics of the leaves and stems of *A. semidentatus*, contributing to species identification and expanding the anatomical knowledge of the genus.

1. METODOLOGY

Plant Material

Fresh leaves and stems, five samples each, of *A. semidentatus* were collected during October 2017 from the Capão do Cifloma region at the Botanical Campus of Federal University of Paraná, Curitiba, Paraná, Brazil (25°26′50"-25°27′33"S, 49°14′16"-49°14′33"W). The voucher specimen was identified by a taxonomist, José Tadeu Weidlich Motta, and deposited in the Curitiba

Municipal Botanical Museum under registry number MBM 304886. Access to the botanical material was authorized and licensed by the Conselho de Gestão do Patrimônio Genético (CGEN/SISGEN) of Ministério do Meio Ambiente, registered under number A13E001.

Light Microscopy Procedure

Portions of mature leaves and fragments of stems cut 5-15 cm from the apex were randomly collected from five specimens and fixed in formalin-acetic acid-alcohol (FAA) (Johansen, 1940) and stored in 70% ethanol after five days (Berlyn; Miksche, 1976). The materials were hand-sectioned using razors in transversal directions. The sections were hydrated and stained with toluidine blue (Ventrella *et al*., 2013) or double-stained with Astra blue and basic fuchsin (Kraus *et al*., 1998). The sections were then placed in a drop of 50% glycerin on a glass slide and covered with a coverslip. To study the epidermal characteristics, leaf fragments were immersed in sodium hypochlorite solution until translucent, washed with distilled water, and stained in safranin (Fuchs, 1963).

Photomicrographs were taken using an Olympus CX31 light microscope equipped with an Olympus C-7070 digital camera in the Laboratory of Pharmacognosy at the University of Ponta Grossa (UEPG), Brazil.

Histochemical Tests

Histochemical tests were conducted to verify the presence of the main classes of secondary metabolites. Freehand sections of FAA-fixed plant materials were performed using razors. The sectioned tissues were treated with various reagents and staining solutions following Ventrella *et al*. (2013), with some modifications. Coomassie brilliant blue was used to identify proteins, 2% solutions of ferric chloride and potassium dichromate to reveal phenolic compounds, 2% iodine solution to detect starch grains, Sudan III, and Sudan black to verify lipophilic compounds. The sections were treated with phloroglucinol-HCl to identify lignified elements, ruthenium red solution to reveal pectins, hydrochloric vanillin-HCl to detect condensed tannins, and Wagner and Dragendorff (Svendsen; Verpoorte, 1983) reagents were used to identify alkaloids. Untreated sections were used as a control. Photomicrographs were prepared as previously described in the light microscopy procedure section.

Scanning Electron Microscopy (Sem) and Energy-Dispersive X-Ray Spectroscopy (Eds) Procedures

The plant materials stored in 70% ethanol were dehydrated in 80%, 90% 100% ethanol series, then dried by CO2 in a critical point dryer (Bal-Tec CPD030). The dried samples were coated with gold using a sputter coater (Quorum SC7620). Electron micrographs were taken with a field-emission SEM (Mira 3, Tescan, Brno-Kohoutovice, Czech Republic) at the Multiuser Laboratory Complex in the State University of Ponta Grossa. An EDS detector (Oxford Instruments, Oxford, UK) attached to the SEM was used for qualitative and quantitative elemental microanalysis of the crystals present in the stem and leaflets; the cells devoid of crystals were used as a control.

2. RESULTS AND DISCUSSION

The leaves of *A. semidentatus* (Fig. 1a) are trifoliolate (Fig. 1b). In frontal view, the lamina presented sinuous anticlinal epidermal cell walls on the adaxial surface (Figs. 1c-d) and wavy walls on the abaxial side (Fig. 1f). The leaves were hypostomatic, and the stomata were anomocytic (Figs. 1f-g). In Sapindaceae, anomocytic stomata are common (Metcalfe; Chalk, 1950; Onuminya; Adediran, 2018; Tirloni *et al*., 2018), yet actinocytic, anisocytic, ciclocytic, and paracytic types were also observed (Pole, 2010; Onuminya; Adediran, 2018). Hypostomatic leaves are frequently found in Sapindaceae (Metcalfe; Chalk, 1950; Pole, 2010), however, in species of *Dodonaea* Mill., amphistomatic leaves were described (Pole, 2010).

Species belonging to the Sapindaceae family can be glabrous or have several types of trichomes (Acevedo-Rodríguez, 2003; Pole, 2010). A range of trichomes (glandular and/or non-glandular) is commonly found in some genera, such as *Allophylus*, *Cardiospermum* L., *Dodonaea*, *Paullinia* L., and *Talisia* Aubl., among others (Pole, 2010; Cunha Neto *et al*., 2017; Tirloni *et al*., 2018).

Glandular trichomes are one of the secretory structures present in Sapindaceae (Cunha Neto *et al*., 2017). Various micromorphologies of glandular trichomes were observed, such as chain (formed by a chain of short cells and a final long cell), balloon (short and uniseriate stalk with a multicellular glandular head), stalked multicellular with mushroom-like head, sessile multicellular, and capitate glandular trichomes (Pole, 2010; Cunha Neto *et al*., 2017). Glandular trichomes are important mainly because they contain many chemical compounds with biological activities. Also, their micromorphologies can be used in species identification (Budel *et al*., 2018; Justus *et al*., 2018; Almeida *et al*., 2020; Almeida *et al*., 2021; Machado *et al*., 2021). In *A. semidentatus*, two types of glandular trichomes were found: peltate and capitate. The peltate glandular trichomes are sessile and composed of a multicellular head with radial disposition; they are found only on the adaxial surface (Fig. 1h). Peltate trichomes were also found in other species, including *Atalaya calcicola* S.T.Reynolds, *Cupaniopsis fleckeri* S.T.Reynolds, *Dodonaea* spp., and *Ganophyllum falcatum* Blume (Pole, 2010). Capitate glandular trichomes are formed by a short uniseriate stalk and an oval head with four cells; they are sparsely distributed on the lamina, petiole, and petiolule (Fig. 1i). Pole (2010) named this capitate trichome a balloon trichome. A similar trichome has also been found in *Allophylus cobbe* (L.) Blume and *A. edulis* (Arambarri *et al*., 2006; Vieira *et al*., 2014) and several other species of Sapindaceae (Pole, 2010; Cunha Neto *et al*., 2017).

Non-glandular trichomes are widely reported in Sapindaceae and are described mainly based on their size and ramification (Metcalfe & Chalk, 1950; Acevedo-Rodríguez, 2003; Pole, 2010).

Pole (2010) classified non-glandular trichomes as simple (small or large sizes) or stellate, the latter being less common.

Simple non-glandular conical trichomes were found in all organs of *A. semidentatus* (Figs. 1e, j-k, 2a, e-f, 3a-c, j, r, and 4a, d), appearing in greater numbers in the stem and petiole. They were unicellular, curved or straight, and varying in size (Figs. 1 e, j); the ones on the midrib were usually larger (Figs. 1e, 3a, and 4d). This kind of trichome was also observed in *A. cobbe* (Pole, 2010) and *A. edulis* (Arambarri *et al*., 2006), and in other Sapindaceae species, such as *Talisia esculenta* Radlk. (Tirloni *et al*., 2018), *Cardiospermum halicacabum* L. (Norfaizal *et al*., 2017), *Dodonaea viscosa* Jacq. (Manfron *et al*., 2010) and *Sapindus trifoliatus* L. (George; Hari, 2020).

The leaflet blade, in cross-section, showed a thin and smooth cuticle (Fig. 3j) that covers the unilayered epidermis (Fig. 1m). The mesophyll presented dorsiventral organization, comprising a layer of palisade parenchyma and three layers of spongy parenchyma (Fig. 1m). Small collateral vascular bundles traverse the mesophyll. Secretory ducts are also found in the mesophyll (Fig. 3j). The dorsiventral mesophyll is also observed in *A. edulis* (Vieira *et al*., 2014) and is usually reported in Sapindaceae species (Metcalfe; Chalk, 1950; Tirloni *et al*., 2018; George; Hari, 2020).

The midrib (Fig. 1k), in cross-section, presented a biconvex shape with prominent convexity on the adaxial surface. The biconvex midrib shape was also reported for *A. edulis* (Arambarri *et al*., 2006). The epidermis was unilayered. The chlorenchyma was interrupted in the midrib region and substituted with angular collenchyma of about eight layers on the adaxial side and 3-4 layers on the abaxial side. In the collenchyma region or near that, idioblasts and secretory ducts are observed in both sides (Fig. 3n). The vascular system was represented by a collateral bundle in an open arc, with additional 4-5 bundles located dorsally (Fig. 1k). A continuous perivascular fiber-sheath surrounded the vascular system (Fig. 3p). Prismatic crystals were found in the cells adjoining fibers (Fig. 1l).

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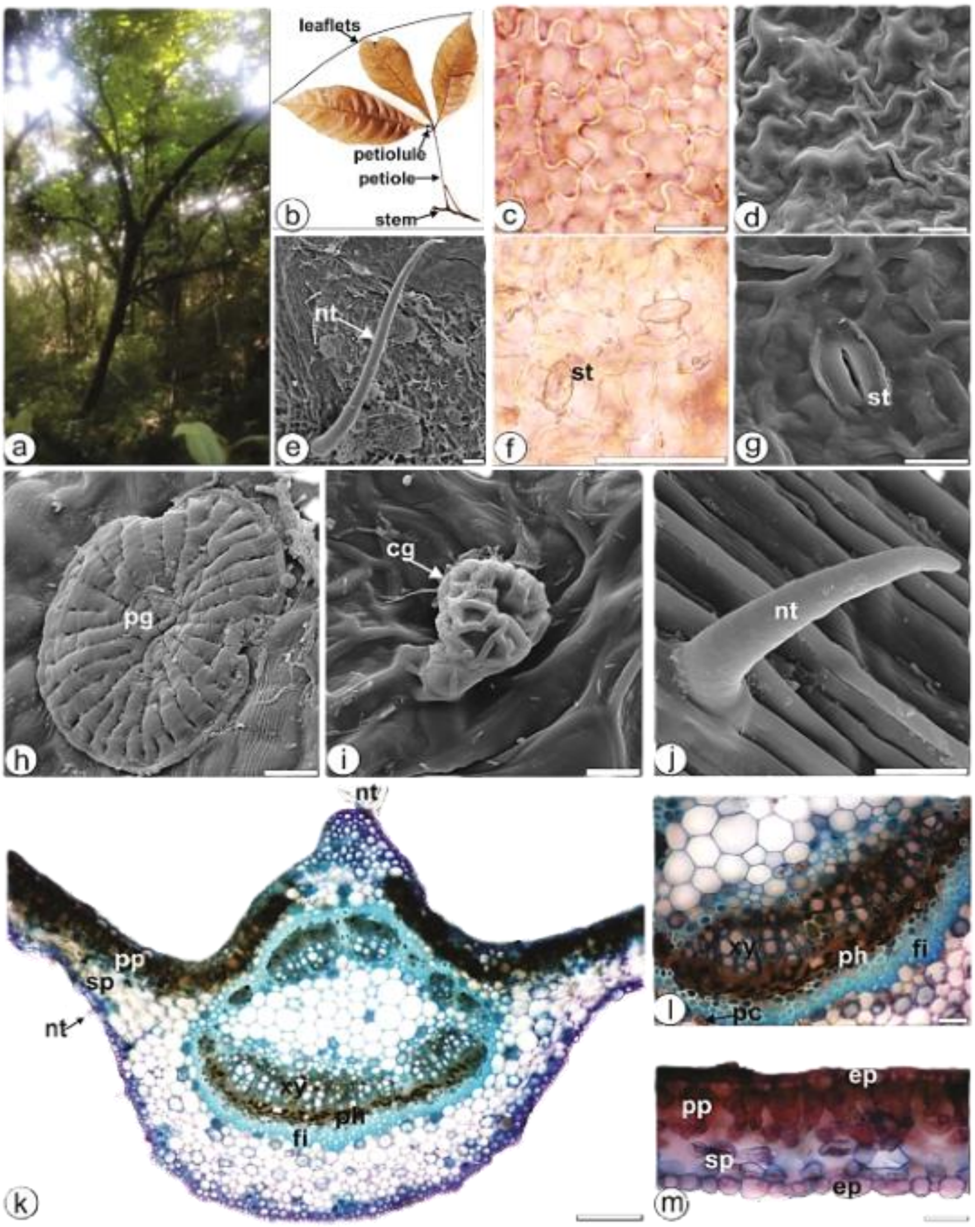
Figure 1a-m. Morpho-anatomy of *Allophylus semidentatus* (c, f, k-m: light microscopy; d, e, g-j: FESEM). (a) *A. semidentatus* in habit. (b) A twig of a plant. (c-j) Leaflets in surface view. (k-m) Leaflet in transverse section. (c, f) Stained in safranin; (m) stained in Astra blue and basic fuchsin; (k-l) stained in toluidine blue



Notes: cg: capitate glandular trichome; ep: epidermis; fi: fibers; nt: conical non-glandular trichome; pc: prismatic crystal; pg: peltate glandular trichome; ph: phloem; pp: palisade parenchyma; sp: spongy parenchyma; st: stomata; xy: xylem. Scale bar: g-j = 10 μm; d = 20 μm; c, e-f, l-m = 25 μm; k = 100 μm.

In cross-section, the petiole was biconvex on the adaxial side and convex on the abaxial side. The vascular system had eleven free collateral vascular bundles surrounding the pith (Fig. 2a).

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A continuous sclerenchymatous ring enclosed the vascular system (Figs. 2a-b and 3r). In Sapindaceae, different petiole shapes were described, such as irregular in *T. esculenta* (Tirloni *et al*., 2018), flat-convex with two wings on the adaxial side in *Cardiospermum* sp. (Solís; Ferrucci, 2006; Norfaizal *et al*., 2017), oval in *S. trifoliatus* (George; Hari, 2020), and flat-convex in *Cupania vernalis* Cambess. (Mundo; Duarte, 2009).

The epidermis had smaller and rounded cells than the leaflet blade epidermis. Beneath the epidermis, angular collenchyma was formed by 4-6 layers. Discontinuous chlorenchyma was observed only on the adaxial side of the petiole (Fig. 2b). Druses and prismatic crystals of calcium oxalate were observed in the petiole (Figs. 2c-d). The petiolule showed a biconvex shape in cross-section with two wings on the adaxial side (Fig. 2e). The epidermis is unilayered, formed by rounded cells (Figs. 2e-f). The vascular system was similar to the petiole, but with ten free collateral vascular bundles, forming a pith (Fig. 2e). Sclerenchymatous fibers were found adjoining the phloem. Prismatic crystals were found near the fibers and in the ground parenchyma (Fig. 2f). Also, idioblasts and secretory ducts, as previously described in the midrib, are present in the petiole and petiolule.

The stem, in the incipient secondary growth, showed a rounded shape in cross-section (Fig. 2g). This stem shape is common in species of Sapindaceae (Mundo; Duarte, 2009; Tirloni *et al*., 2018; George; Hari, 2020). The periderm was present, and the cortex was formed by several layers of parenchyma cells. Idioblasts and secretory ducts are observed in the cortex. The vascular system was arranged with phloem outward and xylem inward. Lignified fibers abutting the phloem formed a narrow ring (Figs. 2g-h). Pith occupies the middle portion of the stem and consists of thin-walled cells (Fig. 2g). Several prismatic crystals were found in the cortex, mainly next to the fibers (Fig. 2i).

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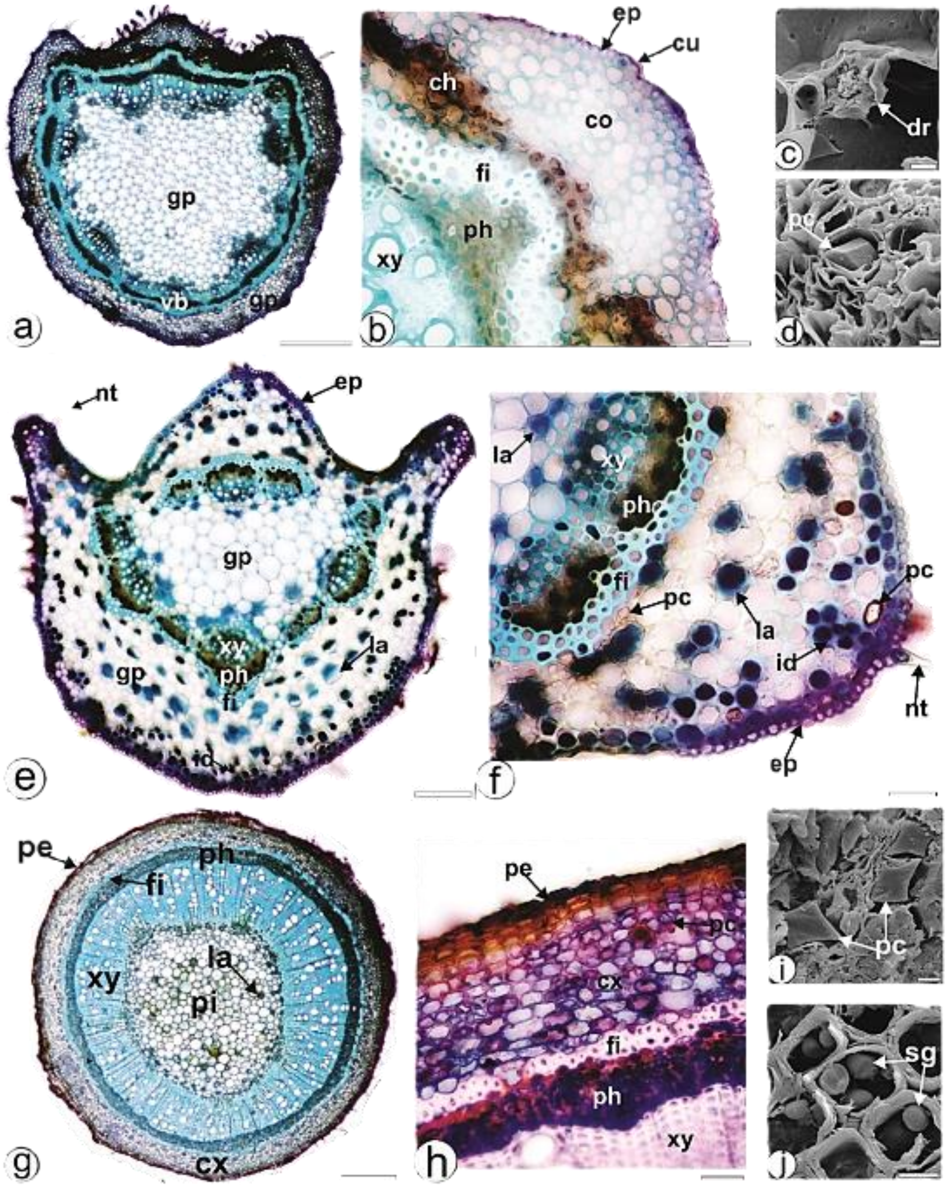
Figure 2a-j. Anatomy of *Allophylus semidentatus* (a-b, e-h: light microscopy; c-d, i-j: FESEM). Petiole (a-d), petiolule (e-f), and stem (g-j) in transverse sections. Stained in Astra blue and basic fuchsin (h); stained in toluidine blue (a-b, e-g)



Notes: nt: conical non-glandular trichome; ch: chlorenchyma; co: collenchyma; cu: cuticle; cx: cortex; dr: druses; ep: epidermis; fi: fibers; gp: ground parenchyma; id: idioblast; la: laticifers; pc: prismatic crystal; pe: periderm; ph: phloem; pi: pith; sd: secretory duct; sg: starch grains; vb: vascular bundle; xy: xylem. Scale bar: c-d, i-j = 5 µm; b, f, h = 25 µm; e = 100 µm; a, g = 250 µm.

The chemical composition of crystals (prismatic and druses) found in *A. semidentatus* was performed through EDS (Fig. 4i), and the spectra showed peaks of carbon (10.17%), calcium (40.73%), and oxygen (49.10%). This chemical composition suggested that the crystals were formed by calcium oxalate. Druses and prismatic crystals are commonly seen in species of Sapindaceae

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family (Metcalfe; Chalk, 1950; Baizhong *et al*., 1995; Mundo; Duarte, 2009). Also, Metcalfe and Chalk (1950) reported the presence of styloid crystals in Sapindaceae.

In addition to glandular trichomes, there are other secretory structures common in the Sapindaceae family, such as idioblasts, secretory ducts, and laticifers (Arambarri *et al*., 2006; Cunha Neto *et al*., 2017; Medina *et al*., 2021). Laticifers containing phenolics, alkaloids, pectins, proteins, and lipophilic compounds were found in the leaves and stems of *A. semidentatus* (Figs. 3d-i, m-o). Idioblasts were found in the ground parenchyma and collenchyma regions in the midrib, petiole, and petiolule (Fig. 2e), containing alkaloids (Fig. 4d), lipophilic compounds (Fig. 3m), phenolics (Fig. 3d), proteins (Fig. 4h), and tannin compounds (Fig. 3i).

Histochemical analysis in plants is an important tool for localization, characterization, and determining the concentration of secondary metabolite groups or chemical compounds within the tissues and cells. In the present study, phenolic compounds were evidenced by treating with ferric chloride and potassium dichromate solutions. They were observed inside the cells of conical non-glandular trichomes (Fig. 3b), palisade parenchyma, phloem of the midrib (Fig. 3a), petiole (Fig. 3c), petiolule (Fig. 3d), stem (Fig. 3e), and laticifers (Fig. 3f). Furthermore, vanillin solution was used for condensed tannins and evidenced this chemical group in the phloem of the midrib (Fig. 3g), petiole (Fig. 3h), petiolule (Fig. 3i), and stem. Laticifers also reacted with this reagent, indicating phenolic compounds (Figs. 3g-i).

Sudan III stained the lipids red or red-orange, and the Sudan black solution stained the lipophilic compounds black in the histochemical tests. Lipids were evidenced in the cuticle (Figs. 3j, k), glandular trichomes (Figs. 3k, l), phloem (Figs. 3k, m-n), secretory ducts (Figs. 3j, n), and in the laticifers (Figs. 3m-o).

Phloroglucinol-HCl stained lignin in the cell walls pink. Consequently, in *A. semidentatus* lignified elements were reacted, such as in the non-glandular trichomes cell walls (Fig. 3r), the fibers and xylem vessels in all analyzed organs (Figs. 3p-r). Starch grains were identified by iodine solution and were present in the perimedullary region of the stem (Figs. 2j and 3s).

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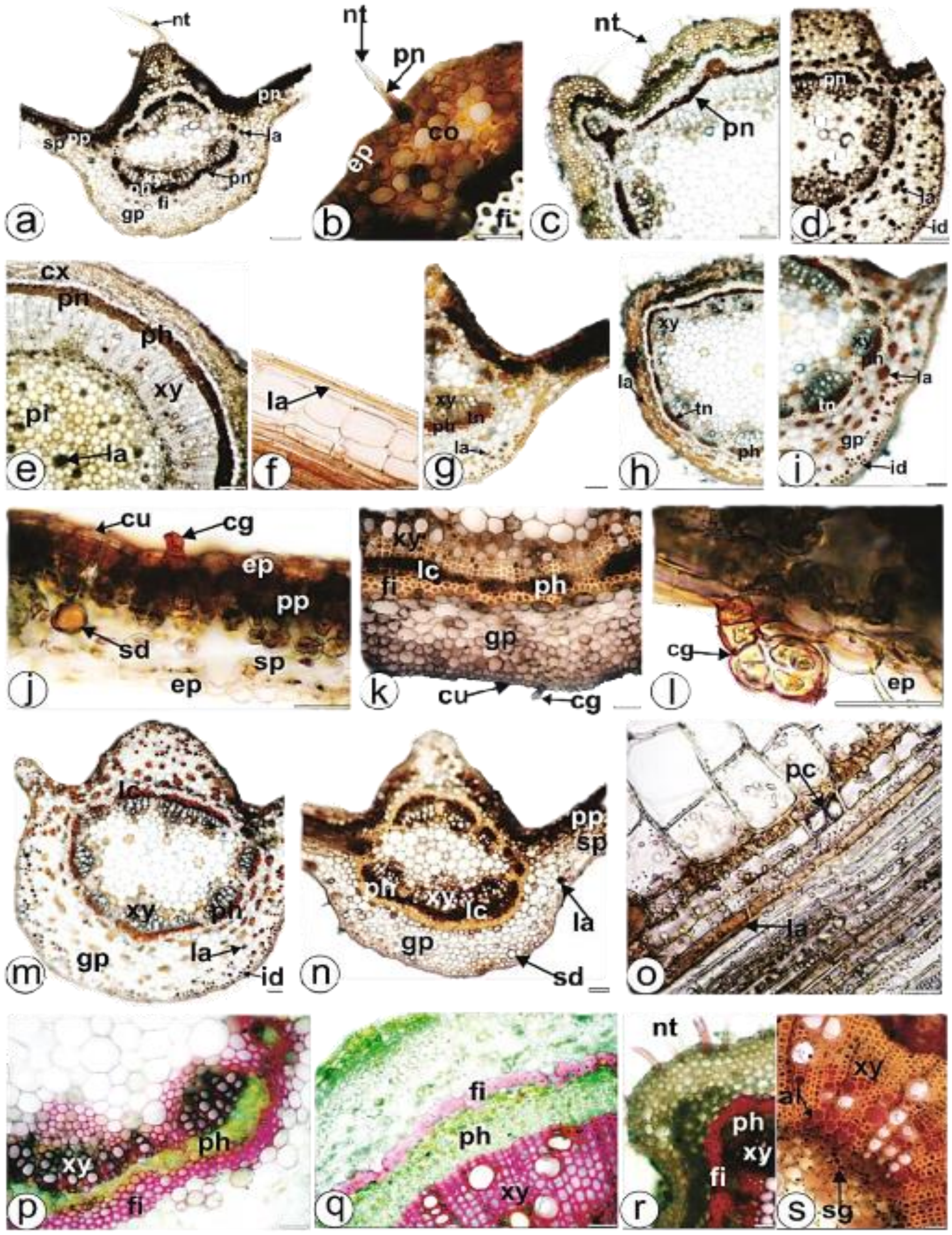
Figure 3a-s. Histochemistry of *Allophylus semidentatus* - light microscopy. Transverse sections of leaflets (a, g, j, l, n, p), petiole (b-c, h, k, r), petiolule (d, i, m), and stem (e, q, s). Longitudinal sections of leaflets (f) and stem (o). Reaction with ferric chloride (a, d), potassium dichromate (b-c, e-f), vanillin (g-i), Sudan black (k, n), Sudan III (j, l, m-o); phloroglucinol-HCl (p-r) and Dragendorff's solution (s)



Notes: al: alkaloids; cg: capitate glandular trichome; nt: conical non-glandular trichome; cx: cortex; co: collenchyma; cu: cuticle; ep: epidermis; fi: fibers; gp: ground parenchyma; id: idioblast; la: laticifers; lc: lipophilic compounds; pc: prismatic crystal; ph: phloem; pi: pith; pn: phenolic compounds; pp: palisade parenchyma; sd: secretory duct; sg: starch grains; sp: spongy parenchyma; tn: tannins; vb: vascular bundle; xy: xylem. Scale bar: b,f, j-l, o-s = 25 µm; g-i, m-n = 50 µm; a, c-e = 100 µm.

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Ruthenium red solution colorized pectin from pink to red. In the present study, pectin was detected in the epidermis, phloem (Fig. 4a), and laticifers (Figs. 4a, b) of *A. semidentatus*. Dragendorff and Wagner reagents stained alkaloids reddish-brown. Alkaloids were evidenced in palisade parenchyma in the leaflets (Fig. 4d), cells next to the xylem, phloem (Figs. 4c-e), in the ground parenchyma of the petiole (Fig. 4c), petiolule (Fig. 4e), stem, and laticifers (Figs. 4c-f). Coomassie brilliant blue stains proteins blue. In *A. semidentatus*, protein was found in cells next to the xylem and in laticifers in the petiole and petiolule (Figs. 4g, h).

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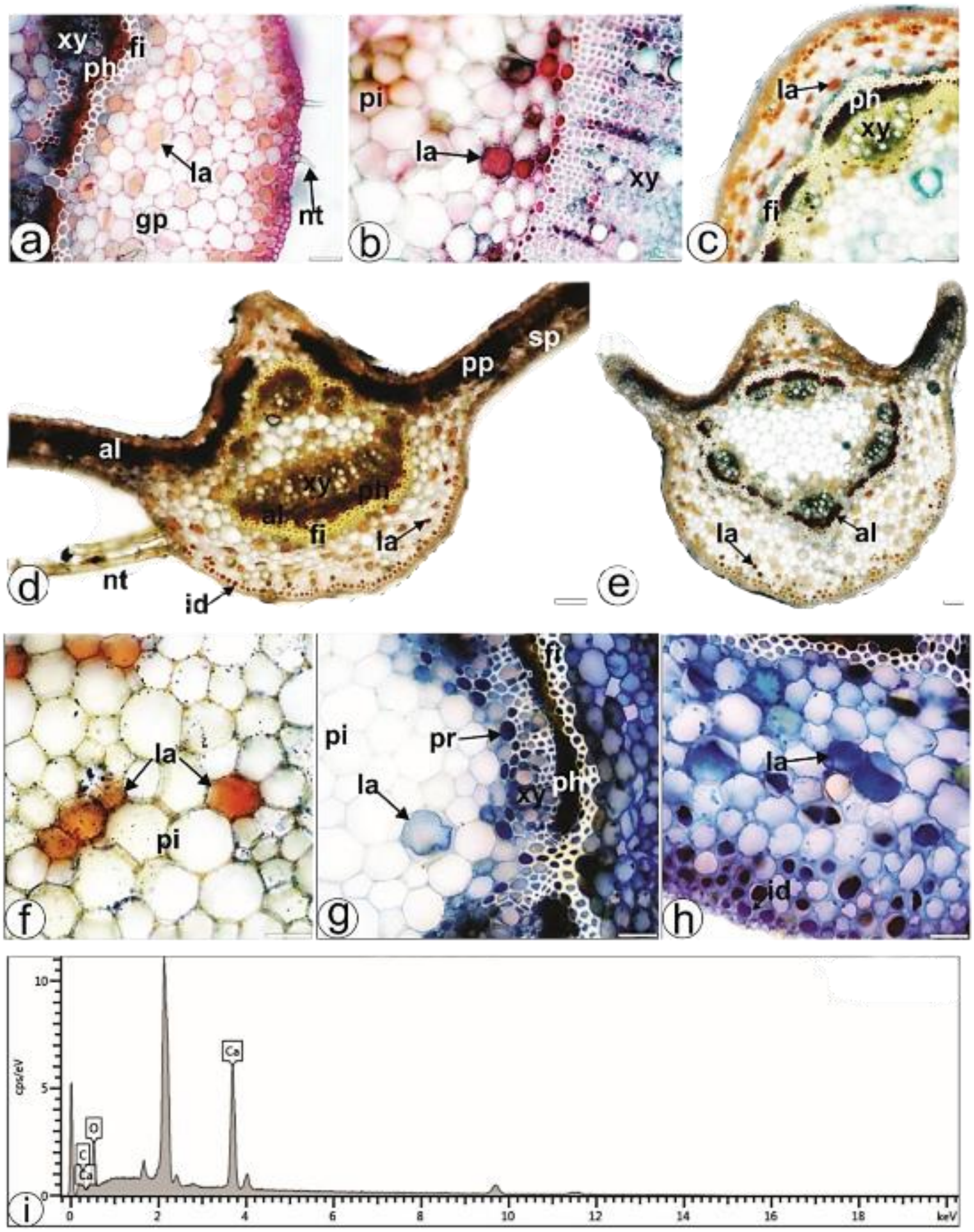
Figure 4a-i. Histochemistry of *Allophylus semidentatus* (a-h: light microscopy; i: EDS spectrum of a crystal). Transverse sections of leaflets (d), petiole (c, g), petiolule (a, e, h), and stem (b, f). Reaction with ruthenium red (a-b); reaction with Wagner (c-e); Dragendorff (f); Coomassie brilliant blue (g-h)



Notes: al: alkaloids; id: idioblast; fi: fibers; gp: ground parenchyma; la: laticifers; nt: conical non-glandular trichome; ph: phloem; pi: pith; pp: palisade parenchyma; pr: protein; sp: spongy parenchyma; xy: xylem. Scale bar: a-b, f-h = 25 µm; d-e = 50 µm.

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3. CONSIDERATIONS

The present work is the first study of the anatomy and histochemistry of *A. semidentatus*. The anatomical and histochemical markers highlighted in this study can be employed to identify and characterize the species. The main anatomical features include the presence of three types of trichomes (peltate and capitate glandular and conical non-glandular), hypostomatic leaves, and the shapes of leaflets, petiole, petiolule, and stem in cross-section. Similar studies are needed for other species of Allophylus to develop comprehensive anatomical data, which can aid in the taxonomy of the genus and family.

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