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Morphoanatomical study of *Clidemia hirta* (L.) D. Don.

Estudo morfoanatômico de *Clidemia hirta* (L.) D. Don.

Estudio morfoanatómico de *Clidemia hirta* (L.) D. Don.

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Abstract
The aims of this study were: to carry out the morphological study of the *Clidemia hirta* (L.) D. Don, the anatomical study of the leaves and young stem and the phytochemical screening of the powder of the leaves. The leaves were collected monthly at Bosque Auguste de Saint-Hilaire, Conservation Unit on Campus II of the Federal University of Goiás (UFG), Goiânia, Goiás, for 12 months. The specie was identified and a voucher specime deposited in the Herbarium of UFG (66 872- UFG). Morphoanatomical analysis was performed according to conventional techniques. It was verified at phytochemical screening, the presence de anthraquinone heterosides, starch, alkaloids, flavonoid and saponins. The leaf blade has uni-stratified epidermis covered by a thin cuticle on the adaxial and abaxial surfaces. The petiole consists of uni-stratified epidermis and cortical parenchyma formed by 4-6 layers of cells. The mesophyll is dorsiventral, the palisade parenchyma has 1 layer of cells and the lacunous parenchyma has 3 layers of cells. The median rib is convex-concave, the cortical parenchyma has 5-6 layers of cells and bicolateral vascular bundles. The young stem has uni-stratified epidermis and a thin cuticle, the cortical parenchyma is formed by 6-8 layers of cells. Flavonoids, saponins, phenols and starch were found. The moisture content was 8.343%, total ash 4.193%, flavonoids 3.13%, total phenols 6.74% and saponins 166.7. The present study contributes to taxonomic identification and provided parameters for the quality control of *C. hirta*.
Keywords: *Clidemia*; Melastomataceae; Morphoanatomy; Medicinal plants.

Resumo
Os objetivos do presente trabalho foram: realizar o estudo morfológico da *Clidemia hirta* (L.) D. Don, o estudo anatômico das folhas e caule jovem e triagem fitoquímica do pó das folhas. As folhas foram coletadas no Bosque Auguste de Saint-Hilaire, Unidade de Conservação no Campus II da Universidade Federal de Goiás (UFG), Goiânia, Goiás mensalmente, por 12 meses. A espécie foi identificada e uma exsicata depositada no herbário da UFG n° 66.872. A análise morfoanatômica foi realizada de acordo com técnicas convencionais. Na triagem fitoquímica foram pesquisados: heterosídeos antraquinônicos, amido; alcaloides; heterosídeos flavonoides e heterosídeos saponínicos. A lâmina foliar possui epiderme uniestratificada revestida por cutícula delgada nas faces adaxial e abaxial. O pecíolo é constituído por epiderme uniestratificada e parênquima cortical formado por 4-6 camadas de células. O mesofilo é dorsiventral, o parênquima paliçádico apresenta 1 camada de células e parênquima lacunoso apresenta 3 camadas de células. A nervura mediana é convexa-côncava, parênquima cortical tem 5-6 camadas de células e feixes vasculares bicolaterais. O caule jovem tem epiderme uniestratificada e cutícula delgada, o parênquima cortical é formado por 6-8 camadas de células. Verificou-se a presença de flavonoides, saponinas, fenóis e amido. O teor de umidade foi de 8,343%, de cinzas totais de 4,193%, de flavonoides de 3,13%, de fenóis totais de 6,74% e saponinas de 166,7. O presente estudo contribui para a identificação taxonômica e forneceu parâmetros para o controle de qualidade da *C. hirta*.
Palavras-chave: *Clidemia*; Melastomataceae; Morfoanatomia; Plantas medicinais.

Resumen
Los objetivos del presente trabajo fueron: realizar el estudio morfológico de la *Clidemia hirta* (L.) D. Don, el estudio anatómico de las hojas y tallo joven y el cribado fitoquímico del polvo de las hojas. as hojas se recolectaron mensualmente en el Bosque Auguste de Saint-Hilaire, Unidad de Conservación en el Campus II de la Universidad Federal de Goiás (UFG), Goiânia, Goiás, mensualmente, durante 12 meses. Se identificó la especie y se depositó un desecante en el herbario de UFG n ° 66.872. El análisis morfoanatómico se realizó según técnicas convencionales. En el cribado fitoquímico, se investigaron los siguientes: heterósidos de antraquinona, almidón; alcaloides; heterósidos de flavonoides y heterósidos de saponina. La lámina de la hoja tiene una epidermis uni-estratificada cubierta por una fina cutícula en las superficies adaxial y abaxial. El pecíolo consta de epidermis uniestratificada y parénquima cortical formado por 4-6 capas de células. El mesófilo es dorsiventral, el parénquima en empalizada tiene 1 capa de células y el parénquima lacunoso tiene 3 capas de células. La vena mediana es convexa-cóncava, el parénquima cortical tiene 5-6 capas de células y haces vasculares bicolaterales. El tallo joven tiene epidermis uni-estratificada y una cutícula delgada, el parénquima cortical está formado por 6-8 capas de células. Se encontraron flavonoides, saponinas, fenoles y almidón. El contenido de humedad fue 8.343%, cenizas totales 4.193%, flavonoides 3.13%, fenoles totales 6.74% y saponinas 166.7. El presente estudio contribuye a la identificación taxonómica y proporcionó parámetros para el control de calidad de *C. hirta*.
Palabras clave: *Clidemia*; Melastomataceae; Morfoanatomía; Plantas medicinales.

1. Introduction

Melastomataceae has 166 genera and about 4,570 species, concentrating predominantly in tropical and subtropical regions of the globe (Clausing & Renner, 2001). In Brazil, it is the sixth-largest family of angiosperms, with approximately 68 genera and more than 1500 species (Romero & Martins, 2002). It is distributed throughout the national territory, being common in the biomes of the Amazon, Cerrado, and Atlantic Forest. Many genera occur in the Cerrado, among them *Cambessedesia* DC., *Chaetostoma* DC., *Clidemia* D Don, *Fritzschia* Cham., *Lavoisiera* DC., *Lithobium Bong., Macairea* DC., *Marcetia* DC., *Microlepis (*DC.) Miq., *Microlicia* D. Don, *Stenodon* Naudin, *Svitramia* Cham. and *Trembleya* DC. (Goldenberg, et al., 2012). The family is characterized by decussed leaves with acrodrome nerves, generally sickle-celled stamens, and poricidal anthers (Romero & Martins, 2002).

The genus *Clidemia* has about 175 species, with a large part occurring in the neotropics, and approximately 50 of them occurring in Brazil, from Santa Catarina to Amazonas (Matsumoto & Martins, 2009). *Clidemia* species are characterized by acrodrome leaves, lateral or pseudo-lateral inflorescences, axillaries, and external calyx lobes larger than the internal ones. The stamens are usually targets, with unappendicular connective tissue or with descending dorsal appendix, inserted at the base of the connective, and the fruits are bacaceous (Goldenberg, et al., 2012). There are five species in the Cerrado, namely *Clidemia biserrata* DC., *Clidemia capitellata (Bonpl.)* D. Don, *Clidemia hirta* (L.) D. Don., *Clidemia octona* (Bonpl.) L. O. Williams e *Clidemia urceolata DC.* (Kuhlmann, 2016).

Clidemia hirta (Melastomataceae) is a pioneer, invasive, and weed species native to the neotropics, occurring from Mexico to southern Brazil. It is a shrub species of 1-2 m in height, characterized by glandular trichomes in the branches, hypanthium indument composed of simple and starry trichome, leaves base cordate to subcordate and ripe purple fruits, with showy white petals (Rocha, et al., 2017). It occurs in the Cerrado region, on the edges of gallery forest, shaded places, blooming and fruiting practically all year round (Goldenberg, et al., 2005; MMA/SBF, 2011).

The *Clidemia hirta* is popularly used for treating a variety of diseases. Its leaves are used in the preparation of teas for the treatment of skin ulcers caused by *Leishmania braziliensis* (França, et al., 1996) and in the treatment of diseases of the nervous and digestive system (Rego, et al., 2016), such as stuck intestine, stomach pain, diarrhea, gastritis, nausea, gas, poor digestion and congestion (Giraldi & Hanazaki, 2010). The infusion of leaves and stem is used in French Guiana in enemas and by women with very heavy menstrual flow, with the infusion having antispasmodic properties (Defilipps, et al., 2007). Tea is used for palpitations, its flowers for kidney diseases, and its fruits for bladder and leukorrhea (Cruz & Kaplan, 2004).

An *in vitro* study carried out with the diluted ethanolic extract of *C. hirta* leaves, demonstrated antibacterial activity against four strains of bacteria: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus hirae* (Lopez, et al., 2016).

Another study, carried out by Narasimham, et al. (2017), demonstrated anti-cancer activity of diluted ethanolic extract of *Clidemia hirta* leaves against cells of the Dalton Lymphoma Ascites (DLA) lineage. Bomfim, et al. (2020) verified antimicrobial activity by the disk diffusion test of the ethyl acetate extract of the aerial parts of *C. hirta* against *M. luteus*, *S. aureus*, and *P. aeruginosa*, with halos of 13.0 ± 1.02, 9.0 ± 0.57, and 10.0 ± 1.52 mm, respectively, antioxidant activity by the DPPH method (63.54 ± 1.46%) and high toxicity against *Artemia salina* (LC50 75.8 µg / ml).

The aims of this study were: to carry out the morphological study of *Clidemia hirta*, the anatomical study of the leaves and young stem, and phytochemical screening of the powder of the leaves not yet reported in the literature.

2. Methodology

2.1 Collection of plant material

The botanical material was collected at Bosque Auguste de Saint-Hilaire, Campus II of the Federal University of Goiás /UFG (16º36'11" S; 49º15'39" W; 695m alt.), in a remnant of seasonal forest, in a shaded place and silica-clay soil. The species was identified by Professor Dr. Heleno Dias Ferreira, and a voucher specimen deposited at the Herbarium of the Federal University of Goiás (number UFG- 66872).

2.2 Morphological description

The macroscopic characterization of the *Clidemia hirt*a was carried out with the naked eye, monthly for 12 months in Bosque Auguste de Saint-Hilaire, Conservation Unit on Campus II of the Federal University of Goiás (UFG) and in the Taxonomy Laboratory, Department of Botany / ICB / UFG with the aid of a SZ-ST OLYMPUS stereoscope microscope and the images were recorded with a CANON EOS T4i digital camera.

2.3 Anatomical study

For the anatomical study, leaves (main rib, vein, maple, and petiole) and fresh young stems between second and third internodes were studied. Paradermical sections were made on the adaxial and abaxial faces of the leaves, and freehand cross-sections. The cuts were clarified with 50% sodium hypochlorite, then washed with distilled water, neutralized with acetic acid and again washed with distilled water, and stained with Alcian / Safranina 9: 1 blue, placed between slide and coverslip containing a drop of glycerin / 50% water (v / v) (Kraus & Arduin, 1997). Histochemical tests were performed with: ferric chloride (Johansen, 1940) (blue-black color indicates the presence of phenolic compounds), Steinmetz - polyvalent reagent (Costa, 2001) (blue-black color presence of starch; the red-orange color presence of lipids; red color shows cutin; golden color shows lignin; calcium oxalate crystals remain colorless) and lugol (Johansen, 1940) (black-blue or dark brown starch). The photographic record of the anatomical structures was performed in a photomicroscope (Zeiss-Axiostar plus) with a digital camera attached (Canon Power Shot G10), using the Axion Vision 4.8 program.

2.4 Phytochemical screening

For phytochemical studies, analyzes of ash and moisture content of the leaves were collected, placed in plastic bags, dried in an oven with air circulation at a temperature of 40 °C, and then pulverized in a knife mill.

The phytochemical composition of leaf powder was screened for the presence of anthraquinone heterosides, saponin heterosides, flavonoid heterosides, starch and alkaloids (Costa, 2001, Cunha, 2009).

The determination of total phenols of the leaves of *C. hirta* was carried out according to the methodology described by Hagerman and Butler (Mole & Waterman, 1987) and for flavonoids content was used the method described by Rolim et al. (2005).

The leaves powder (0.75 g) were extracted with distilled water (150 mL). The mixture was heated to boiling, after kept in water bath at between 80 and 90 °C during 30 min. The contents of the flask were transferred to a 250 mL volumetric flask and the volume was made up with distilled water. The extract was filtered through qualitative filter paper, with first 50 mL discarded. The aqueous extract obtained were used to quantification of total phenols (TP).

To extract the flavonoids, 0.5 g of the vegetable drug was weighed and transferred to a 250 mL round-bottom flask. 100 ml of 0.02M methanol: acetic acid (99: 1) solution was added and the mixture was heated in a water bath under reflux at 90-100 ºC for 40 min. The flask was cooled under running water and the extract was filtered through filter paper.

In a test tube, 2 ml of the extract were added and the absorbance reading was performed at 361nm. The methanol solution: 0.02M acetic acid (99: 1) was used as white. To prepare the standard curve, 5 mg of rutin were weighed in a 50 mL volumetric flask whose volume was completed with a 0.02M methanol: acetic acid (99: 1) solution. 200 aliquots were withdrawn; 400; 600; 800 and 1000 µl, transferred to test tubes previously identified and made up to 2 ml with the methanol: 0.02M acetic acid (99: 1) solution. The mixture was homogenized and the absorbance reading was performed at 361 nm. In this way, an absorbance x concentration curve was plotted. From the equation obtained from the standard curve, it was possible to calculate the concentration (mg / mL) of total flavonoids in the extract and the percentage present in the plant drug. The experiment was carried out in triplicate.

The moisture analyzer (Ohaus model MB35) determined the moisture content leaf powder (Brazil, 2010). Total ash content was determined according to the Brazilian Pharmacopoeia (Brasil, 2019). The experiment was carried out in triplicate.

To determine the swelling index, 1 g of the pulverized plant material was weighed and transferred to an Erlenmeyer flask containing 50 mL of boiling water. The solution was kept under moderate boiling for 30 min. Then, it was cooled and filtered into a 100 mL volumetric flask. Extractions of the same material were carried out using successive 10 ml portions of boiling water until the volume of 100 ml was completed. The decoction obtained was distributed in 10 test tubes with a lid, in a successive series of 1, 2, 3 to 10 mL and the volume of the liquid adjusted to 10 mL with water. The tubes were capped and shaken with vertical movements for 15 seconds. The tubes were left to stand for 15 min and the foam height was measured to calculate the foam index.

3. Results

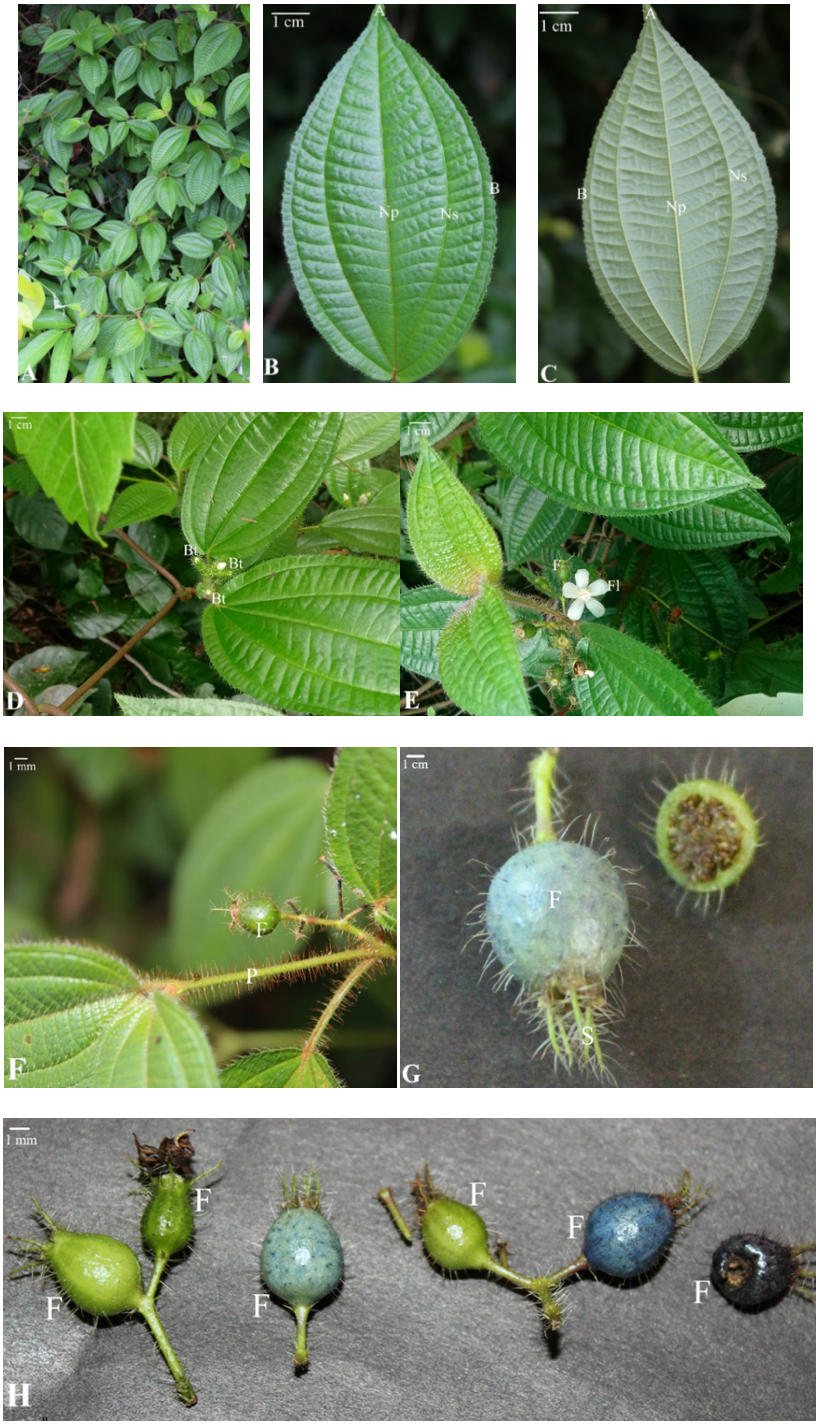
Clidemia hirta occurs in partially shaded, damp, and warm areas. During the month of September, it was verified the presence of green fruits. From October to June, flowering and fruiting are observed, with both flower buds and open flowers. It was observed during the monthly visits, the presence of ants and larvae on leaves.

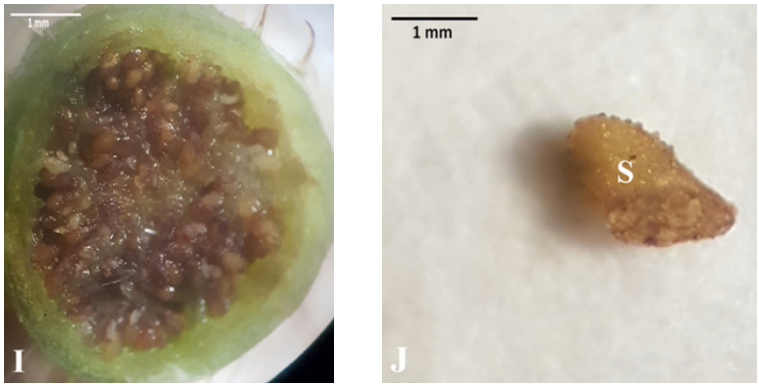
3.1 Morphological description

C. hirta is a shrub with a branched, green stem, up to 1.5 meters high (Figure 1A). Green, simple, opposite, petiolate, hirsute leaves on both sides and along the leaf margin, trichomes sparsely arranged on the adaxial face and along the ribs on the abaxial face, young leaves with pink hairiness and elliptical leaf blade, 5-17 cm x 3-8 cm, acuminate apex, rounded base, toothed-ciliated margin, basal acromodal venation. The dark green color on the adaxial face and light green on the abaxial face (Figures 1B and 1C). The hirsute petiole is 2 to 5 cm long and 1 to 3 mm in diameter (Figure 1F). White flowers (Figures 1D and 1E). Persistent calyx with 5 free, filiform, hirsute sepals, 0.5 mm long. Corolla with 5 free petals, glabrous and oblong, 0.80 to 1 mm x 0.50 to 0.60 mm, rounded apex; androecium with 10 stamens of 5 mm long, of the same shape and size, white, dialistemonous,

inserted in the hypanthium. Monothecous anthers with two chambers. Inferior ovary, glabrous, with 5 locus multiovulate. Terminal stylus, white; 1 stigma. Berry type fruit, 8 mm x 7 mm, depending on the stage of maturation, the color of the fruit can vary from green to dark purple, rounded to oblongoid in shape, rounded apex, externally hirsute, and with a large number of seeds (Figure 1G, 1H and 1I). Cuneiform seed, rough, 1 mm long (Figure 1J).

Figure 1. *Clidemia hirta*. **A**- General aspect of the species. **B**- Adaxial face of the leaf. **C**- Abaxial face of the leaf. **D**- Flower buds. **E**- Open flower. **F**- Green fruit. **G**- Fruit. **H**- Fruits in different ripening stages. **I**-Fruit in cross-section showing seeds. **J**- Detail of the seeds. **B**- Board. **Bt** -Flower buds. **Fl**- Flower. **F**- Fruit. **NP**- Midrib. **NS**- Secondary vein. **P**-Petiole.





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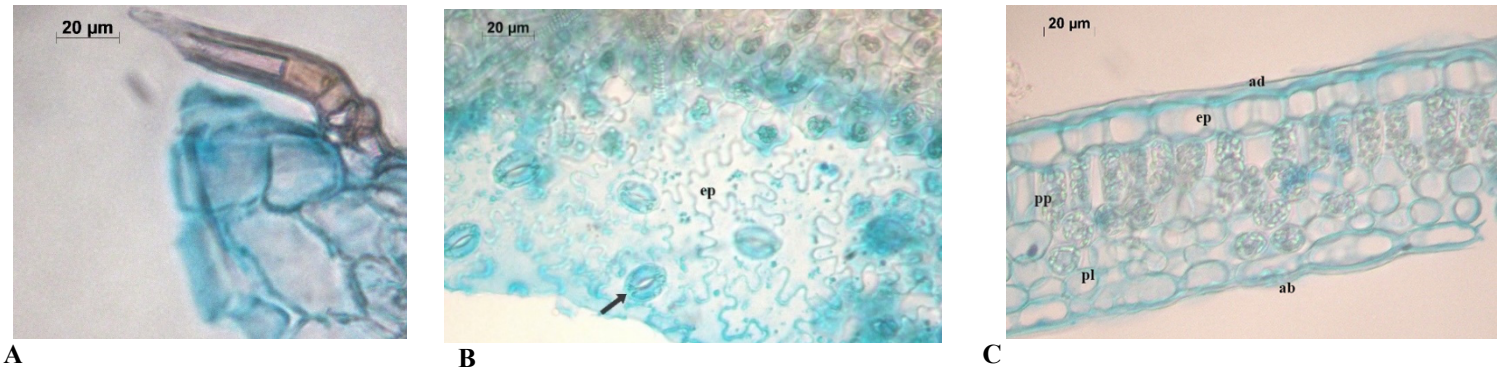
3.2 Anatomical study

The leaf, in the cross section, is hypoestomatic, has a uni-stratified epidermis covered by a thin cuticle on the adaxial and abaxial surfaces. The cell walls of the epidermis of the adaxial face are straight (Figure 2A). The cell walls of the epidermis of the abaxial face are sinuous with stomata that are usually anomocytic (Figure 2B). Presence of simple trichomes, distributed sparsely on both sides of the leaf blade (Figure 2A).

The mesophyll is dorsiventral. The epidermal cells on the adaxial face are rectangular and more voluminous than those on the abaxial face (Figure 2C). The palisade parenchyma is composed of 1 layer of cells and the lacunous parenchyma is composed of 3 layers of cells. Occurrence of idioblasts with druses both in the palisade parenchyma and in the lacunous parenchyma.

Figure 2. Section of the leaf blade of *C. hirta*. **A**- Adaxial epidermis - paradermic section. **B**- Abaxial epidermis - paradermic section showing anomocytic stomata. **C**- Mesophyll in cross section. **A, B** and **C**- Alcian / safranin blue staining. **ad-** Adaxial face, **ab**- Abaxial face, **ep**- Epidermis, **pl**- Lacunous parenchyma, **pp-**palisade parenchyma, arrow-stoma.

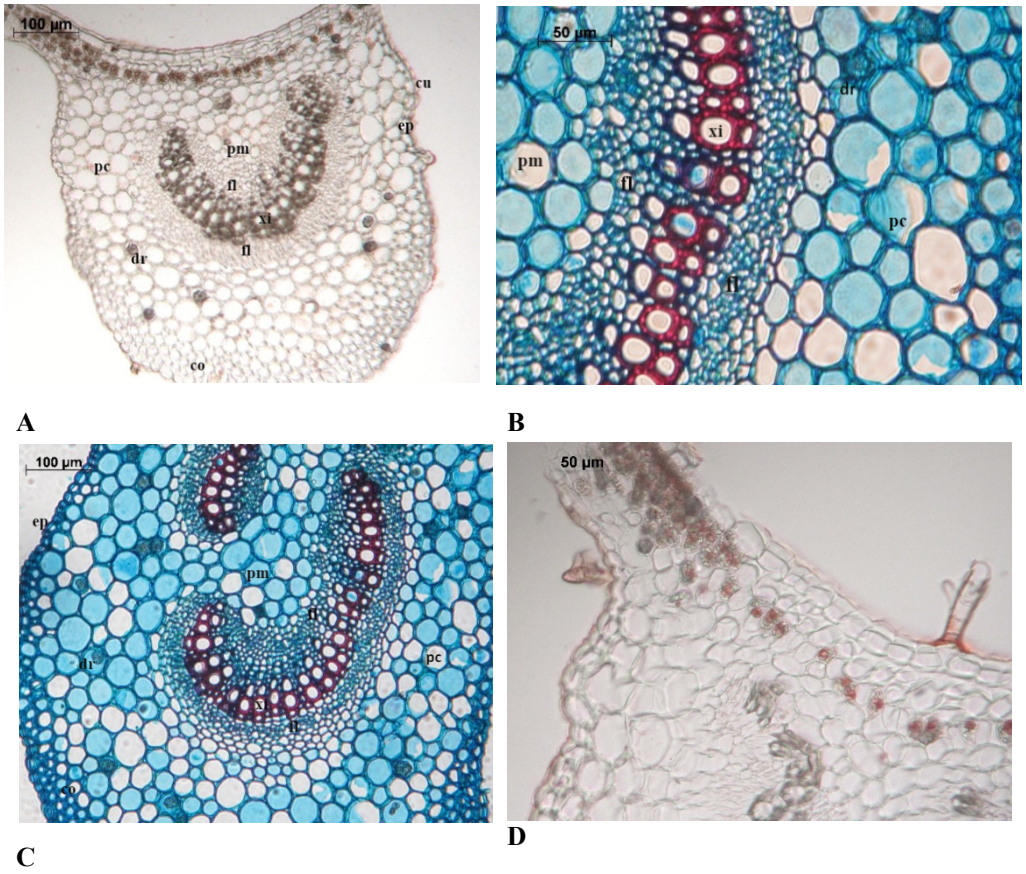


A **B** **C**

Source: Authors.

In cross-section, the main rib has a convex-concave outline (Figure 3A). It has a thin cuticle and consists of a uniseriate epidermis. The collenchyma is angular, with 2-3 layers of cells. The cortical parenchyma consists of 5-6 layers of cells (Figure 3B). Idioblasts with druse occur both in the cortical parenchyma and in the medullary parenchyma (Figure 3B). The vascular bundles are bicollateral, in the shape of an open arch or horseshoe (Figures 3C and 3D). Presence of smaller vascular bundles facing the adaxial face.

Figure 3. Cross-section of the main rib. **A**. General aspect. **B**. Detail of the phloem and xylem cortical parenchyma. **C**. Overview of the vascular system and medullary parenchyma. **D**. Epidermis with thin cuticle. **A**- Steinmetz. **B** and **C**-Alcian Blue / Safranin. **D**- Sudan. **Cu**- cuticle, **co**- Collenchyma, **dr**- Druses, **ep**- Epidermis, **fl**- Phloem, **xi**- Xylem, **pc**- Cortical parenchyma, **pm**- Medullary parenchyma, trichrome.



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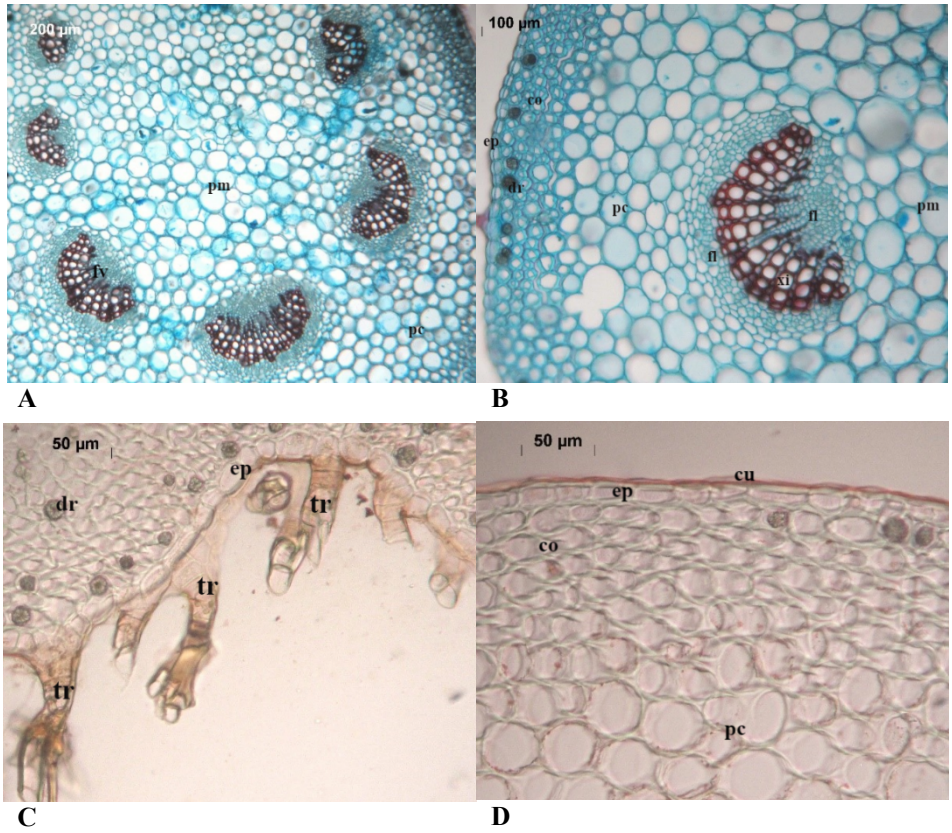
In cross-section, the contour *C. hirta* petiole is concavo-convex. The petiole has a thin cuticle (Figure 4D), consists of the uniseriate epidermis (Figures 4B and 4D) and, under the epidermis, an angular collenchyma formed by 4-5 layers of cells occurs and the cortical parenchyma is made up of 4-6 cell layers (Figure 4B). Both in the collenchyma and in the cortical parenchyma, idioblasts with druses were found (Figures 4B and 4C). The vascular system consists of 7 bicollateral vascular bundles (Figures 4A and 4B) with layers of parenchyma between them. In the central region, medullary parenchyma consisting of cells of varying sizes (Figure 4A). Both faces have simple and branched trichomes (Figure 4C).

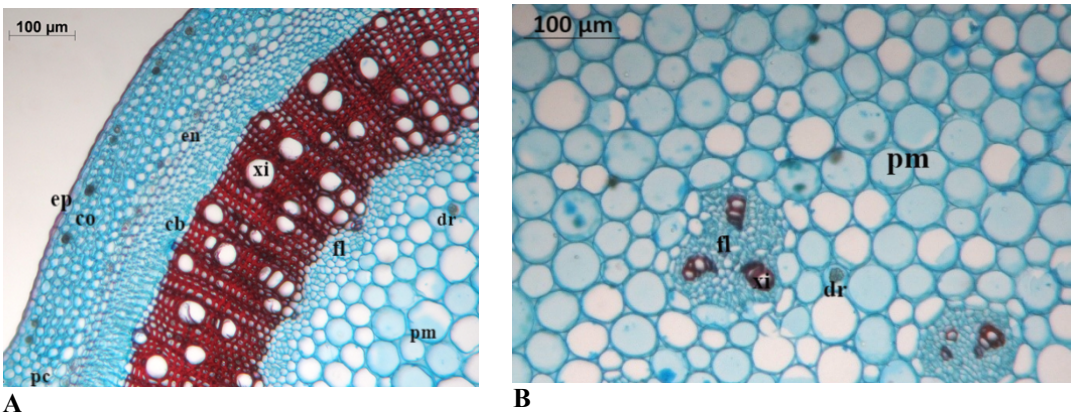
Figure 4. Petiole. **A**. General aspect. **B.** Detail of the epidermis, collenchyma containing prismatic crystals, cortical parenchyma, vascular bundle, and medullary parenchyma. **C.** Detail of the trichomes and druses. **D**. detail of the thin cuticle covering the epidermis. **A** and **B**- Alcian blue / safranin. **C** and **D**- Steinmetz. **Co**- Collenchyma, **dr**- Idioblast containing druses, **fv**- Vascular bundles, **pc**-Cortical parenchyma, **pm**- Medullary parenchyma.



Source: Authors.

The young stem has a single-celled epidermis covered by a continuous, thin cuticle (Figure 5A). The collenchyma has 3-4 layers of cells, with some idioblasts with druse. Presence of endoderm layer. The cortical parenchyma with 6-8 layers of cells (Figure 5A). The vascular bundles are amphiphilic in the medullary region (Figure 5B) with 1-2 xylemic beam rays. The medullary parenchyma consisting of cells of varying sizes (Figure 5B).

Figure 5. The young stem in cross-section. **A**. Detail of the cortical region. **B.** Detail of the medullary region with. Druses and vascular bundle. **Cb**- Cambio, **dr**- Druses, **en-** Endoderm, **ep**- Epidermis, **co**- Collenchyma, **ph**- Phloem, **pc-** Cortical parenchyma, **pm-** Medullary parenchyma.



A **B**

Source: Authors.

3.4 Phytochemical screening

In phytochemical screening, is detected the presence of flavonoids (3.13% content), saponins, and total phenols (6.74% content). The content of volatile compounds was 8.34% ± 0.22. The total ash content was 4.193% ± 0.000173. The mucilage content was 1.71 mL.

4. Discussion

The leaves of Melastomataceae are generally hairy, opposite or opposite-crossed, and petiolate, with whole, lanceolate, ovate or oblong blades and with smooth or serrated margins (Metcalfe & Chalk, 1950). The external morphological characteristics of *C. hirta* have common characteristics in species of Melastomataceae. Macro and microscopic analyzes of the leaves are important for pharmacognostic studies aiming at the correct identification of the material (Caldas, et al., 1986).

According to Cogniaux (1891) and Wurdack, et al. (1993), *Clidemia* is distinguished from the other genera of Melastomataceae by the bacaceous fruits, by the lateral or pseudo-lateral inflorescences, calyx with long and subulate external lacinia and petals with a rounded apex. *C. hirta* presents 2 inflorescences per node. It can be easily recognized by the glossy and patent leaves, by the hirsute trichomes, and urceolate hypanthium.

Regarding the species of *Clidemia* found in the savannah such as *Clidemia octona* (Bonpl.) L.O. Williams, *Clidemia biserrata* DC., *Clidemia capitellata* (Bonpl.) D. Don and *Clidemia urceolata* DC., *C. hirta* has in common the isophilic leaves, petiole present with more than 5 mm, secondary basal vein, inflorescences axillary or pseudo-terminal, and corolla with 5 petals, with the exception of *Clidemia octona* (Bonpl.) L.O. Williams with more than six petals. Unlike *C. biserrata* and *C. urceolata*, which present the adaxial surface of the bulged leaf, *C. hirta* has a flat surface, as do *C. capitellata* and *C. octona*. In addition, the bracts or bracteoles of *C. hirta*, *C. biserrata* and *C. octona* are deciduous, while *C. capitellata* and *C. urceolata* have persistent bracts. *C. biserrata*, *C. capitellata*, *C. octona* and *C urceolata* have one inflorescence per node (Michellangeli & Reginato, 2015). The inflorescences of *C. hirta* are smaller, congested and the flowers are larger than those observed in *C. biserrata* (Araujo & Lima, 2013).

The epidermis of *C. hirta* is uni-stratified, which is consistent with other genera of the Miconieae tribe, which includes the genus *Clidemia*. In Melastomataceae leaves, the coating system is highly specialized, and the presence of trichomes, of varied and complex shapes, constitutes an important aid in the identification of genera and species (Metcalfe & Chalk, 1950). In the studied species, the cuticle is thin, although Cassiano, et al. (2010) state that the presence of leaf epidermis covered by a thick

cuticle in the Melastomataceae family is common. The leaf blade of *C. hirta* presented only simple trichomes, as well as *C. biserrata*. *C. capitelatta* presented glandular trichomes, simple trichomes and stellate trichomes, *C. octona* presents simple and stellate trichomes, and C. *urceolata*, glandular and stellate trichomes. Metcalfe & Chalk (1950) state that in the Melastomataceae family, stomata are, in general, anomocytic or anisocytic. In *C. hirta*, only anomocytic stomata were found. In the mesophyll, epidermal cells on the adaxial face larger than those on the abaxial face predominate, thus corroborating the description made by Reis (2005) for species belonging to the Miconieae tribe.

In the leaves of the Melastomataceae family, the presence of hypodermis, sclereids, calcium oxalate crystals, and phenolic compounds is frequent, as highlighted by Metcalfe & Chalk (1950) and Baumgratz & Ferreira (1980).

In *C. hirta*, no hypodermis and sclereids were found, but crystals of calcium oxalate in the form of druses are present in the leaf blade, petiole, and stem, and are sometimes grouped in the cortical parenchyma of the median rib and petiole. The functions of calcium oxalate crystals, according to Duarte and Lopes (2005), are related to ion balance and osmoregulation, storage of calcium or oxalate, mechanical support, and protection against herbivory. The crystals also allow the best use of the light, which reaches the adaxial face, extending it over the chlorophyll parenchyma, since they occur in shaded places (Edwards & Wratten, 1981).

In the region of the central rib, the contour of the adaxial face may be ridged or flat, while the abaxial face may be convex or rounded. The vascular bundles may appear immersed in the mesophyll or be abruptly differentiated from it, they may be collateral or bicollateral, with the latter occasionally presenting the most developed phloem on the adaxial side (Keating, 1984). *C. hirta*, had a convex shape on the abaxial face of the main vein and bicollateral vascular bundles in the shape of an open arch and a U-shape.

The petiole of *C. hirta* is formed by seven bicollateral vascular bundles which have xylem surrounded by phloem on both sides, characteristic of Melastomataceae (Reis, 2004)

One of the important anatomical features in the stem of Melastomataceae is the intraxylemic phloem, as seen in *C. hirta*. Many species of Melastomataceae have vascular bundles in the medulla (Metcalfe & Chalk, 1950), usually formed by phloem, as in *Clidemia blepharodes* DC. (Reginato, et al., 2009). According to Graciano-Ribeiro, et al. (2009), the internal phloem improves the conduction of organic sap in the plant.

It was found for the leaves of *C. hirta* 8.343% of moisture content, 4.193% of total ash, 3.13% of flavonoids, 6.74% of total phenols and saponins were quantified in 166.7.

The moisture content is an important parameter since raw materials that are very desiccated lead to the loss of their chemical constituents and samples with excess water allow enzymatic deterioration and proliferation of microorganisms (WHO, 1998; Farias, 2004). The determination of total ash allows verifying the presence of non-volatile inorganic impurities such as carbonates, phosphates, silicates, and silica that may be present as contaminants in the vegetable raw material (Farias, 2004). Considering that in the researched literature no values of total ash and volatile content were found for *C. hirta*, these data may provide subsidies for establishing parameters for the quality control of this plant raw material.

The content of flavonoids is of considerable importance since these substances have a series of pharmacological properties that allow them to act in biological systems and thus favor human health (Peterson, et al., 1998). These compounds with beneficial health action, act as antioxidants, cell proliferation inhibitors, antiestrogens, and intracellular mediators, exercising protection mainly against cancer and cardiovascular diseases (Huber, 2008). Regarding phenols, studies have confirmed their benefits, namely their antioxidant, anti-sclerotic, and anti-thrombotic activities, prevention of the effects of hyperglycemia, anticarcinogenic, anti-aging, and antimicrobial action, as well as, cardioprotective effect (Giada & Mancini Filho, 2006). In *C. hirta*, satisfactory values of flavonoids and phenols were found, which demonstrates its potential with herbal medicine.

The foam index was used to quantify the saponins present in the leaves. Saponins are bioactive compounds that are generally produced by plants to neutralize pathogens and herbivores. In addition to their role in plant defense, saponins are of great interest because they are active components of medicines and for their valuable pharmacological properties. Among the biological actions attributed to saponins, there are reports of hypocholesterolemic, antifungal, antiviral, antibacterial, immunostimulating, anticancer, anti-inflammatory, antioxidant and antipyretic properties (Güçlü-üstündağ & Mazza, 2007). The foam index quantified the saponins in the leaves of *C. hirta* at 166.7, which indicates a number of saponins that do not present a danger to human health.

The intumescence index used to assess the presence of mucilage, is a measure of the volume occupied by the swelling of one gram of the plant sample by adding water or another intumescent agent, under defined conditions (Barroso & Oliveira, 2009). Mucilages have emollient, moisturizing, and thickening properties, so they can be used for the production of moisturizing creams, expectorants, among others. After measuring and calculating the volume of the swelling of the drug, the presence of mucilages was found, with which the intumescence index was determined, being 1.71.

5. Conclusions

Information on the genus *Clidemia* is quite scarce in the literature, and further studies on it are necessary for a better characterization of the species of the genus. Therefore, the present study contributes to the correct identification of the species and provides data for the quality control of the vegetable raw material.

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