

Centrolobium tomentosum: macro- and microscopic diagnosis of the leaf and stem

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Abstract: *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae, known as "araribá-rosa", is a large tree that is widespread throughout Brazil. The bark of its stem is used in folk medicine as an astringent and the leaves are employed as a poultice for wounds and bruises. The aim of this study was to contribute to the pharmacognostic quality control of this medicinal species through a macro- and microscopic diagnosis of its leaves and stems. Mature leaves and young stems were collected at Embrapa (Colombo, PR) and analyzed using standard microtechniques. The leaves are opposite or alternate, compound, imparipinnate, oblong-lanceolate and hypostomatic with paracytic stomata. The mesophyll is dorsiventral and the midrib is biconvex with a collateral vascular bundle in a circular arrangement. The petiolute is circular and its vascular system is composed of two major vascular bundles in the middle, with smaller bundles between them. The rachis has a structure similar to the petiole, and these parts have a sclerenchymatic sheath shaped in multiple arcs, which surrounds a collateral vascular bundle that is centrally located. The stem has a uniseriate epidermis with peripheral phellogen and a sclerenchymatic sheath composed of many arcs, which surrounds the phloem. The stems and leaves also have non-glandular and glandular trichomes, phenolic compounds and idioblasts made of calcium oxalate crystals. This manuscript has an important role in the morpho-anatomical diagnosis of the Brazilian flora.

Introduction

The family Fabaceae has three major subfamilies: Mimosoideae, Caesalpinioideae and Faboideae (Judd et al., 2008). In Brazil there are approximately 178 genera and 1550 species (Bertoluzzi et al., 2006) of Fabaceae, and many of these taxa are used in landscaping, foods, folk medicines and by the pharmaceutical industry (Souza & Lorenzi, 2005; Judd et al., 2008).

The genus *Centrolobium* Mart. is exclusive to the Neotropics (Lima, 1983-1985), and mainly occurs in Bolivia and Brazil (Tropicos, 2009). *Centrolobium tomentosum* Guillemain ex Benth., which belongs to subfamily Faboideae, is a tree (Figure 1A) that can reach 35 m tall (Lima, 1983-1985), and is native to South-Central Brazil (Tropicos, 2009). This species is popularly known as "araribá-rosa" or "araruva" and it is employed in afforestation (Lorenzi, 2002), in dyes and in folk medicine. The bark of its stem is used as an astringent and the leaves as a poultice for wounds and bruises (Corrêa, 1984).

Chemical studies of *C. tomentosum* have found tannins in the bark (Bastos, 1952), and Aragão-Craveiro et al. (1970) isolated diaryl-heptanoid compounds from

the stem, such as centrolobine, des-*O*-methyl-centrolobine and centrolobol. These compounds have been shown to be active against the promastigote form of *Leishmania amazonensis*, which is the causative agent of cutaneous leishmaniasis, an endemic disease in some regions of Brazil (Araujo et al., 1999).

Due to the few studies concerning the morphological and anatomical profile of *C. tomentosum*, the goal of this study was to produce macroscopic and microscopic diagnosis of the leaf and stem of this medicinal species, in order to contribute to the pharmacognosy quality control of this species and to what is known about the Brazilian flora.

Material and Methods

Plant material

In September 2008, the plant material *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae, was collected from specimens growing in sunny areas at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), located in Colombo, Paraná, Brazil (25°17'S, 40°13'W, 950 m elevation). The



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voucher specimen was identified and deposited in the Fernando Cardoso Herbarium as HFC 1197. The external morphology of the leaves was based on Hickey (1974) and the leaf dimensions were calculated based on the average measurements of at least twenty leaflets and the anatomical morphology was based on Metcalfe & Chalk (1950).

Assays

Mature leaves and young stem fragments (5-25 cm from the stem apex) were fixed in FAA70 (Johansen, 1940) and stored in 70% (v/v) ethanol (Berlyn & Miksche, 1976). To prepare semi-permanent slides, freehand sections (transverse, longitudinal and paradermal) of the material previously fixed in FAA70 were stained either with astra blue and basic fuchsine (Roeser, 1972) or with toluidine blue (O'Brien et al., 1964). Permanent slides were prepared

from the fixed material, dehydrated in an ethanol series (80 and 95% v/v), embedded in synthetic resin, sectioned with a rotary microtome and stained with toluidine blue (Kraus & Arduin, 1997).

For the microchemical tests, the following reagent solutions were used: Sudan III to verify cutin, oil and fat (Sass, 1951); lugol for starch (Berlyn & Miksche, 1976); ferric chloride for phenolic compounds (Johansen, 1940); phloroglucin for lignin (Foster, 1949); and sulphuric acid for calcium salt crystals (Oliveira et al., 1991).

The ultrastructural analysis of the leaf surface, using scanning electron microscopy, was performed with samples fixed in FAA70. The studied material was dehydrated twice, first in an increasing ethanol series (80, 90 and 100% v/v) and then in a critical point dryer. The samples were then coated with gold, and examined and photographed with a scanning electron microscope (Souza, 2007).

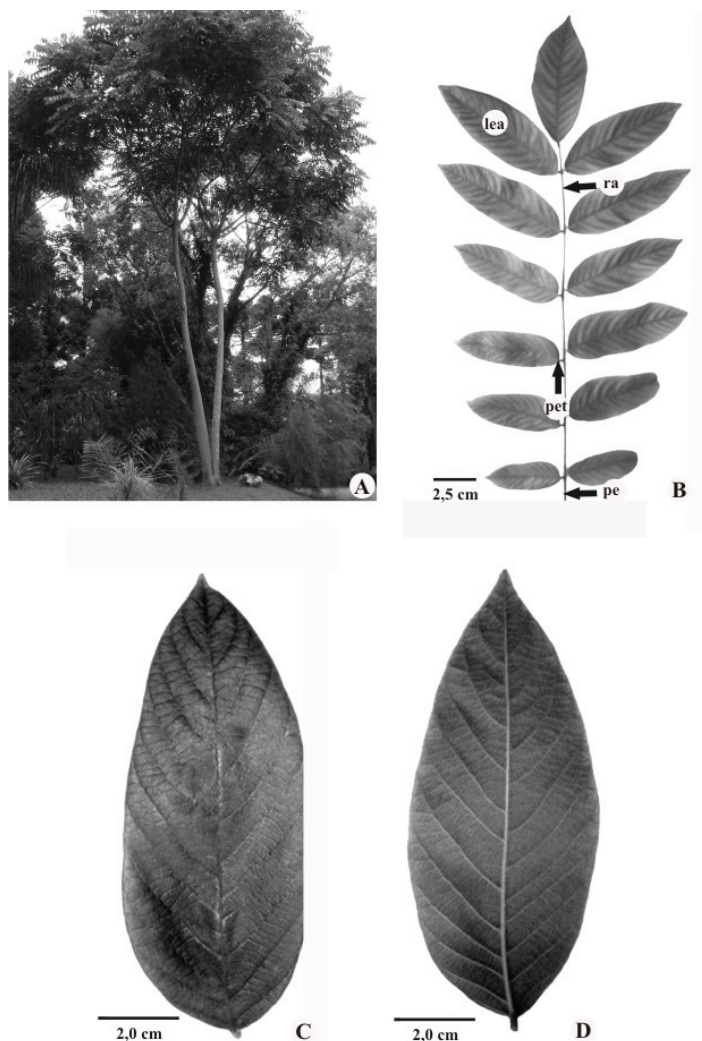


Figure 1. *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae. Leaf: A. In the natural habitat; B. Overall aspect of the composite leaf; C. Adaxial surface of the leaflet; D. Abaxial surface of the leaflet. Abbreviations: lea: leaflet, pe: petiole, pet: petiole, ra: rachis.

Results

Macroscopic diagnosis

The leaves are opposite or alternate, compound, imparipinnate, and have 13-19 leaflets (Figure 1B). The petiole is about 8 cm long. The rachis, which is the main axis of the leaf, is approximately 36 cm and the petiolute has mean length of 0.5 cm (Figure 1B). The leaflets are opposite or alternate, symmetrical to slightly asymmetrical, with a smooth margin (Figures 1B-1D). The average size of the leaflets is 10 cm long x 4 cm wide, which are usually ovate to oblong-lanceolate (Figures 1B-1D). Most leaflets have an acute apex (Figure 1D); however, there are some that have indentations, forming an emarginate apex. The base of the leaflets varies from obtuse to round (Figures 1C, 1D). The surface of the leaflets is tomentose, as well as other parts of the leaf, and the laminar texture is subcoriaceous.

The venation pattern is pinnate (Figure 1D), with secondary veins departing from the midrib, and is subclassified as camptodromous-brochidodromous venation, in which the veins are interconnected in a series

of prominent arcs near the margin.

Microscopic diagnosis

Leaf

In face view of the leaflet blade, the epidermal cells are polygonal to slightly wavy on both surfaces (Figures 2A, 2B). The cuticle, which has lipophilic substances, is smooth for almost the whole length, with slightly striated ornamentation near the base of each trichome (Figure 2C).

Multicellular, long and thin walled, trichomes (Figures 2D, 2E) occur on both epidermal surfaces. The base of these trichomes is composed of relatively small cells, and the apical cells are elongate and acute (Figure 2D). On the abaxial surface there are multicellular, peltate, glandular trichomes. These are inserted in epidermal depressions (Figure 2E).

The stomata are paracytic. They have two subsidiary cells parallel to the guard cells (Figure 2B). These epidermal appendices have external cuticular ledges (Figure 2C) and the stomata are restricted to the abaxial surface, characterizing the leaf as hypostomatic.

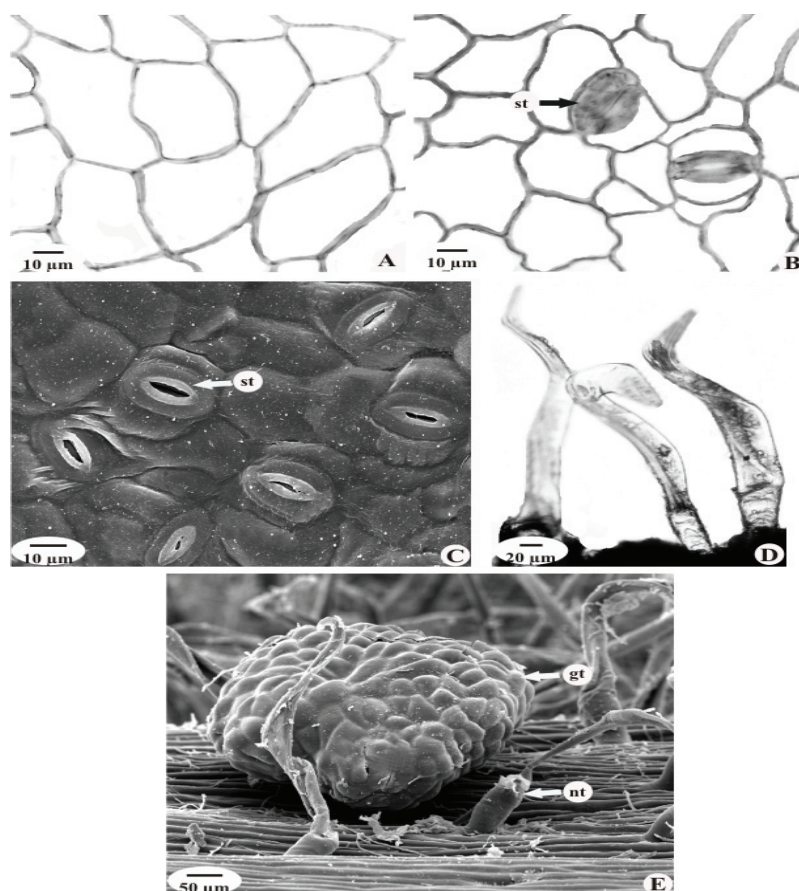


Figure 2. *Centrolobium tomentosum* Guillemín ex Benth., Fabaceae. Leaf, in face view: A. Epidermal cells of the adaxial surface; B. Epidermal cells of the abaxial surface with stomata; C. Stomata of the abaxial surface; D. Non-glandular trichomes; E. Non-glandular and glandular trichomes. Abbreviations: gt: glandular trichome, nt: non-glandular trichome, st: stomatum.

In transverse section, the epidermis is uniseriate, revealing adaxial cells comparatively larger than those of the opposite surface (Figures 3A, 3B), and covered with a thin cuticle. The stomata are found at the same level as the epidermal cells (Figure 3A).

The mesophyll is dorsiventral, consisting of approximately two layers of palisade cells along the adaxial side, representing 60% of the height of the photosynthetic parenchyma, and two to four layers of spongy parenchyma on the opposite side (Figures 3A, 3B). Vascular bundles of small and medium size are distributed throughout the mesophyll (Figure 3B), and surrounded by a sclerenchymatic sheath.

The midrib is biconvex in cross section, with two or three small projections on the abaxial side (Figure 3C). Below the epidermis, there are few layers of collenchyma (Figure 3D). Several vascular bundles are centrally arranged (Figure 3C) and all of the bundles are surrounded by a thick sclerenchymatic sheath consisting of fibers (Figures 3C-3E).

The petiolule (Figures 4A, 4B), in cross section, is circular in shape. The epidermis is uniseriate and covered with a slightly thickened cuticle (Figure 4A). There are multicellular, long and thin walled trichomes and, inserted in epidermal depressions, there are multicellular peltate glandular trichomes (Figure

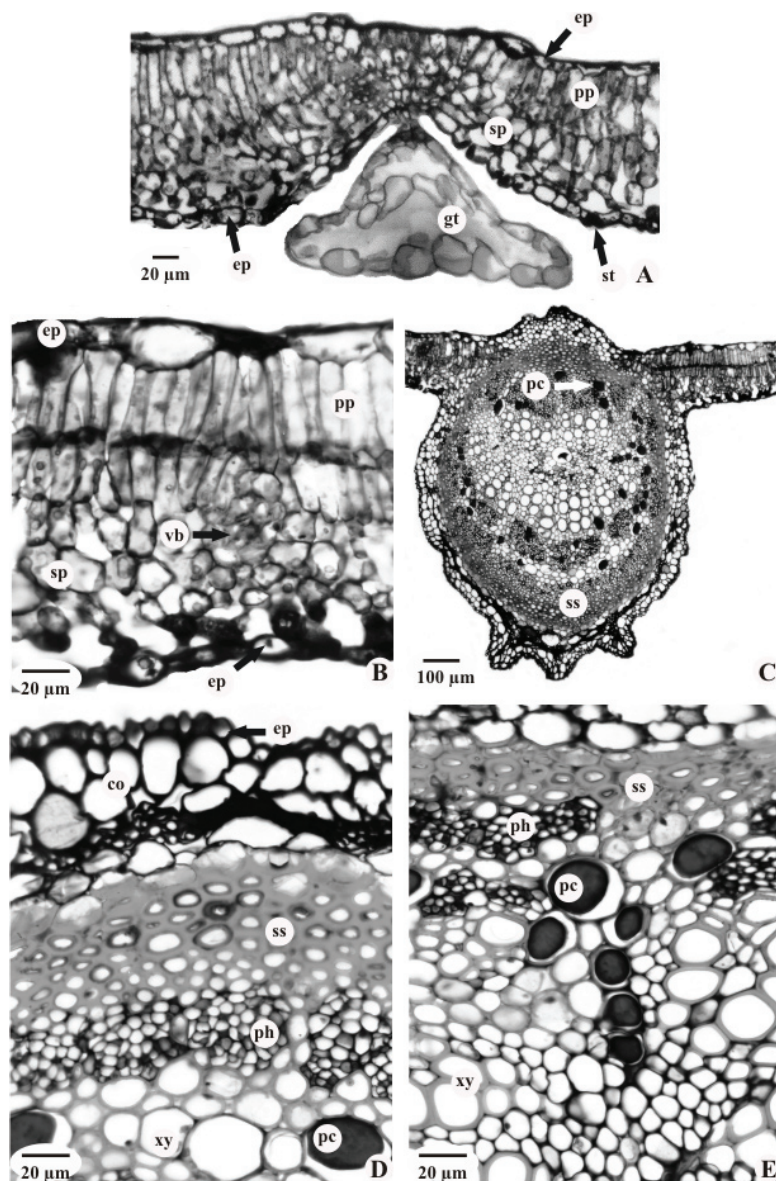


Figure 3. *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae. Leaf, in transverse section. A. Intervain region, showing glandular trichome; B. Detail of dorsiventral mesophyll and a minor vascular bundle; C. Overall appearance of the midrib; D, E. Detail of the sclerenchymatic sheath, collateral vascular bundle and idioblasts with phenolic compounds. Abbreviations: co: collenchyma; ep: epidermis; gt: glandular trichome; pc: phenolic compounds; ph: phloem; pp: palisade parenchyma; sp: spongy parenchyma; ss: sclerenchymatic sheath; vb: vascular bundle; xy: xylem.

4A). There are few layers of annular collenchyma and various strata of ground parenchyma (Figures 4A, 4C). The vascular system of the petiolule has a sclerenchymatic sheath (Figures 4A, 4B), and the system (Figure 4D) is composed of two major vascular bundles in the middle, with smaller bundles between them (Figure 4A).

The rachis has a structure similar to the petiole. The rachis (Figure 5A) and petiole, in cross section, are circular in shape. The epidermis is uniseriate and covered with a thin cuticle. Throughout the rachis and petiole there are multicellular trichomes with acute apices and thin walls. The collenchyma, below the epidermis, has a few cell layers of the annular type (Figure 5B). The vascular

system of the rachis and petiole is entirely surrounded by a lignified sclerenchymatic sheath composed of many arcs (Figure 5A). The vascular system is collateral, centrally arranged and has phloem with sieve elements and parenchyma. The xylem has tracheary elements and lignified ray parenchyma (Figure 5A). Near the periphery of the pith there are some parenchyma cells in early stages of lignification.

Phenolic compounds (Figures 3C-3E, 4A, 5A) are present mainly in the vascular system of all parts of the leaf. Amyloplasts are generally found in the ground parenchyma and phloem. Druses and prisms of calcium oxalate are also observed throughout the leaf.

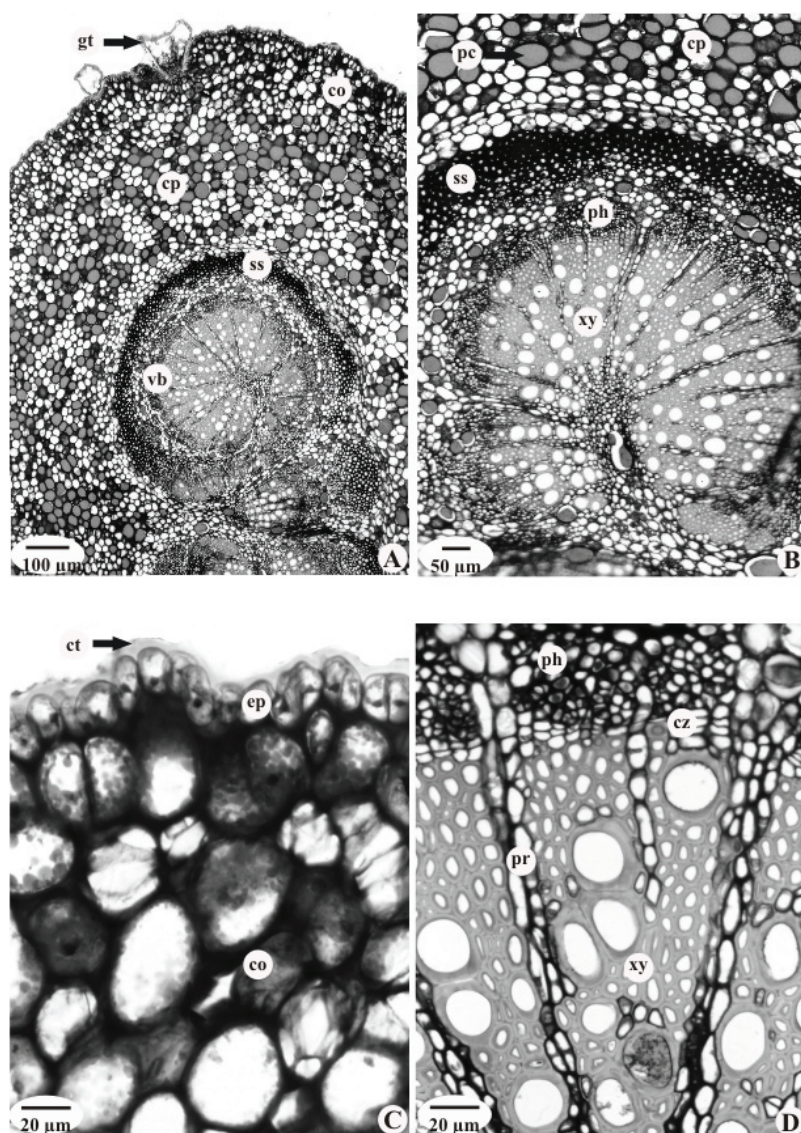


Figure 4. *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae. Petiolule, in transverse section: A. Overall appearance; b. Detail of the petiolule; C. Detail of the cuticle, epidermis and collenchyma; D. Collateral vascular bundle. Abbreviations: co: collenchyma, cp: cortical parenchyma, ct: cuticle, cz: cambial zone, ep: epidermis, gt: glandular trichome, pc: phenolic compounds, ph: phloem, pr: parenchymatic ray, ss: sclerenchymatic sheath, vb: vascular bundle, xy: xylem.

Stem

In cross section, the stem (Figure 5C) is circular in shape. The epidermis is uniseriate and covered with a thin cuticle which has lipophilic substances. Trichomes on the stems are structurally similar to those found on the leaves. Phellogen is also present on the outer portion of the stems.

In the cortex, there are annular collenchyma and a multistratified cortical parenchyma (Figure 5C). The collateral vascular cylinder is surrounded by a sclerenchymatic sheath that is composed of fibers and formed of continuous multiple arcs (Figures 5C, 5D). Narrow parenchymatic rays traverse both the phloem and xylem cylinders (Figures 5C, D).

Idioblasts containing phenolic compounds (Figure 5C) are present in the cortical parenchyma vascular system and pith. Prisms of calcium oxalate are also observed.

Discussion

Considering the external leaf morphology observed in this study, *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae, has features similar to those reported by Von Martius et al. (1840-1906), Corrêa (1984), Lorenzi (2002) and Filardi et al. (2007) for this species.

The species analyzed in this investigation reveals structural aspects consistent with the Faboideae (Fabaceae), presenting glandular and non-glandular trichomes, paracytic stomata, dorsiventral mesophyll and prisms of calcium oxalate in the leaves (Metcalf & Chalk, 1950). Metcalfe & Chalk (1950) mention typical features of *Centrolobium*, such as the occurrence of multicellular uniseriate trichomes, with short basal cells and an elongated apical cell, and peltate glandular trichomes, which is in correspondence with what was observed in the present study. The figure presented by Metcalfe & Chalk (1950) of

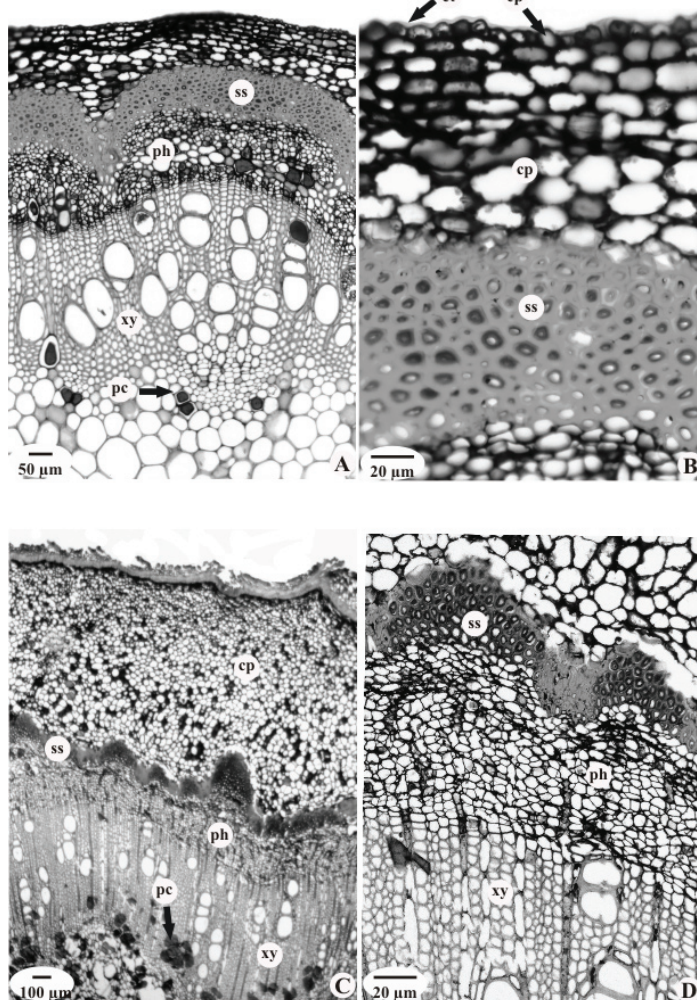


Figure 5. *Centrolobium tomentosum* Guillemain ex Benth., Fabaceae. A. Overall appearance of the petiole, in transverse section; B. Detail of the cuticle, epidermis, collenchyma and sclerenchymatic sheath; C. Overall appearance of the stem; D. Detail of the stem, showing part of the sclerenchymatic sheath and vascular system. Abbreviations: co: collenchyma, cp: cortical parenchyma, ct: cuticle, ep: epidermis, pc: phenolic compounds, ph: phloem, ss: sclerenchymatic sheath, xy: xylem.

peltate glandular trichomes located in a small depression of the epidermis of *C. robustum* Mart. resembles the same structure that was observed in *C. tomentosum*.

When comparing the anatomy of other medicinal taxa of the Faboideae, *Indigofera microcarpa* Desv. "anileira" has leaflets with lignified filiform trichomes, different categories of stomata on both epidermal surfaces, styloid crystals and a vascular bundle in an open arc in the midrib (Lima et al., 2003), which are characteristics that are not found in *C. tomentosum*. Additionally, for *Indigofera suffruticosa* Mill. and *I. truxillensis* Kunth. the presence of forked trichomes and a granular cuticle are also important distinguishing features (Barros & Teixeira, 2008).

Marquiafável et al. (2007) analyzed the content of secretory cells of species of *Indigofera* L. and observed that these cells contain alkaloids and phenolic compounds. The finding of this second group of compounds, comprising mainly tannins and flavonoids, was also found in *C. tomentosum* in the present investigation.

For *Erythrina velutina* Willd. "mulungu", the distinguishing characters are amphistomatic leaves, stellate trichomes and vascular bundles centrally arranged in the midrib (Ventura et al., 2006). Except for the trichomes, these characteristics are also found in *E. cristagalli* L. (Gratieri-Sossella, 2005), which has a distinctly papillose epidermis on the abaxial surface.

In relation to the stem, peripheral phellogen and a continuous sclerenchymatic sheath are mentioned for *Centrolobium* by Metcalfe and Chalk (1950), and the present study confirmed that these characteristics occur in *C. tomentosum*.

This diagnosis of *C. tomentosum* aimed to identify and characterize morpho-anatomical and microchemical characteristics of this species in order to contribute to the quality control of this medicinal plant.

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