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Gochnatia polymorpha: macro- and microscopic identification of leaf and stem for pharmacognostic quality control

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Abstract: *Gochnatia polymorpha* (Less.) Cabrera, Asteraceae, is popularly known as cambará and cambara-de-folha-grande in Brazil. It is used in traditional medicine to treat respiratory and gastrointestinal disorders. Pharmacological studies revealed anti-inflammatory, antispasmodic, antibacterial and antiviral activities. The goal of this paper was to carry out morphological and anatomical studies in order to describe the aerial parts of *G. polymorpha*. The botanical material was collected, fixed, and prepared according to usual light and scanning electron microtechniques. The leaves are simple, oblong-lanceolate to elliptical-lanceolate in form with mucronate acute apex, rounded base, entire or slightly toothed margin, and short petiole. In transection, the epidermis is uniseriate along the leaf blade. A subepidermal layer next to the adaxial side is present. Anomocytic stomata are seen only on the abaxial surface. Capitate glandular trichomes and T-shaped non-glandular trichomes occur on the leaves. The mesophyll is dorsiventral and minor collateral vascular bundles are enclosed by a sheath of thick-walled parenchymatic cells. The midrib is biconvex and the petiole has a circular shape. The epidermis of the stem consists of a single layer of cells with glandular and non-glandular trichomes. The vascular cylinder shows typical structure and perivascular fiber caps are next to the phloem.

Article

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Introduction

Gochnatia Kunth belongs to Asteraceae and shows about seventy species occurring in America from Mexico to Argentina and in Asia. This genus consists of trees or shrubs that particularly grow in Brazil where there are 22 species distributed in Rio Grande do Sul, Paraná, São Paulo, Goiás, Minas Gerais, Bahia, and Ceará (Freire et al., 2002). Chemical studies have reported the presence of sesquiterpenes lactones, diterpenes, triterpenes, flavonoids, coumarins, and essential oil in species of *Gochnatia* (Catalan et al., 1996; Silva et al., 2011).

Gochnatia polymorpha (Less.) Cabrera is a representative of Mutisieae and is popularly known as cambará (Rossato & Kolb, 2010) and cambará-de-folha-grande (Lorenzi, 2002) in Brazil. It is a medium sized tree (Schlemper et al, 2011) with a twisted and branched trunk of sympodial growth. It can reach 10 m in high (Lorenzi, 2002) and is distributed in the regions of neotropical savanna, mainly in Southeastern Brazil (Lorenzi, 2002; Rossato & Kolb, 2010).

It has been used in folk medicine for treating respiratory problems such as colds and coughs and for avoiding gastrointestinal diseases (Alice et al., 1995; Mors et al., 2000; Stefanello et al., 2006b; Schlemper et al., 2011). Pharmacological studies have demonstrated that *G. polymorpha* showed anti-inflammatory (Moreira et al., 2000), antimutagenic (Horn & Vargas, 2008), antispasmodic (Schlemper et al., 2011), and antimicrobial activities (Stefanello et al., 2006b).

Various terpene compounds were isolated from flowers of *G. polymorpha* such as: lupeol, lupeol acetate, lupeol palmitate, taraxasterol, taraxasteryl acetate, pseudotaraxasterol, pseudotaraxasterol acetate, α-amyrin, α-amyril palmitate, β-amyrin, and β-amyril palmitate (Silva et al., 2011). The essential oil from flowers showed (*E*)-nerolidol, eugenol, and phenylacetaldehyde as major constituents while the volatile oil from roots presented a higher content of β-bisabolene and bisabolol (Stefanello et al., 2006a). Two *ent*-kaurenediterpenes were also isolated from the aerial parts of *G. polymorpha* (Sacilotto et al., 1997).

An accurate identification of the investigated

species is remarkable required for pharmacognostic purposes. In that sense, morpho-anatomical studies are essential tools to provide low cost and reliable data. In general, medicinal plants are mainly sold as fragments or powders and morpho-anatomical descriptions can be used as the first parameters for their quality control.

Considering the lack of available data on the morphology and anatomy of *G. polymorpha*, the goal of the present work was to carry out the macro- and microscopic identification of its aerial parts as a contribution to the pharmacognostic studies involving *Gochnatia*.

Materials and Methods

The aerial parts of *Gochnatia polymorpha* (Less.) Cabrera, Asteraceae, were collected in São Maximiano Farm located in Guaíba, Rio Grande do Sul (30° 10' S and 51° 20' W, 27 m high), in December 2010. The species was identified by the voucher ICN 996231 lodged at the herbarium from the Instituto de Ciências Naturais at the Universidade Federal do Rio Grande do Sul.

The plant material was fixed in FAA 70 (Johansen, 1940) and kept in 70% ethanol solution (v/v) (Berlyn & Miksch, 1976). This material was sectioned by hand or using rotary microtome to obtain semipermanent and permanent slides for microscopic studies, respectively. Transverse and longitudinal sections were stained either with toluidine blue (O'Brien et al., 1964) or astra blue and basic fuchsine (Roeser, 1972).

For microchemical tests, transections of the previously fixed material were prepared by freehand. The following standard solutions were used for microchemical

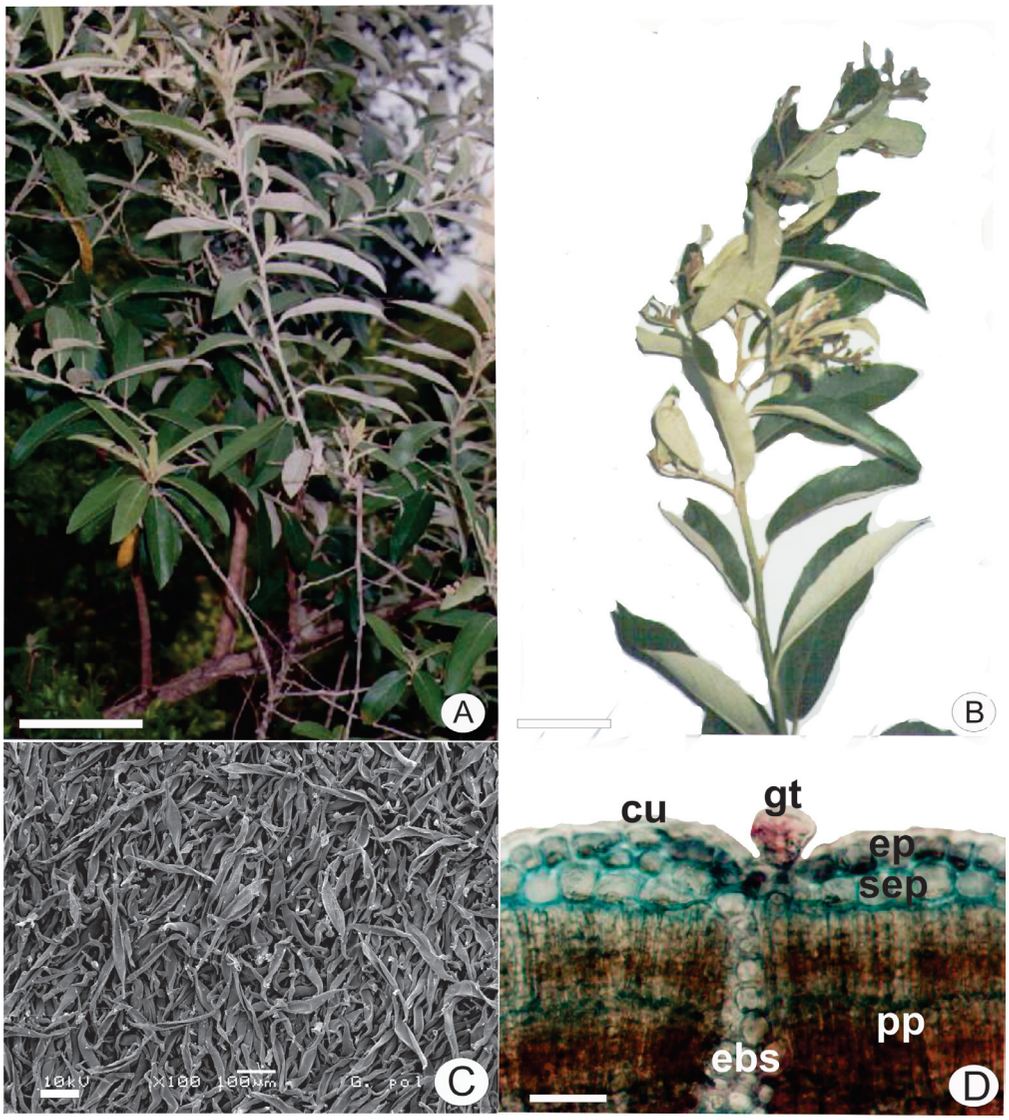

Figure 1. *Gochnatia polymorpha* (Less.) Cabrera, Asteraceae. A. General aspect; B. Aspect of aerial parts; C. Numerous trichomes on the abaxial side; D. Transection of the leaf blade showing capitate glandular trichome (gt), epidermis (ep), cuticle (cu), subepidermal layer (sep), palisade parenchyma (pp), bundle sheath extension (ebs). Bars = 15 cm (A), 5 cm (B), 20 µm (D).

tests: hydrochloric phloroglucin for lignified elements (Foster, 1949), Sudan III for lipophilic compounds (Sass, 1951), ferric chloride for phenolic substances (Johansen, 1940), and lugol to detect starch (Berlyn & Miksch, 1976). Photos were taken using light microscope with different magnifications.

For the analysis of scanning electron microscopy (SEM) (Souza, 1998), the samples were fixed in FAA 70, dehydrated in a graded ethanol series and CO_2 critical point drying apparatus (Bal-Tec CPD-030), coated with gold (Balzers SCD-030) and examined using the Jeol JSM- 6360LV microscope.

Results

Morphological analysis of *Gochnatia polymorpha* (Figure 1A) reveals simple leaves varying from oblong-lanceolate to elliptical-lanceolate in form and measuring 8-12 cm in length and 3-5 cm in width, with alternate phyllotaxy, mucronate acute apex, rounded base, entire or slightly toothed margin, pinnate venation and short petiole. The adaxial surface is bright green as compared to the gray abaxial side (Figure 1B). In addition, the leaves show coriaceous consistency.

In surface view, the leaf blade shows that the

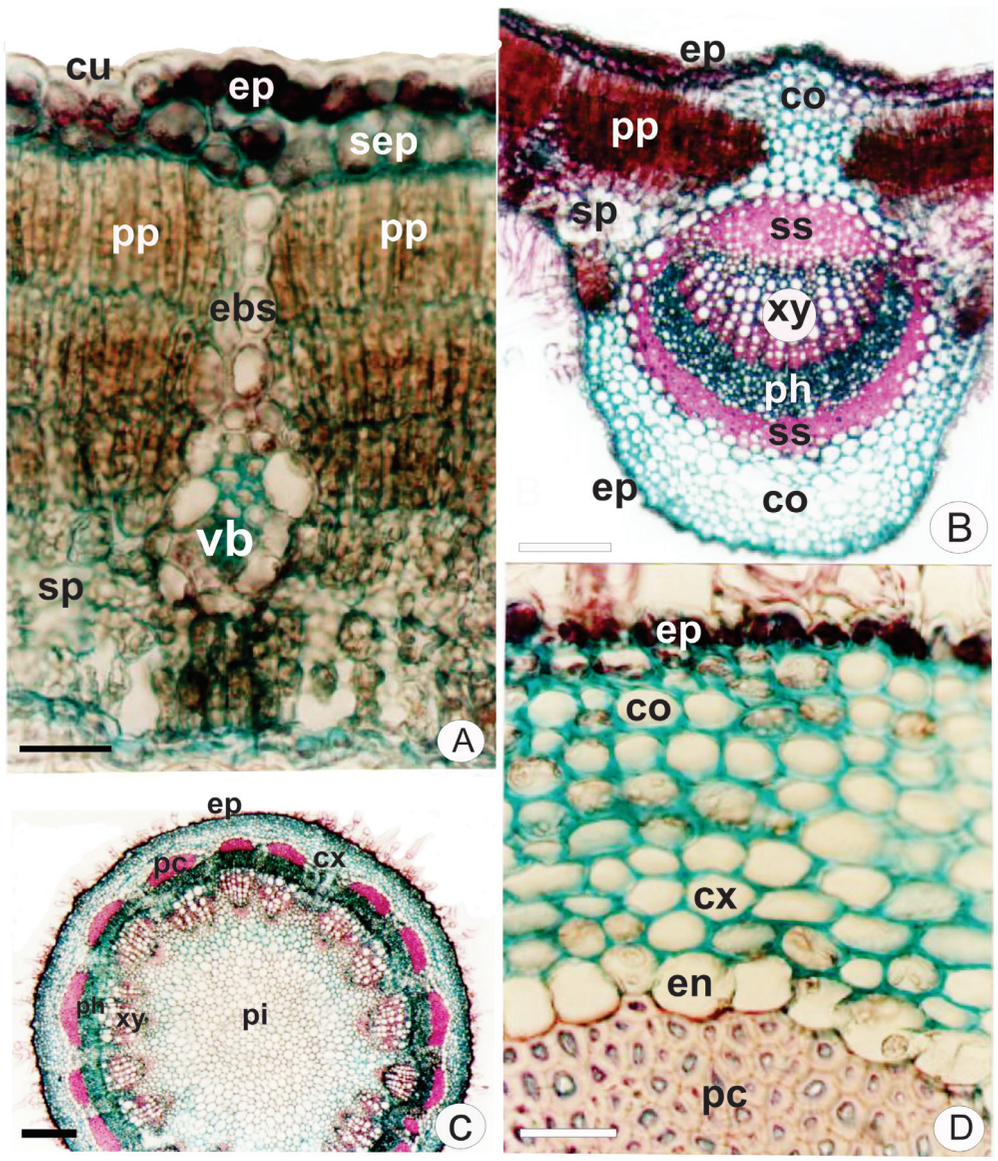

Figure 2. *Gochnatia polymorpha* (Less.) Cabrera, Asteraceae. Transection. A. Leaf blade with cuticule (cu), epidermis (ep), subepidermal layer (sep), bundle sheath extension (ebs), vascular bundle (vb), palisade parenchyma (pp) and spongy parenchyma (sp); B. Midrib showing epidermis (ep), collenchyma (co), sclerenchymatic sheath (ss), xylem (xy), phloem (ph), palisade parenchyma (pp), spongy parenchyma (sp); C. Stem showing epidermis (ep), cortex (cx), perivascular fiber cap (pc), phoem (ph), xylem (xy) and pith (pi); D. Detail of the stem indicating epidermis (ep), collenchyma (co), cortex (cx), endodermis (en), perivascular fiber cap (pc) and phoem (ph). Bars = 20 µm (A, D), 100 µm (B), 200 µm (C).

anticlinal walls of epidermal cells are straight and relatively thin on both sides. Anomocytic stomata are only observed on the abaxial surface. The stomata are at the same level of other cells of epidermis or slightly raised above the surface.

In transection, the epidermis is uniseriate along the leaf blade. An adaxial subepidermal cell layer is observed (Figure 1D) which can represent a hypodermis. However no ontogenetic study was performed to confirm this hypotesis. The epidermis is coated with a thick and smooth cuticle (Figures 1D, 2A) on both sides. The cuticle is slightly thicker on the adaxial surface.

Several non-glandular trichomes occur only on the abaxial surface (Figure 1C) and some glandular trichomes inserted in small depressions are found on both sides (Figures 1D, 2A). The non-glandular trichomes are branched with T-shaped apical cell (Figures 1C, 3B). The capitate glandular trichomes are stalked or sessile and the head consists of one or two cells (Figures 1D, 2A, 3C, 3D).

The mesophyll reveals dorsiventral organization comprising 2-3 layers of palisade parenchyma and 5-6 layers of spongy parenchyma (Figures 2A, B). Minor collateral vascular bundles are embedded in the mesophyll and they are enclosed by a sheath of thick-walled parenchymatic cells and fibers. This sheath also presents extensions towards the epidermis (Figures 1D, 2A).

The midrib shows a biconvex shape in transection, being slight convex on the adaxial surface. The uniseriate epidermis is covered by a thick and smooth cuticle. There are 8-10 layers of angular collenchyma on the adaxial side and 5-6 layers on abaxial side. In the ground parenchyma, *G. polymorpha* exhibits a single collateral vascular bundle which is surrounded by sclerified sheath (Figure 2B).

The petiole is circular in transection and its epidermis has the same characteristics previously described for the leaf blade. The vascular system shows an interrupted arch shape with two collateral bundles in an adaxial position. The vascular system is surrounded by sclerified cells. Sclerified cells are common in the petiole cortex.

The stem presents an incipient secondary growth and has a circular shape in transection (Figure 2C). The epidermis consists of a single layer of cells (Figure 2D) with glandular and non-glandular trichomes (Figures 3B, C, D) similar to those found in the leaf. The collenchyma

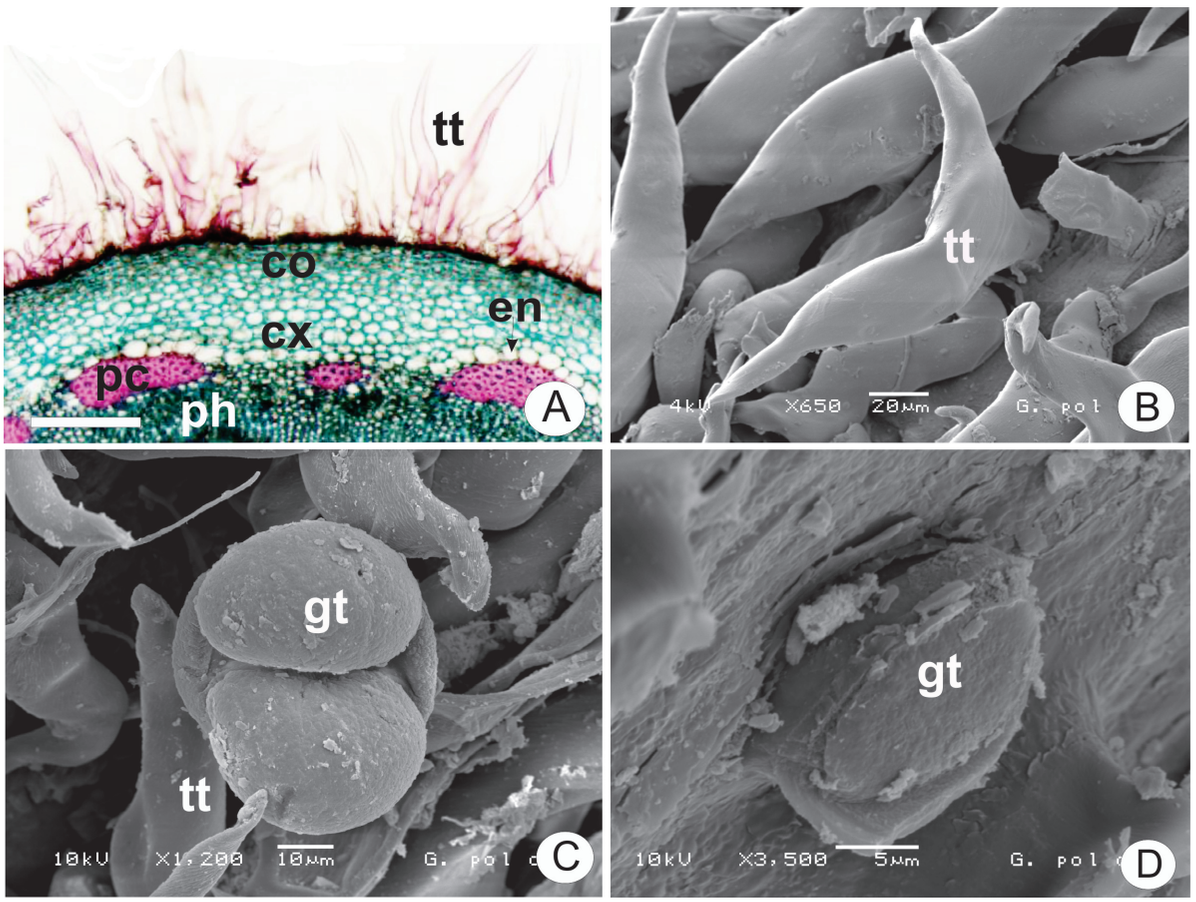

Figure 3. *Gochnatia polymorpha* (Less.) Cabrera, Asteraceae. A. Stem detail with non-glandular trichome (tt), collenchyma (co), cortex (cx), endoderm (en), perivascular fiber caps (pc) and phoem (ph); B. Surface view of stem: non-glandular trichome (tt) with T-shaped apical cell; C e D. Surface view of stem: capitate glandular trichomes (gt). Bar = 100 µm (A).

forms a continuous ring and contains chloroplasts (Figure 2D). An endodermis containing amyloplasts bounds the cortex internally (Figures 2D). The vascular cylinder shows a typical structure (Figure 3A) and a vascular cambium is evident. Perivascular fiber caps are next to the phloem (Figures 2C, 3A). The pith cells show pitting and are slightly lignified. In transection, elongated pith cells are verified close to the perimedular zone while they are circular in the central zone (Fig. 2C).

Discussion

Gochnatia spp. have simple leaf morphology but its leaves demonstrate great variation in shape from linear to suborbicular. The venation is predominantly pinnate, although in taxa such as *Gochnatia arequipensis* Sandwith, *Gochnatia glutinosa* Klatt, *Gochnatia rotundifolia* Less. the leaves are three-veined (Freire et al., 2002). In this study, the leaf morphology of *Gochnatia polymorpha* (Less.) Cabrera is in accordance with the previous reports of Alice et al. (1995) and Freire et al. (2002) for this species.

An adaxial hypodermis is observed in a few number of *Gochnatia* species whereas it is absent in *G. amplexifolia* (Gardner) Cabrera, *G. cardenasii* S.F. Blake, *G. discoidea* (Less.) Cabrera, *G. foliolosa* (D. Don) D. Don & Arn. ex Hook., *G. glutinosa* and *G. vernonioides* Kunth. Occasionally, a descontinuous hypodermis is reported, *e.g. G. argentina* Cabrera, *G. discolor* Baker and *G. orbiculata* (Malme) Cabrera (Freire et al., 2002). In this investigation, the term subepidermal layer is used since no ontogenetic approach was performed for elucidating whether this layer has its origin from epidermis or mesophyll.

The anatomical characters observed in the leaf blade, mainly the presence of a subepidermal layer, correspond to that described by Rossato & Kolb (2010; 2012) for *G. polymorpha*. However, these authors demonstrate that this species can show up to three layers of adaxial hypodermis in higher luminosity environments.

According to Metcalfe & Chalk (1950), the presence of stomata in surface view has diagnostic value for Asteraceae. In addition, several genera of Asteraceae can present anomocytic (predominantly) and anisocytic stomata as observed in *Calea* (Farago et al., 2006; Budel et al., 2006), *Baccharis* (Oliveira et al., 2011; Souza et al., 2011), *Lucilia* (Duarte et al., 2011), *Mikania* (Budel et al., 2009; Gasparetto et al., 2010). In this work, anomocytic stomata are verified in *G. polymorpha* on the abaxial surface. However, *G. barrosii* Cabrera shows anomocytic stomata on both sides (Rossato & Kolb, 2012).

Various secretory structures such as ducts, cavities and glandular trichomes can occur on leaves of Asteraceae (Castro et al., 1997; Budel et al., 2013). However, ducts and cavities are not present in *G. polymorpha*. The glandular trichomes of many Asteraceae species are multicellular, biseriate, stalked or sessile (Fahn, 1988). The glandular trichomes of *G. polymorpha* present all these usual characteristcs of Asteraceae. In particular, the glandular trichomes of *G. polymorpha* demonstrate a head consisting of one or two cells as reported by Bieras & Sajo (2009).

The non-glandular trichomes are diverse in anatomy, morphology, and microstructure, but they are mainly classified by the morphology, resulting in descriptive terms such as stellate, T-shaped, dendritic, and spiral (Werker, 2000). In this study, *G. polymorpha* presents T-shaped non-glandular trichomes. Simple and T-shaped non-glandular trichomes were previously described for *G. polymorpha* by Alice et al. (1995) and Bieras & Sajo (2009). Stellate non-glandular trichomes are found in *G. barrosii* (Bieras & Sajo, 2009).

In particular, the two apical cells of the non-glandular trichomes of *G. polymorpha* are sharp-pointed. This characteristic is widely reported in several genera of Asteraceae as *Acanthospermum* (Martins et al., 2006), *Ageratum* (Oliveira et al, 1993, Tavares et al, 2000; Procopio et al., 2003), *Baccharis* (Budel & Duarte, 2007; Budel & Duarte, 2008a; Budel & Duarte, 2008b; Budel et al., 2012), *Bidens* (Procopio et al., 2003), *Calea* (Budel et al., 2006), *Conyza, Galinsoga* (Procopio et al., 2003) and *Gochnatia* (Bieras & Sajo, 2009).

Several species of Asteraceae show mesophyll distinguished in palisade and spongy parenchyma (Budel et al., 2005; Budel & Duarte, 2010; Oliveira et al., 2011; Souza et al., 2011). In this sense, *G. polymorpha* exhibits a dorsiventral mesophyll as expected. Similar structural arrangement was observed for this species in the paper of Rossato & Kolb (2010; 2012). Bieras & Sajo (2009) also verified a dorsiventral mesophyll for *G. barrosii.*

The minor collateral vascular bundles of leaves of *G. polymorpha* are enclosed by a sheath of thick-walled parenchymatic cells and fibers. This sheath also presents extensions towards the epidermis as indicated for this species by Rossato & Kolb (2012). According to Oliveira et al. (1993), the presence of a fiber cap in the vascular system is a remarkable attribute concerning the diagnosis of herbal drugs. In this investigation, the stem of *G. polymorpha* demonstrates perivascular fiber caps next to the phloem. In addition, fibers around the vascular bundles are a characteristic of Mutisieae which suggests a phylogenetic relationship (Rossato & Kolb, 2010).

The endodermis is formed by a layer of cells that bounds internally the cortical region. In Asteraceae, the endodermis can exhibit Casparian strips which is characterized as a typical endodermis, or else can contain starch which is reported as a starch sheath (Metcalfe & Chalk, 1950; Esau, 1976). In *G. polymorpha*, a starch sheath is observed.

Conclusion

Considering the previously investigated macro- and microscopic aspects of the aerial vegetative organs of *Gochnatia polymorpha* (Less.) Cabrera, the morpho-anatomical characters of uniseriate epidermis, adaxial subepidermal cell layer, anomocytic stomata on the abaxial surface, capitate glandular trichomes, T-shaped non-glandular trichomes, dorsiventral mesophyll, and perivascular fiber caps next to the phloem in the stem should be taken into account as quality control parameters for its pharmacognostic study.

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Authors' contributions

JY contributed in collecting the plant sample and its identification, running the laboratory work, analyzing of data and drafting the paper. PMDB and PVF contributed in performing the scanning electron microscopy (SEM) analysis and critical reading of the manuscript. MRD contributed to the critical reading of the manuscript. JMB supervised the laboratory work and contributed in plant identification and herborizing procedure. All the authors have read the final manuscript and approved the submission.

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