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Original Article

Pharmacobotanical study of *Baccharis pentaptera*

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ABSTRACT

Baccharis L. sect. *Caulopterae*, Asteraceae, comprises thirty species in Brazil that show stems represented by cladodes, which are very similar in morphology. These species are popularly known as "carqueja" in Brazil and Argentina and are used in popular medicine as diuretic and stomachic. The aim of this work was to examine the morpho-anatomical characters of cladodes of *Baccharis pentaptera* (Less.) DC. for diagnosis purposes. The plant material was prepared by light and scanning electron microscopy. *B. pentaptera* shows opposite and spread wings in the two-winged cladode axis and irregular arrangement in the three-winged cladode. The wings have a uniseriate epidermis with palisade parenchyma next to both sides of epidermis. The spongy parenchyma crossed by minor collateral vascular bundles is observed in the central region of wings. The glandular trichomes are capitate and biseriate and the non-glandular trichomes are uniseriate and flagelliform with 2–3 cells that extend from the base. In caulinar axis, there are uniseriate epidermis, chlorenchyma alternating with angular collenchyma and perivascular fiber caps adjoining the phloem which is outside the xylem. Prismatic and styloid crystals are verified in the perimedullary zone. These combined characters can assist the diagnosis of *Baccharis* species sect. *Caulopterae*.

Introduction

Baccharis L. sect. *Caulopterae* includes *Organensis*, *Trimera* and *Genistelloides* groups (Heiden et al., 2009). This section occurs in Southern America and comprises thirty species in Brazil that show stems represented by cladodes (Heiden et al., 2009) which are similar in morphology (Budel et al., 2008). In that sense, a great effort has been devoted to examine the anatomical characters of aerial vegetative organs from *Baccharis* for diagnosis purposes. In addition, these species are used in popular medicine as diuretic and stomachic and are popularly known as "carqueja" in Brazil and Argentina (Budel et al., 2008).

Baccharis pentaptera (Less.) DC., Asteraceae, is a shrub that inhabits exclusively Southern America. The scientific names *Baccharis stenocephala* Baker and *Baccharis fastigiata* Baker are considered synonyms of *B. pentaptera* due to several updates provided by taxonomists (Schneider et al., 2009). In spite of *B. pentaptera* is not officially accepted in national pharmacopoeias, *Baccharis trimera* (Less.) DC., a quite similar taxon that is widely sold in pharmacies, herbal and street shops, is claimed to be the most popular and investigated species of "carquejas" and is included in Farmacopeia Brasileira V (2010).

B. pentaptera is used in folk medicine similarly to other "carquejas" (Barroso and Bueno, 2002). However, it has been usually mistaken for *B. crispa* Spreng., *B. microcephala* (Less.) DC., *B. opuntioides* Mart. ex Baker, *B. trimera* (Less.) DC., and *B. usterii* Heering (Barroso and Bueno, 2002; Alonso and Desmarchelier, 2006; Rodrigues et al., 2008). Moreover, few studies were performed in order to investigate chemical differences among species from *Caulopterae* section. Regarding *B. pentaptera*, Bona et al. (2002) showed that its essential oil yielded 1.8% and Simões-Pires et al. (2005) identified β-pinene (41.3%) as its main volatile compound.

Considering that medicinal plants are widely sold as fragments or powders, morpho-anatomical description is the first parameter that can be used for diagnosis. In this context, morphological and anatomical characterization can be a simple and fast tool for identification of herbal drugs. Thus, the goal of this paper was to discuss these features for cladodes of *B. pentaptera* in order to provide further data about *Caulopterae* section.

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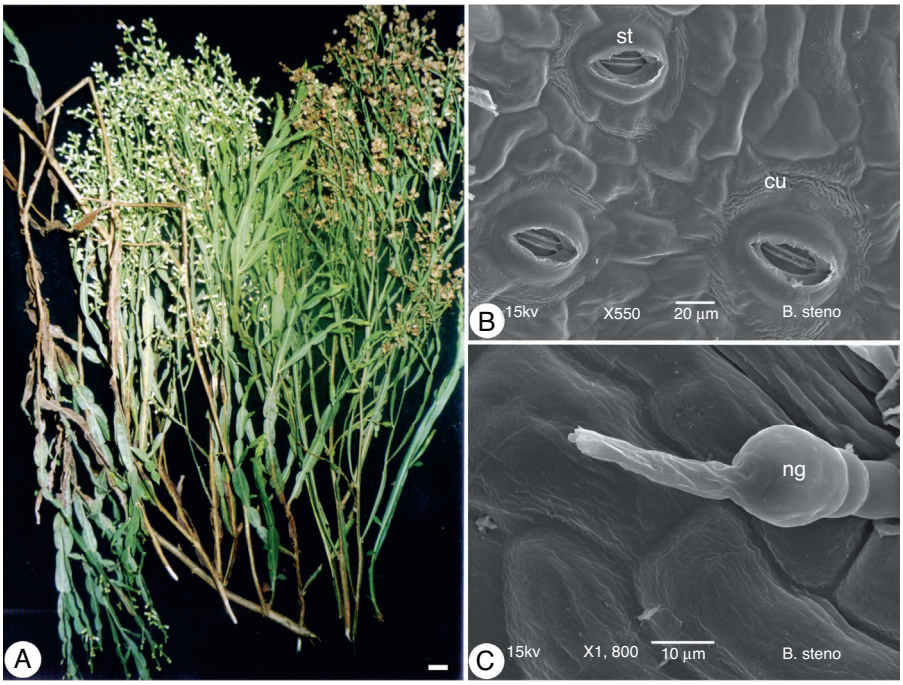

Fig. 1. *Baccharis pentaptera* (Less.) DC., Asteraceae. (A) General aspect; (B) face view of epidermal cells, stoma (st) and striated cuticle (cu) by SEM; (C) surface view of epidermis showing flagelliform non-glandular trichome (ng) (SEM). Bar = 0.5 cm (A).

Materials and methods

Plant material

Baccharis pentaptera (Less.) DC., Asteraceae, was collected from specimens of sunny areas at Fazenda São Maximiano in Guaíba (coordinates 30°10′ S and 51°20′ W, and 27 m altitude), Serra do Sudoeste, Rio Grande do Sul, Brazil during December 2003. A voucher was identified by taxonomists and registered under the number ICN 128450 in the herbarium at the Institute of Biosciences, Federal University of Rio Grande do Sul.

Morpho-anatomical assays

Cladode fragments were collected 5–15 cm from apex. At least five specimens were fixed in FAA 70 (Johansen, 1940), which was replaced by 70% ethanol (v/v) (Berlyn and Miksche, 1976). The plant material was sectioned by hand or dehydrated, embedded in glycolmethacrylate (Leica historesin) and sectioned using the Leica RM-2145 microtome. Cross-sections and longitudinal sections were stained with toluidine blue (O'Brien et al., 1964) or astra blue and basic fuchsine combination (Roeser, 1972).

The following standard solutions were used for microchemical tests: hydrochloric phloroglucin to reveal lignin (Sass, 1951), Sudan III for lipophilic compounds (Foster, 1949), ferric chloride for phenolic substances (Johansen, 1940), sulphuric acid for calcium oxalate crystals (Oliveira et al., 2005) and iodine-iodide for starch (Berlyn and Miksche, 1976). Photomicrographs were taken with the Olympus BX-40 light microscope equipped with a digital camera.

For the analysis by scanning electron microscopy (SEM) (Souza, 1998), the fixed material was dehydrated using increasing amounts of ethanol and the CO_2 critical point apparatus (Balzers CPD-030) and coated with gold (Balzers Sputtering SCD-030). Electron micrographs were taken with the Jeol JSM-6360LV scanning microscope.

Results

The stem of *B. pentaptera* (Less.) DC. (Fig. 1A) usually grows to a height of 0.5–1 m and shows three wings (Fig. 2C) in the vegetative axis and two wings in the reproductive branches (Fig. 2B). The axis is rigid and the wings are discontinuous. The wings are opposite and spread in the axis of the two-winged cladode (Fig. 2B); they are irregularly arranged in the cladode with three wings (Fig. 2C).

In the wings, the epidermal cells are polygonal (Fig. 1B and C) and have thick anticlinal walls with evident primary pits in frontal view. Anomocytic stomata (Fig. 1B) are located at the same level or slightly above the surrounding cells. In cross-section, the epidermis is uniseriate (Fig. 2D–F) and coated with a thin and slightly striated cuticle (Figs. 1B, C and 2E). In particular, cuticle is striated around stomata (Fig. 1B).

Glandular (Fig. 2A) and non-glandular trichomes (Figs. 1C and 2A) are inserted in the same small epidermal depressions and are either isolated or in clusters (Fig. 2A and D). The glandular trichomes (Fig. 2A) are capitate and biseriate and have 8 cells at the base. The non-glandular trichomes are uniseriate and flagelliform with 2–3 cells that extend from the base (Fig. 1C). Both glandular and non-glandular trichomes show cells at the base with dense cytoplasm and an evident nucleus that were stained positively for lipophilic substances.

The chlorenchyma consists of a palisade parenchyma, comprising approximately three strata of short cells below both sides of the epidermis, and a spongy parenchyma in the central region (Fig. 2C, D and F). Minor collateral vascular bundles surrounded by an endodermis cross the spongy parenchyma (Fig. 3A). Some minor vascular bundles have xylem to the upper side and others show xylem to the lower side. One or more secretory ducts (Fig. 2D and F) composed by a uniseriate epithelium, dense cytoplasm, evident nucleus and lipophilic content can be found next to endodermis, in direction to phloem. At the wing edges, there are 2–3 layers of angular collenchyma below the epidermis, a collateral vascular bundle with a perivascular fiber cap adjoining the phloem and secretory ducts (Fig. 2D).

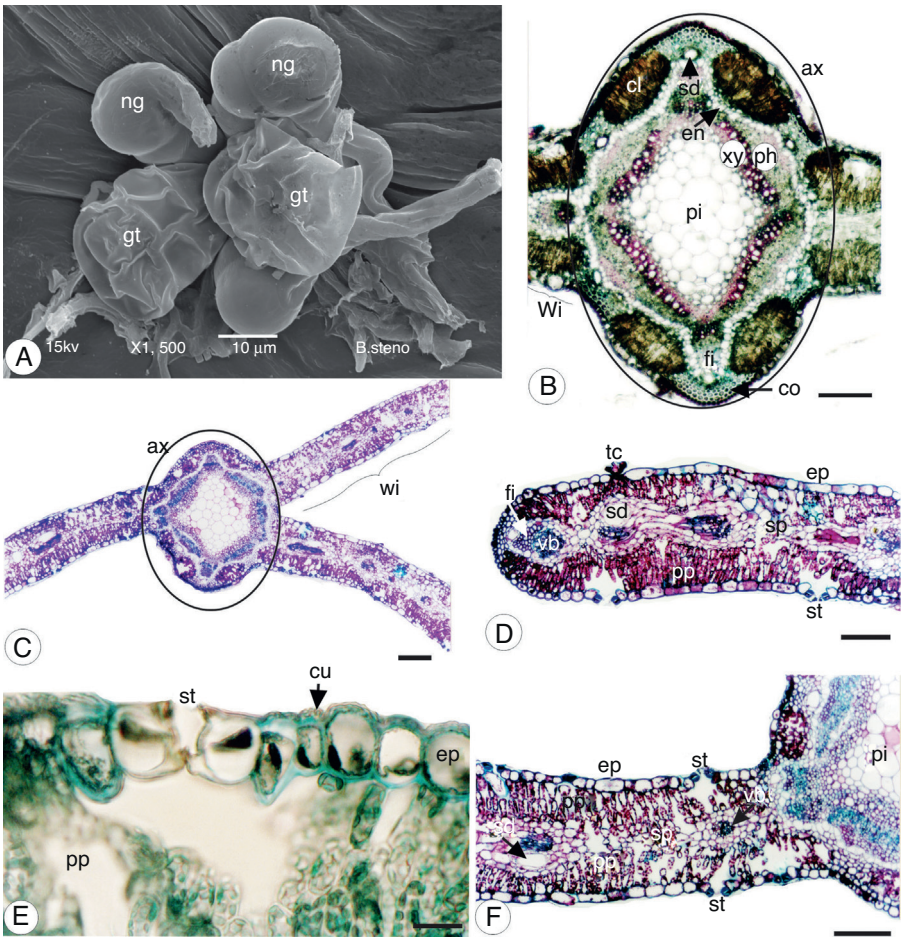

Fig. 2. *Baccharis pentaptera* (Less.) DC., Asteraceae. (A) Surface view of epidermal cells exhibiting biseriate glandular trichomes (gt) and non-glandular trichomes (ng) in cluster by SEM; (B) cladode in cross-section showing axis (ax), chlorenchyma (cl), collenchyma (co), endodermis (en), fibers (fi), pith (pi), phloem (ph), secretory ducts (sd), xylem (xy) and two wings (wi); (C) cladode in cross-section indicating axis (ax) and three wings (wi); (D) wing in cross-section near to the border showing epidermis (ep), fibers (fi), palisade parenchyma (pp), spongy parenchyma (sp), stoma (st), trichomes in clusters (tc) secretory duct (sd) and vascular bundle (vb); (E) wing in cross-section showing cuticle (cu), epidermis (ep), stoma (st) and palisade parenchyma (pp); (F) wing and axis indicating epidermis (ep), palisade parenchyma (pp), spongy parenchyma (sp), stoma (st), secretory duct (sd), vascular bundle (vb) and pith (pi). Bar = 20 µm (E), 100 µm (B, D, F), 200 µm (C).

In stem axis, the epidermis exhibits similar features to the wings and, below it; the chlorenchyma alternates with angular collenchyma, which has 2–3 rows in the ribs (Figs. 2B and 3B, F). Secretory ducts, similar to those viewed in the wings, can be found next to endoderm that bounds the internal part of the cortex (Figs. 2B and 3B, C, F) and its walls are impregnated with lipophilic compounds.

The vascular cylinder shows cambium that produces inward xylem and outward phloem (Fig. 3B and C). Perivascular fiber caps are adjoining to phloem and some fibers can also appear in phloem (Fig. 3B, C and F). The pith is composed by relatively large parenchymatous cells with thin walls (Fig. 3B–D). Prismatic and styloid crystals (Fig. 3D and E) of calcium oxalate occur in the perimedullary region.

Discussion

Baccharis L. sect. *Caulopterae* comprises shrubs with winged stems (Heiden et al., 2009) that can be either continuous or discontinuous and form different-sized segments (Barroso and Bueno, 2002). In this section, there are several species which show three-winged vegetative and reproductive branches, such as *B. apicifoliosa* A. A. Schneid. & Boldrini, *B. crispa* Spreng., *B. flexuosiramosa* A. A. Schneid., *B. glaziovii* Baker, *B. jocheniana* G. Heiden & Macias, *B. junciformis* DC., *B. microcephala* (Less.) DC., *B. milleflora* (Less.) DC., *B. myriocephala* Baker and *B. penningtonii* Heering. The species *B. articulata* (Lam.) Pers. presents two/three-winged vegetative branches and two-winged reproductive cladodes (Heiden et al., 2009). In this study, *B. pentaptera* (Less.) DC. showed three wings in the vegetative axes and two wings in the reproductive branches.

The epidermal characteristics stablished for *B. pentaptera* are observed in most of "carquejas" (Cortadi et al., 1999; Budel et al., 2003, 2004; Budel and Duarte, 2010; Pereira et al., 2014; Jasinski et al., 2014), including the anatomical pattern of anticlinal epidermal cells walls. Anomocytic and anisocytic stomata are usually described for several species of *Baccharis* (Souza et al., 2011, 2013; Budel et al., 2013; Jasinski et al., 2014). However, Pereira et al. (2014) verified actinocytic and anomocytic stomata for *B. milleflora* DC. Actinocytic stomata were also found only in the non-winged *B. conferta* Kunth and *B. boliviensis* (Weed) Cabrera (Freire et al., 2007). Freire et al. (2007) reported that the stomatal type and epidermal cell walls can be used to differentiate *Baccharis* species when they have similar trichomes.

Biseriate capitate glandular trichomes are typically registered for "carquejas". Similar trichomes were reported in many species of *Baccharis*, such as *B. coridifolia* DC. (Budel and Duarte, 2007), *B. curitybensis* Heering ex Malme, *B. spicata* (Lam.) Baill. (Oliveira et al., 2011), *B. boliviensis*, *B. crispa*, *B. dracunculifolia* DC., *B. glaziovii* (Jasinski et al., 2014), *B. milleflora* (Pereira et al., 2014),

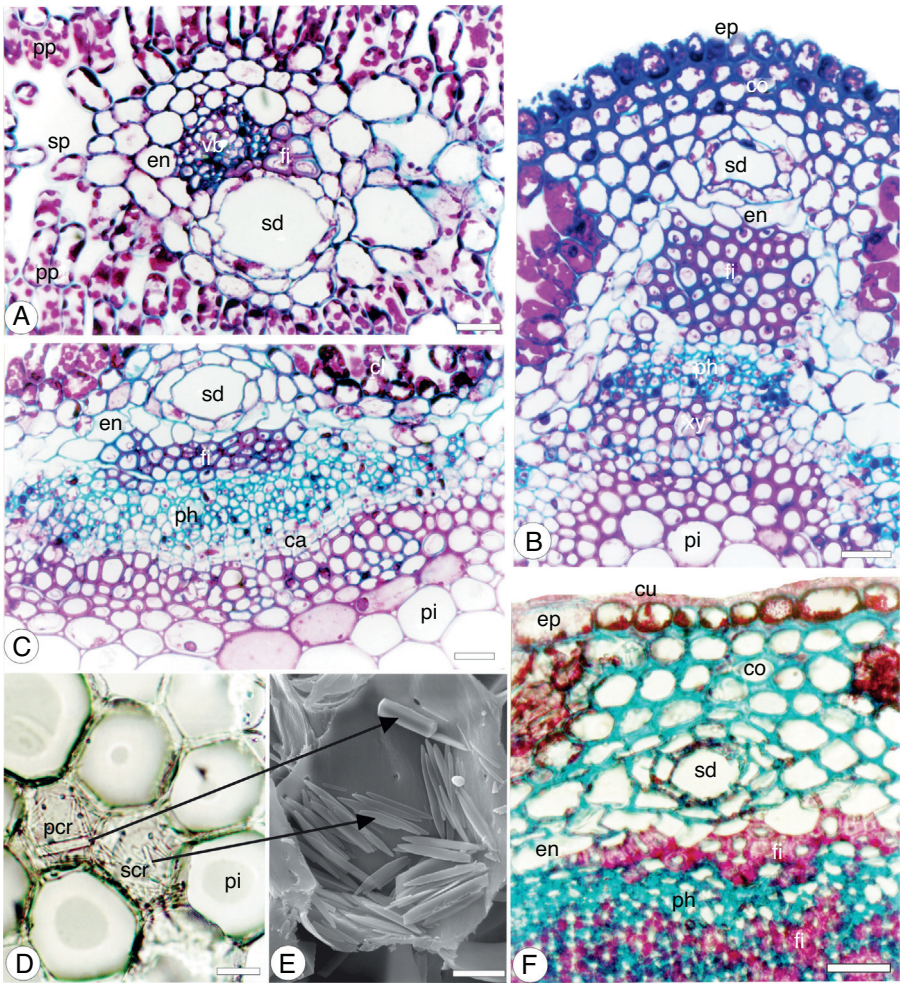

Figure 3. *Baccharis pentaptera* (Less.) DC., Asteraceae – Cladode in cross-section. (A) Detail of wing, showing a minor vascular bundle (vb), endodermis (en), fibers (fi), palisade parenchyma (pp), spongy parenchyma (sp) and secretory duct (sd). (B) Axis in direction to collenchyma (co) exhibiting epidermis (ep), endodermis (en), fibers (fi), secretory duct (sd), phloem (ph), xylem (xy) and pith (pi). (C) Axis in direction to chlorenchyma (cl) indicating cambium (ca), endodermis (en), fibers (fi), secretory duct (sd), phloem (ph), xylem (xy) and pith (pi). (D) Prismatic (pcr) and stiloid (scr) crystals of calcium oxalate in the pith (pi). (E) Prismatic and styloid crystals of calcium oxalate in pith (SEM). (F) Axis showing fibers (fi) in phloem (ph), cuticle (cu), collenchyma (co), secretory duct (sd), endodermis (en) and epidermis (ep). Bar = 20 μm (A–E).

B. notosergila Griseb., *B. pedunculata* (Mill.) Cabrera (Heiden et al., 2009), *B. teindalensis* Kunth, *B. tola* Phil. (Freire et al., 2007), and *B. usterii* Heering (Budel and Duarte, 2010). Moreover, other genera of Asteraceae have these glandular trichomes, mainly *Gochnatia* (Youssef et al., 2013), *Calea* (Budel et al., 2006; Camilotti et al., 2014) and *Mikania* (Gasparetto et al., 2010; Amorin et al., 2014; Araújo et al., 2015). Furthermore, other types of glandular trichomes were also described for "carquejas" as uniseriate capitate glandular trichomes in *B. sagittalis* (Less.) DC. and *B. triangularis* Hauman (Petenatti et al., 2007) and non-capitate glandular trichomes in *B. usterii* (Budel and Duarte, 2010).

The non-glandular trichomes observed in this study were found for both winged and non-winged species, such as *B. coridifolia* (Budel and Duarte, 2007), *B. anomala* DC. (Budel and Duarte, 2008a), *B. singularis* (Vell.) G.M. Barroso (Souza et al., 2011), *B. spicata* (Oliveira et al., 2011), *B. cognata* DC. (Budel et al., 2013), *B. glaziovii* (Jasinski et al., 2014) and *B. milleflora* (Pereira et al., 2014). In addition, simple and flagelliform non-glandular trichomes with uniseriate isodiametric cells at the base were described for the non-winged *B. uncinella* DC. (Budel and Duarte, 2008b).

As usual, the trichomes morphology was used as a taxonomic standard for their classification in this investigation. However, the non-glandular trichomes of *B. pentaptera* demonstrated basal cells stained for lipophilic compounds. Hence, these trichomes can be also established as a mixed type owing to their functional properties for protection and secretion.

Similar chlorenchyma organization was reported for several "carquejas" (Sá and Neves, 1996; Cortadi et al., 1999; Budel et al., 2003; Rodrigues et al., 2008; Budel and Duarte, 2009, 2010; Pereira et al., 2014). The wing edge arrangement of *B. pentaptera* that presented vascular bundles and secretory ducts was also found in *B. milleflora* (Pereira et al., 2014), *B. myriocephala* (Sá and Neves, 1996), *B. trimera* (Budel and Duarte, 2009), and *B. usterii* (Budel and Duarte, 2010). However, *B. crispa* showed only secretory ducts on the wing edges, which were not accompanied by vascular bundles (Cortadi et al., 1999).

Several members of *Baccharis* are high producers of essential oils (Simões-Pires et al., 2005; Molares et al., 2009; Budel et al., 2012; Florão et al., 2012; Lage et al., 2015). These volatile oils are usually found in glandular trichomes and secretory ducts of Asteraceae species (Budel et al., 2012; Jasinski et al., 2014) and have some well-known medicinal properties as anti-inflammatory, antiprotozoal, schistosomicidal, and antimicrobial activities (Parreira et al., 2010; Florão et al., 2012; Valarezo et al., 2015). The remarkable presence of these secretory structures confirms some previous literature data that demonstrated a yield of 1.8% for the essential oil of *B. pentaptera*, in which β-pinene was its main volatile component (Bona et al., 2002; Simões-Pires et al., 2005).

In spite of their responsiveness to changes in the environmental calcium concentration, the occurrence of calcium oxalate crystals is strongly dependent of a genetic control. In addition, crystal type and distribution may be considered a taxonomic character for classification of species (Franceschi and Nakata, 2005). *B. pentaptera* showed prismatic and styloid crystals of calcium oxalate in the perimedullary region. These types are typically encountered in the perimedullary region of cladodes of "carquejas", such as *B. articulata*, *B. crispa*, *B. trimera* (Cortadi et al., 1999), *B. myriocephala* (Sá and Neves, 1996), *B. microcephala* (Budel and Duarte, 2009), *B. glaziovii* (Jasinski et al., 2014), *B. sagittalis*, and *B. triangularis* (Petenatti et al., 2007). However, *B. milleflora* revealed no crystal in the investigation performed by Pereira et al. (2014).

As expected, the morpho-anatomical characters of the cladodes of *B. pentaptera* corresponded with those of the section *Caulopterae*. These features need to be evaluated as a whole for species diagnosis. However the following structures should be emphasized as distinguishing characters of other *Caulopterae* species: opposite and spread wings in the axis of the two-winged cladode, irregular arrangement in the three-winged cladode, uniseriate epidermis, palisade parenchyma next to both sides of the epidermis, spongy parenchyma traversed by minor collateral vascular bundles in the central region, capitate and biseriate glandular trichome, uniseriate and flagelliform non-glandular trichome with 2–3 cells that extend from the base, caulinar axis with chlorenchyma alternating with angular collenchyma, perivascular fiber caps adjoining the phloem which is outside the xylem, and prismatic and styloid crystals in the perimedullary zone.

However, these diagnostic elements are not usually detected or preserved for highly fragmented commercial samples. In that sense, further analyses should be performed in order to a final differentiation of this species from other "carquejas", including quantitative micrograph (Petenetatti et al., 2007) and additional histochemical reaction as reported by Ventrella and Marinho (2008).

Authors' contributions

JMB developed the research and prepared the manuscript. JPP, VLPS, CRCF and PVF contributed in performing the scanning electron microscopy (SEM) analysis, describing the anatomical data of the species and providing a critical reading of the manuscript. MRD created the project and supervised the laboratory work. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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