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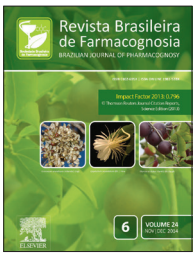

Original article

Morpho-anatomical characteristics of *Baccharis glaziovii* in support of its pharmacobotany

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A B S T R A C T

Baccharis glaziovii Baker, Asteraceae, also known as *carqueja* or *carqueja-arbustinho*, is a native shrub of Brazil that reaches 0.5-2.5 m in height. It is a dioecious species that blossoms from September to December. This species has cladodes, which are winged stems that belong to the "*carquejas*" and are widely used indiscriminately by the population due to their gastric and diuretic properties. *Carquejas* are included in section *Caulopterae* and are difficult to identify even for taxonomists or *Baccharis* specialists. In the present study, a morpho-anatomical (cladodes and leaves) analysis of the medicinal plant was undertaken to improve its identification and add to the knowledge of section *Caulopterae*. Fragments of cladodes and leaves were collected and prepared in accordance with standard optical and scanning electron microscopy techniques. The morpho-anatomical characteristics found in *B. glaziovii*, include three-winged stems showing wings in a regular arrangement around the stem axis, short and petiolate leaves, flagelliform and simple non-glandular trichomes, concave-convex midrib, petioles with a concave shape and a slight projection on the adaxial face and convex with three projections on the abaxial surface, and calcium oxalate crystals in the form of raphides, styloids and pyramidal in the perimedullary region of the cladode, when evaluated as a whole, provide support for the quality control.

Introduction

The genus *Baccharis* comprises herbaceous perennials, subshrubs and shrubs found in the Americas. Currently, 175 species of *Baccharis* have been recorded in Brazil, and 83 of these are found in Paraná State (Heiden and Schneider, 2013; Heiden et al., 2014), which is one of the major centers of species diversity of this genus in Brazil (Heiden and Ribas, 2012; Heiden et al., 2014). Regarding its chemical features, more than 150 compounds have been isolated and identified (Abad and Bermejo, 2007), and approximately 120 species have been chemically analyzed (Verdi et al., 2005). The studied compounds include diterpenoids, triterpenoids, flavonoids, coumarins and essential oils (Verdi et al., 2005; Abad and Bermejo, 2007). However, no chemical marker has been established to characterize the *Baccharis* species (Lonni et al., 2003; 2005;

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Simões-Pires et al., 2005). Several species of this genus that possess cladodes are popularly known as *carquejas*, and are traditionally used as anti-inflammatory, stomachic and diuretic remedies (Abad and Bermejo, 2007; Lemos et al., 2007; Budel et al., 2008; Boller et al., 2010).

The properties of *carqueja* tea have been confirmed by pharmacological studies, and these activities have been correlated with the effects of the compounds isolated from the extracts. Some toxicological studies suggest that *carqueja* tea should not be consumed by pregnant women and patients undergoing medical treatment for blood pressure problems (Anvisa, 2011). These studies emphasize the need to better understand the uses of these medicinal plants both to confirm the value of their traditional use and to ensure their safe use in the future.

Baccharis glaziovii Baker, Asteraceae, is popularly known as *carqueja* and *carqueja-arbustinho*. It is a shrub native to Brazil and reaches 0.5-2.5 m in height. It is found in Eastern Paraguay, Southeastern and Southern Brazil, and Southeastern Argentina. It grows in damp places, such as swamps and hillside slopes, and it blossoms from June to March with a peak flowering season from September to December (Heiden et al., 2012).

In view of the difficulty to identify *Baccharis*, particularly the *carqueja* species, several studies have examined its morpho-anatomical characteristics to supply pharmacobotanical characters to the taxon and support the quality control of vegetable drugs (Budel et al., 2004, 2005; Budel and Duarte, 2009, 2010). In addition, there has been substantial confusion between different representative species known by the same popular name, used arbitrarily for the same therapeutic purposes (Degen et al., 2005).

From this perspective, since there have been very few studies of *B. glaziovii* and because it is similar to other *carquejas*, the present study undertook morpho-anatomical analysis of cladodes and leaves with the aim of assisting in the identification of the medicinal plant and providing further information about the section *Caulopterae*.

Materials and methods

Plant material

Baccharis glaziovii Baker, Asteraceae, samples (cladodes and leaves) were collected in November 2012, at Sitio Mr. Vitor Jasinski, located in Dulcio countryside (Marechal Mallet City), in Paraná State, Brazil, whose approximate coordinates are 25°48'26.99'' S and 50°52'54.91'' W, elevation 881 m. The identification of the botanical material and the deposit of voucher specimens were supervised by Osmar dos Santos Ribas of the Municipal Botanic Museum of Curitiba (MBM381048-Fem/MBM381049-Masc), Dr. Ivo Nelson Matzenbacher of the Federal University of the Rio Grande do Sul (ICN191190-Fem/ICN191189-Masc) and Dr. Gustavo Heiden, Department of Botany, Federal Rural University of Rio de Janeiro (RBR35685-Fem/RBR35686-Masc). The access to the botanical material was authorized and licensed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Conselho de Gestão do Patrimônio Genético (CGEN/MMA) registered under Nº 010304/2013-4.

Morpho-anatomical analysis

Cladodes and leaves fragments were fixed in formalin-acetic acid-alcohol (FAA) and kept in 70% (Johansen, 1940) and kept in 70% ethanol solution (Berlyn and Miksche, 1976). Transverse and longitudinal free-hand sections were stained either with basic fuchsine and astra blue dye (Roeser, 1972) or with toluidine blue (O' Brien et al., 1964). Histochemical reactions were performed with ferric chloride to detect phenolic compounds (Johansen, 1940), Sudan IV to detect lipophilic substances (Foster, 1949), phloroglucin to detect lignified elements (Sass, 1951), iodine-iodide to detect starch (Berlyn and Miksche, 1976) and sulfuric acid to verify the nature of the crystals (Oliveira and Akisue, 1997). Photographs were taken using an Olympus CX 31 light microscope coupled to a C7070 camera. For scanning electron microscopy (SEM) analysis (Souza, 1998), cladodes and leaves fixed in FAA 70 were dehydrated in graded ethanol series and critical point-dried in a Bal-Tec CPD-030 dryer, coated with gold using a Balzers SCD-030 coating device and examined using a JEOL JSM-6360 LV microscope.

Results and discussion

According to Heiden et al. (2012), the section *Caulopterae* corresponds to the *Organensis* and *Trimera* groups by Barroso (1976) and *Baccharis genistelloides* group by Müller (2006). The *Baccharis* species with winged stems (cladodes) are popularly called *carquejas* (Budel et al., 2005). Certain species, including *B. flexuosiramosa* A. A. Schneid and Boldrini (Schneider and Boldrini, 2008b), *B. opuntioides* Mart. ex Baker (Schneider and Boldrini, 2008a; Schneider et al., 2011), *B. trimera* (Less.) DC. (Rodriguez et al., 2008) and *B. usterii* Heering (Budel and Duarte, 2010), display both cladodes and leaves. The leaves, if present, are morphologically different and assist in the identification and differentiation of *carquejas*.

Baccharis glaziovii (Fig. 1A) is an erect shrub measuring 0.5 to 2 m in height. The cladode (winged stem) has three discrete flat or slightly wavy wings that measure 1-19 cm long × 0.5-1.3 cm wide (Fig. 1B). The leaves are shortly petiolate, oblong in shape, and have entire and revolute margins, an obtuse apex and cuneate base, as well as being penninerved measuring 0.5 to 2.7 in length × 0.5 to 6 cm in width.

Carquejas may have cladodes with 2 or 3 wings arranged regularly or irregularly to the stem axis (Barroso and Bueno, 2002). In a cross-sectional view, the three wings of *B. glaziovii* are arranged almost regularly, and there are small ribs between them (Fig. 1C).

Anatomically, the epidermis is uniseriate in the wings (Fig. 1D), and coated with a thin and slightly striated cuticle (Fig. 2B). From a surface view, the epidermal cells are polygonal (Figs. 2A-C) and have thin anticlinal walls. Anomocytic stomata (Fig. 2A) are located slightly above the other epidermal cells. These characteristics are in agreement with the data obtained from other studied *carquejas* (Budel et al., 2005; Petenatti et al., 2007; Budel and Duarte, 2010).

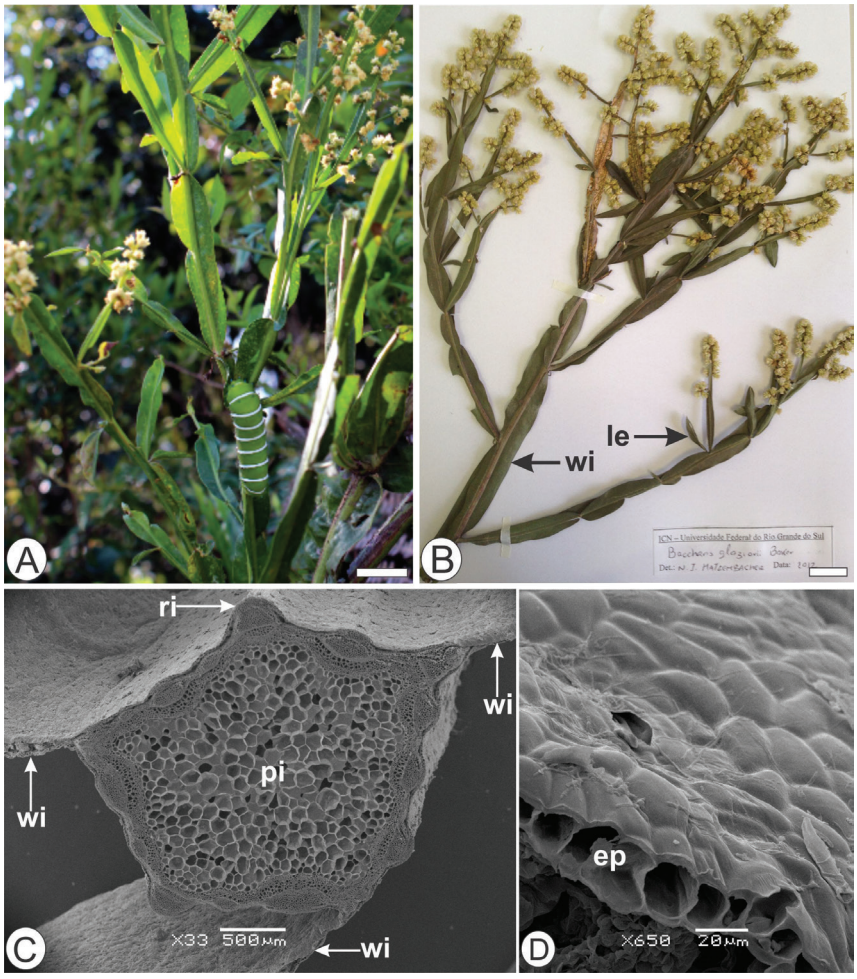

Figure 1 – *Baccharis glaziovii* Baker, Asteraceae. **A**. General appearance; **B**. Appearance of cladodes, indicating leaves (le) and wings (wi); **C**. Front view of cladode, exhibiting pith (pi), ribs (ri) and wings (wi) in SEM (scanning electron microscopy); **D**. Cross-section of epidermis (ep) (SEM). Bar: 1.5 cm (A), 1 cm (B).

The glandular trichomes of *B. glaziovii* are capitate (Fig. 2E) composed of 8-12 cells from the base. Similar glandular trichomes were found in *B. articulata* (Lam.) Pers. and *B. gaudichaudiana* DC. (Rodriguez et al., 2010), *B. crispa* (Less.) DC. (Budel et al., 2004) and *B. microcephala* (Less.) DC. (Budel and Duarte, 2009). Additionally, capitate uniseriate glandular trichomes were found in *B. sagittalis* (Less.) DC. and *B. triangularis* Hauman (Petenatti et al., 2007), although capitate glandular trichomes were not observed in *B. usterii* (Budel and Duarte, 2010).

The essential oil present in *carquejas* can be found in glandular trichomes and secretory ducts (Budel et al., 2012). Budel et al. (2013) reported that the base cells of the non-glandular trichomes in *B. cognata* DC. reacted positively to lipophilic compounds with Sudan IV. These authors suggested that the cells may store essential oil. In this study, only the biseriate and capitate glandular trichomes reacted with Sudan IV.

The chlorenchyma consists of a palisade parenchyma, composed of approximately two strata of short cells on both sides of the epidermis, and a spongy parenchyma in the central region (Fig. 2F). This arrangement has also been found in some *carquejas* (Budel et al., 2004; Petenatti et al., 2007; Budel and Duarte, 2009). However, only palisade parenchyma was observed in the wings of the cladodes of *B. trimera* (Cortadi et al., 1999). In this study, minor collateral vascular bundles encircled by an endodermis are located in the spongy parenchyma (Fig. 2F) and have an alternating distribution. Each bundle directed to the upper and lower sides of the xylem, alternately. These same features were found in *B. myriocephala* DC. (Sá and Neves, 1996), *B. usterii* (Budel and Duarte, 2010) and *B. milleflora* (Less.) DC. (Pereira et al., 2014). Additionally, a well-developed perivascular fiber cap adjoining the phloem (Fig. 2F) can be observed.

In *B. glaziovii*, the secretory ducts (Fig. 2F) are located near the vascular bundles. They display uniseriate epithelium consisting of 8-12 cells with dense cytoplasm containing essential oil. Similar secretory ducts are often found in *carquejas* (Sá and Neves, 1996; Cortadi et al., 1999; Rodriguez et al., 2008; Budel and Duarte, 2009, 2010). However, *B. crispa* only has secretory

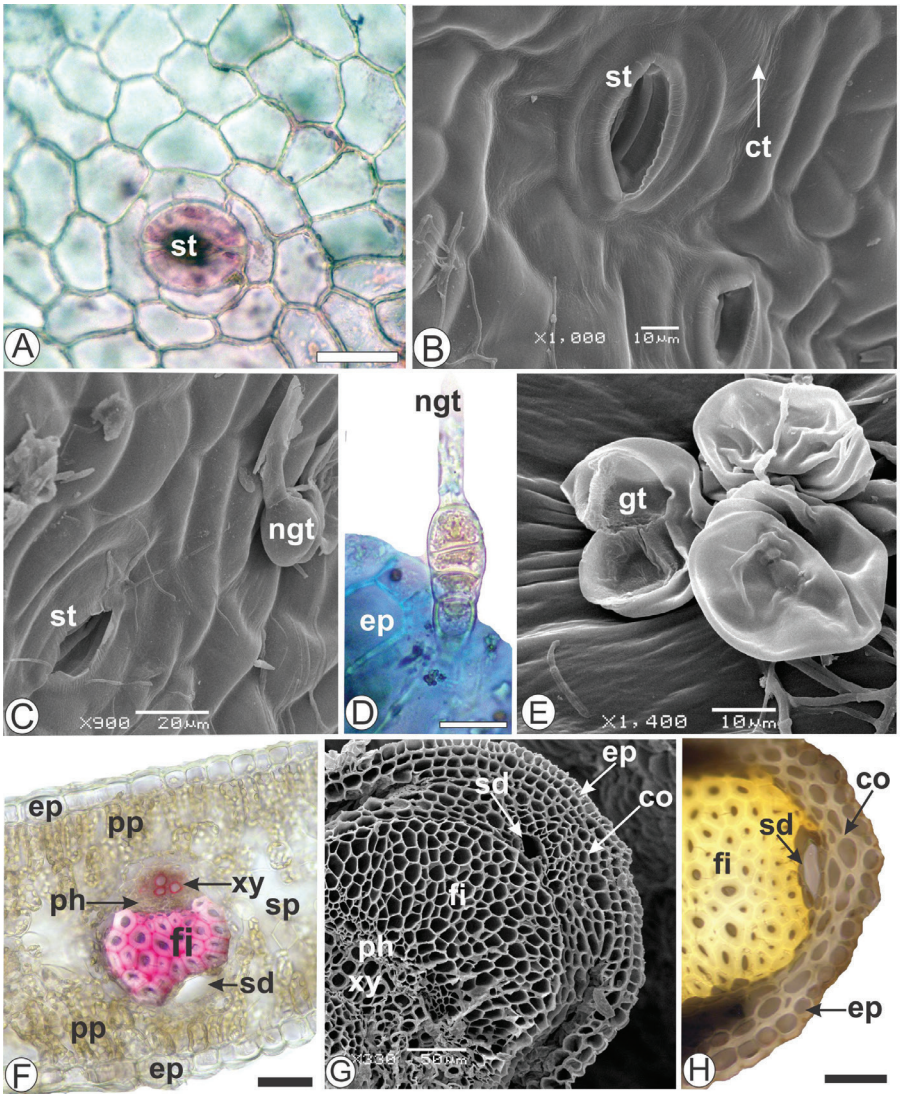

Figure 2 – *Baccharis glaziovii* Baker, Asteraceae – Wing of cladodes. **A**. From surface view of epidermis exhibiting anomocytic stomata (st); **B**. From surface view showing stomata and cuticles (ct) in SEM (scanning electron microscopy); **C**. Front surface view indicating stomata (st) and non-glandular trichome (ngt); **D**. Front surface view showing a non-glandular trichome (ngt); **E**. Front surface view indicating glandular trichome (gt) in clusters; **F**. Cross-section showing epidermis (ep), fibers (fi), palisade parenchyma (pp), phloem (ph), secretory ducts (sd), spongy parenchyma (sp), xylem (xy) with phloroglucin reaction; **G**. Wing in cross-section near the border, showing epidermis (ep), collenchyma (co), fibers (fi), secretory duct (sd), phloem (ph) and xylem (xy) in SEM; **H**. Wing in cross-section near the border, showing epidermis (ep), collenchyma (co), fibers (fi), and secretory duct (sd). Bar: 20 µm (A, D, F), 50 µm (H).

ducts at the edge of the wings, and they do not accompany the vascular bundles (Cortadi et al., 1999).

The edges of the wings of *B. glaziovii* exhibit some layers of collenchyma below the epidermis, a collateral vascular bundle with a perivascular fiber cap adjoining the phloem, and secretory ducts (Figs. 2G, 2H). These features are widely reported in *carquejas* (Rodriguez et al., 2008; Budel and Duarte, 2009, 2010).

In the axis of the cladode of *B. glaziovii*, the epidermis (Fig. 3A) shows similar characteristics to that of the wings (Fig. 3A). The cuticle reacted in the presence of Sudan IV (Fig. 3B). Underlying the coating system, there is alternating chlorenchyma with collenchyma (Fig. 3C). When this is facing the direction of the vascular bundles, it has approximately 1-5 layers (Figs. 3A-C). Secretory ducts, similar to those in the wings, are found near the endodermis (Figs. 3B, 3D, 3F). There are impregnations of lipophilic compounds (Fig. 3B) in the endodermal cells (Figs. 3B-3D).

The vascular cylinder presents cambia forming phloem on the outward, and xylem inward (Figs. 1C, 3C-E). The pith occupies most of the cladode volume (Fig. 1C) and is formed by relatively large parenchymal cells with thin walls. In the phloem, fibers can be observed with different degrees of lignifications (Figs. 3D-E). Well-developed perivascular fiber caps are found adjoining the phloem (Figs. 3A-E). These fiber caps reacted positively to phloroglucin (Fig. 3E). These

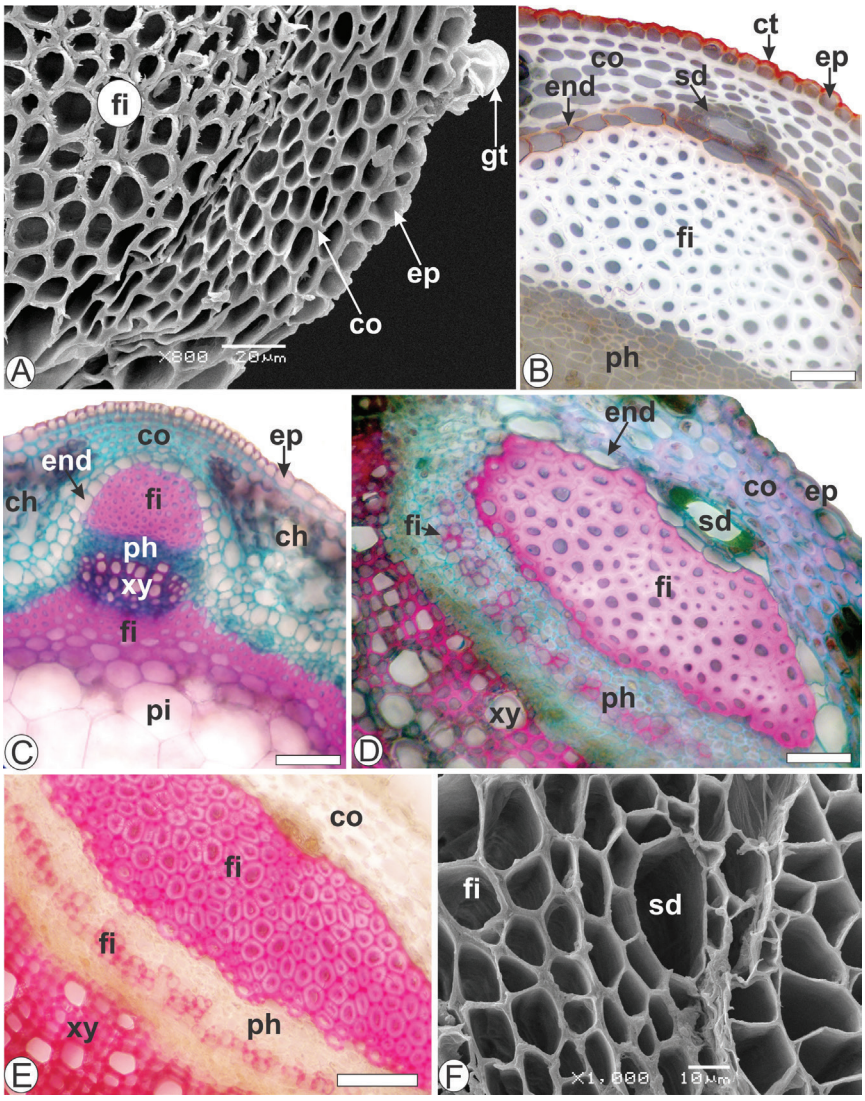

Figure 3 – *Baccharis glaziovii* Baker, Asteraceae – Axis of cladode in cross-section. **A**. Detail of the axis, showing collenchyma (co), epidermis (ep), fiber (fi) and glandular trichome (gt) in SEM; **B**. Axis in reaction with Sudan IV, indicating collenchyma (co), cuticle (ct), endodermis (end), epidermis (ep), fibers (fi), phloem (ph), and secretory ducts (sd); **C**. Detail of the axis, exhibiting chlorenchyma (ch), collenchyma (co), epidermis (ep), endodermis (end), fibers (fi), phloem (ph), xylem (xy), and pith (pi); **D**. Axis in collenchyma (co) direction, exhibiting epidermis (ep), endodermis (end), fibers (fi), secretory duct (sd), phloem (ph), and xylem (xy); **E**. Axis in reaction with phloroglucin, indicating collenchyma (co), fibers (fi), phloem (ph) and xylem (xy); **F**. Detail of the fiber (fi) and secretory duct (sd). Bar: 50 µm (B, D, E), 100 µm (C).

characteristics are consistent with the *Baccharis* pattern (Sá and Neves, 1996; Cortadi et al., 1999; Petenatti et al., 2007; Budel and Duarte, 2009, 2010). In *B. glaziovii*, the pith is composed of cells of various sizes and with thin walls. These cells occupy the largest volume of the axis (Figs. 1C, 3C).

The calcium oxalate crystal shapes differ and are frequently described as druses, styloids, raphides, prisms and crystal sand (Franceschi and Nakata, 2005). The type, presence or absence of crystals may be characterized as a taxonomic feature (Lersten and Horner, 2000; Meric, 2009). The occurrence of calcium oxalate crystals in the pith is common in *carquejas*; however, different forms can help in the characterization of species.

Several types of calcium oxalate crystals were found in *carquejas*, including raphides and hexagonal and tetragonal prisms in *B. triangularis*; raphides and tetragonal prisms in *B. sagittalis* (Petenatti et al., 2007); crystal sand and square dipyramids in *B. usterii* (Budel and Duarte, 2010); elongated square dipyramids in *B. articulata*; elongated square dipyramids, square dipyramids, cubes, tetragonal prisms in *B. crispa* (Cortadi et al., 1999) and *B. trimera* (Cortadi et al., 1999; Budel and Duarte, 2009); and square dipyramids in *B. microcephala* (Budel and Duarte, 2009). In this study, the types of calcium oxalate crystals identified are raphides (Figs. 4A-B), styloids (Fig. 4A) and elongated square dipyramids (Figs. 4A, 4C).

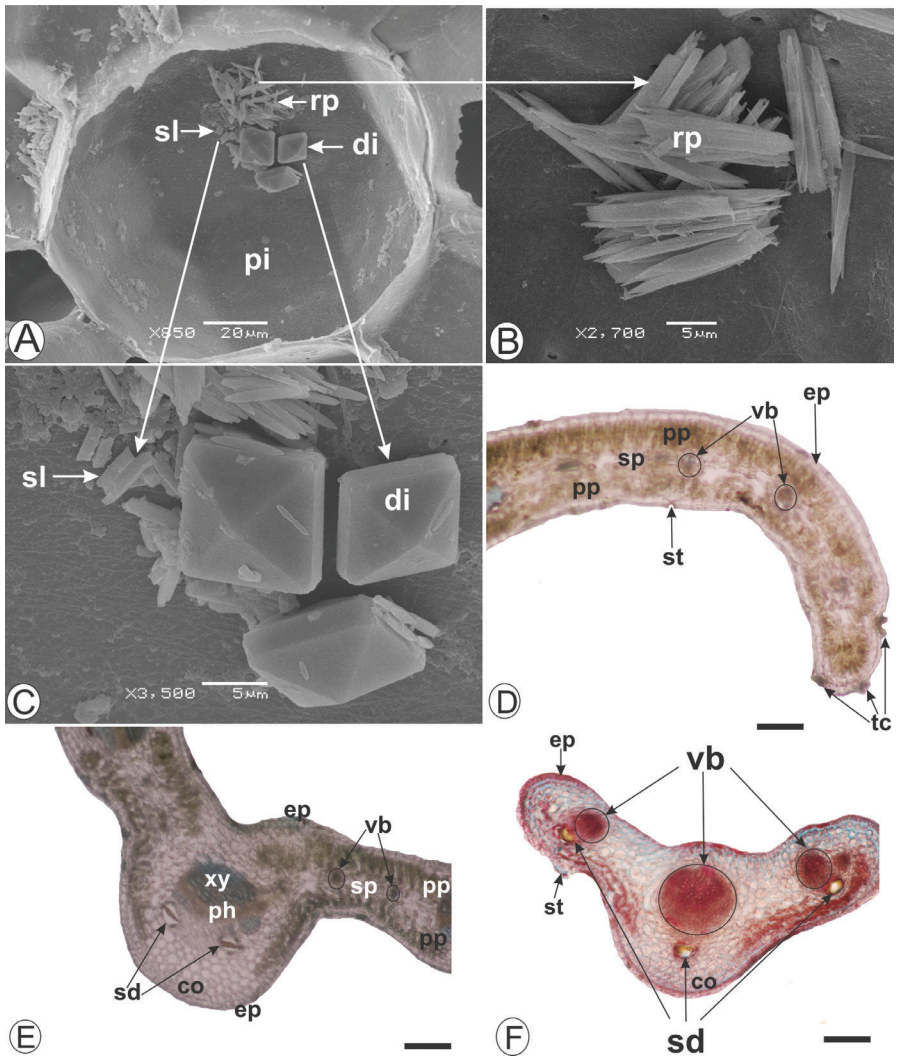

Figure 4 – *Baccharis glaziovii* Baker, Asteraceae. **A-C**. Axis in SEM. A. Styloids (sl) and elongated square dipyramid (di) crystals in the pith; **B**. Detail of figure A, indicating raphides (rp); **C**. Detail of figure **A**. showing an elongated square dipyramid crystal (di); **D**. Blade organization, revealing isobilateral mesophyll and showing epidermis (ep), stomata (st), palisade parenchyma (pp), spongy parenchyma (sp), trichomes in clusters (tc), and a vascular bundle (vb); **E**. Midrib, showing collenchyma (co), epidermis (ep), phloem (ph), secretory ducts (sd), xylem (xy) and vascular bundles (vb); **D**. Petiole in cross-section, indicating, collenchyma (co), stomata (st), epidermis (ep), secretory ducts (sd), and vascular bundles (vb). Bar: 50 µm (D, E), 100 µm (F).

The leaf blade of *B. glaziovii* has an epidermis with the same characteristics described for the wing. In relation to the presence of stomata, the leaf is amphistomatic. The mesophyll is isobilateral forming 2-3 layers of palisade parenchyma and approximately three layers of spongy parenchyma in the middle region. Vascular bundles are distributed in the spongy parenchyma (Fig. 4D) surrounded by an endodermis that may be accompanied by secretory ducts in the external phloem position. These features are also found in the leaves of *B. sagittalis* (Pettenati et al., 2007) and *B. usterii* (Budel and Duarte, 2010).

From a cross-sectional view, the midrib is concave-convex. A single epidermal layer is covered by a thin and slightly striated cuticle. Underlying the coating system on the abaxial side, there are two layers of angular collenchyma (Fig. 4E). A single collateral vascular bundle is located in the ground parenchyma. The endodermis includes the vascular bundle, which may be accompanied by secretory ducts positioned externally to the phloem (Fig. 4E). In contrast with this finding, *B. usterii* proved to be slightly convex on the adaxial side and convex on the opposite face, according to data obtained from Budel and Duarte (2010).

In *carquejas*, the leaves have been described in the literature are sessile (Pettenati et al., 2007; Budel and Duarte, 2010). In *B. glaziovii*, the leaves are petiolate and the petiole, when observed in a cross-section, is concave with a slight projection on the adaxial face and convex with three projections on the abaxial surface, with the central part being more prominent. The epidermal coating displays the same characteristics as those observed in the leaf. The collenchyma is angular and occurs

in continuous stripes of 1-3 sets of cells. Secretory ducts are present in an external phloem position (Fig. 4F).

Conclusion

In general, the cladodes of the species of *carquejas* have similar characteristics. In this study, the morpho-anatomy of *B. glaziovii* was used to improve the differentiation of other *Baccharis*, including those that have leaves. In addition, when evaluated as a whole, it was established that the main features found in the cladodes and leaves of *B. glaziovii* supply pharmacobotanical characteristics to the taxon and give support for quality control of vegetal drugs. For this study, the main features used were: three-winged stems showing wings arranged in a regular way from the stem axis; short and petiolate leaves, oblong shape, entire and revolute margins, obtuse apex, cuneate base and penninerved; flagelliform and simple non-glandular trichomes formed by four base cells that extend toward the apex, midrib with concave-convex shape, a petiole with concave shape and slight projection on the adaxial face and convex with three projections on the abaxial surface, and the perimedullary region of the cladode showing calcium oxalate crystals in the form of raphides, styloids and pyramidals.

Authors' contributions

VCGJ contributed in collecting the plant samples and identification, maintenance of the herbarium, running the laboratory work analysis of the data and drafted the paper. FRC, RP, RZS and JMB designed the study, supervised the laboratory work and contributed to critical reading and writing of the manuscript. 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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REFERENCES

Abad, M.J., Bermejo, P., 2007. *Baccharis* (Compositae): a review update. Caldasia, Bogotá 7, 76-96.

Anvisa, 2011. Formulário de Fitoterápicos da Farmacopéia Brasileira, Agência Nacional de Vigilância Sanitária, p. 126.

Barroso, G.M., 1976. Compositae-subtribo Baccharidineae Hoffmann. Estudo das espécies ocorrentes no Brasil. Rodriguesia 28, 1-273.

Barroso, G.M., Bueno, O.L., 2002. *Compostas. Subtribo: Baccharidinae*. Flora Ilustrada Catarinense. Itajaí: Herbário Barbosa Rodrigues.

Berlyn, G.P., Miksche, J.P., 1976. *Botanical microtechnique and cytochemistry.* Ames: Iowa State University.

Boller, S., Soldi, C., Marques, M.C.A., Santos, E.P., Cabrini, D.A., Pizzolatti, M.G., Zampronio, A.R., Oturi, M.F., 2010. Anti-inflammatory effect of crude extract and isolated compounds from *Baccharis illinita* DC in acute skin inflammation. J. Ethnopharmacol. 130, 262-266.

Budel, J.M., Duarte, M.R., Santos, C.A.M., 2004. Stem morpho-anatomy of *Baccharis cylindrica* (Less.) DC., Asteraceae. Rev. Bras. Cienc. Farm. 40, 93-99.

Budel, J.M., Duarte, M.R., Santos, C.A.M., Farago, P.V., Matzenbacher, N.I., 2005. O progresso da pesquisa sobre o gênero *Baccharis*, Asteraceae: I - estudos botânicos. Rev. Bras. Farmacogn. 15, 268-271.

Budel, J.M., Matzenbacher, N.I., Duarte, M.R., 2008. "Genus *Baccharis* (Asteraceae): a review of chemical and pharmacological studies", in "Recent Progress in Medicinal Plants - Phytopharmacology and Therapeutic Values" (Houston: Studium Press LLC, ed.) p. 1-18.

Budel, J.M., Duarte, M.R., 2009. Análise morfoanatômica comparative de duas espécies de carqueja: *Baccharis microcephala* DC e *B. trimera* (Less.) DC., Asteraceae. Braz. J. Pharm. Sci. 45, 75-85.

Budel, J.M., Duarte, M.R., 2010. Macro and microscopic characters of the aerial vegetative organs of carqueja: *Baccharis usterii* Heering. Braz, Arch. Biol. Technol. 53, 123-131.

Budel, J.M., Duarte, M.R., Döll-Boscardin, P.M., Farago, P.V., Matzenbacher, N. I., Sartoratto, A., Sales Maia, B.H.L.N., 2012. Composition of essential oils and secretory structures of *Baccharis anomala, B. megapotamica* and *B. ochracea*. J. Essent. Oil Res. 24, 19-24.

Budel, J.M., Farago, P.V., Duarte, M.R. 2013. Pharmacobotanical study of *Baccharis cognata* DC. (Asteraceae: Astereae). Lat. Am. J. Pharm. 32, 550-554.

Cortadi, A., Di Sapio, O., Mc Cargo, J., Scandizzi, A., Gattuso, S., Gattuso, M. 1999. Anaomical studies of *Baccharis articulata, Baccharis crispa* and *Baccharis trimera,* "Carquejas" used in folk medicine. Pharm. Biol. 37, 357-365.

Degen, R., Soria, N., Ortiz, M., Basualdo, I., 2005. Problemática de nombres communes de plantas medicinales comercializadas en Paraguay. Dominguezia 21, 11-16.

Foster, A.S., 1949. *Practical plant anatomy.* 2. ed. Princeton; D. Van Nostrand.

Franceschi, V.R., Nakata, P.A., 2005. Calcium oxalate in plants: formation and function. Annu. Rev. Plant Biol. 56, 41-71.

Heiden. G., Baumgratz, J.F.A., Esteves, R.L., 2012. *Baccharis* subgen. *molina* (*Asteraceae*) no Estado do Rio de Janeiro, Brasil. Rodriguesia 63, 649-687.

Heiden. G., Leoni, L.S., Nakajima, J.N., 2014. *Baccharis magnifica* (Asteraceae, Astereae): a striking new species endemic to the summits of Serra do Caparao, southeastern Brazil. Phytotaxa 162, 211216.

Heiden, G., Ribas, O.S., 2012. *Baccharis umbellata* (Asteraceae, Astereae): a new species endemic to the highest summits of Paraná, Southern Brazil. Phytotaxa 49, 23-28.

Heiden, G., Schneider, A.A., 2013. *Baccharis*. In: Lista de Espécies da Flora do Brasil. Jardim Botanico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB5151, accessed 28 April 2014.

Johansen, D.A., 1940. *Plant microtechnique*. New York: McGraw Hill Book.

Lemos, M., Barros, M.P., Souza. J.P., Silva-Filho, A.A., Bastos, J.K., Andrade, S.F., 2007. *Baccharis dracunculifolia*, the main botanical source of Brazilian green propolis, displays antiulcer activity. J. Pharm. Pharmacol. 59, 603-608.

Lersten, N.R., Horner, H.T., 2000. Calcium oxalate crystals types and trends in their distribution patterns in leaves of *Prunus* (Rosaceae: Prunoideae). Plant Syst. Evol. 224, 83-96.

Lonni, A.A.S.G., Scarminio, I.S., Silva, L.M.C., Ferreira, D.T., 2003. Differentiation of species of the *Baccharis* genus by HPLC and chemometric methods. Analyt. Sci. Tokyo. 19, 1013-1017.

Lonni, A.A.S.G., Scarminio, I.S., Silva, L.M.C., Ferreira, D.T., 2005. Numerical taxonomy characterization of *Baccharis* genus species by ultraviolet-visible spectrophotometry. Analyt. Sci. Tokyo. 21, 235-239.

Meric, C., 2009. Calcium oxalate crystals in some species of the tribe inuleae (Asteraceae). Acta Biol. Cracov. Series Botanica 51, 105-110.

Müller, J., 2006. Systematics of Baccharis (Compositae- Astereae) in Bolivia, including an overview of the genus. Syst. Bot. Monog. 76, 1-341.

O' Brien, T.P., Feder, N., Mccully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59, 368-373.

Oliveira, F., Akisue, G., 1997. *Fundamentos de farmacobotânica*. 2. ed. São Paulo: Atheneu.

Petenatti, E.M., Petenatti, M.E., Cifuente, D.A., Gianello, J.C., Giordano, O.S., Tonn, C.E., Del-Vitto, L.A., 2007. Medicamentos herbarios en el centro-oeste Argentino. VI. Caracterización y control de calidad de dos espécies de "carquejas": *Baccharis sagittalis* y *B. triangularis* (Asteraceae). Lat. Am. J. Pharm. 26, 201-208.

Pereira, C.B., Farago, V.P., Budel, J.M., De Paula, J.P., Folquitto, D.G., Miguel, O.G., Miguel, M.D., 2014. A new contribution to the pharmacognostic study of carquejas: *Baccharis milleflora* DC., Asteraceae. Lat. Am. J. Pharm. 33, 841-847.

Rodriguez, M.V., Gattuso, S., Gattuso, M., 2008. *Baccharis crispa* y *Baccharis trimera* (Asteraceae): revisión y nuevos aportes para su normalización micrográfica. Lat. Am. J. Pharm. 27, 387-397.

Rodriguez, M.V., Martínez, M.L., Cortadi, A.A., Bandoni, A., Giuliano, D.A., Gattuso, S.J., Gattuso, M.A., 2010. Characterization of three sect. Caulopterae species (*Baccharis*-Asteraceae) inferred from morphoanatomy, polypeptide profiles and spectrophotometry data. Plant. Syst. Evol. 286, 175-190.

Roeser, K.R., 1972. Die Nadel der Schwarzkiefer-Massenprodukt und Kunstwerk der Natur. Mikrokosmos, Stuttgart. 61, 33-36.

Sá, M.F.A., Neves, L.J., 1996. Contribuição ao estudo das plantas medicinais *Baccharis myriocephala* DC. Rev. Bras. Farm. 77, 88-96.

Sass, J.E., 1951. *Botanical microtechnique*. 2 ed. Ames: Iowa State College.

Schneider, A.A., Boldrini, I.I., 2008a. Ocorrência de *Baccharis opuntioides* Mart. ex Baker (Asteraceae: Astereae) para a região Sul do Brasil. Rev. Bras. Bioc. 6, 137-139.

Schneider, A.A., Boldrini, I.I., 2008b. Two new species of *Baccharis* sect. *Caulopterae* (Asteraceae: Astereae) from Southern Brazil. J. Bot. Res. Inst. Texas. 2, 45-51.

Schneider, A.A., Heiden, G., Boldrini, I.I., 2011. *Baccharis scopulorum*, a new species of section Caulapterae (Asteraceae: Astereae) from rocky cliffs of southern Brazil. Phytotaxa 15,9-14.

Simões-Pires, C.A., Queiroz, E.F., Henriques, A.T., Honstettmann, K., 2005. Isolation and on-line identification of antioxidant compounds from three *Baccharis* species by HPLC-UV-MS/MS with post-column derivatization. Phytochem. Analysis 16, 307-314.

Souza, W., 1998. *Técnicas básicas de microscopia eletrônica aplicadas às Ciências Biológicas.* Rio de Janeiro: Sociedade Brasileira de Microscopia Eletrônica.

Verdi, L.G., Brighente, I.M.C., Pizzolatti, M.G., 2005. Gênero *Baccharis* (Asteraceae): Aspectos químicos, econômicos e biológicos. Quim. Nova 28, 85-94.