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Original Article

Comparative analytical micrographs of "vassouras" (*Baccharis*, Asteraceae)

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ABSTRACT

Baccharis belongs to the Asteraceae family and comprises a number of medicinal species. *Baccharis brevifolia* DC., *B. microdonta* DC., *B. pauciflosculosa* DC., and *B. trilobata* A.S. Oliveira & Marchiori, which are popularly known in Brazil as "vassouras" ("broom"), are all found in Southern Brazil. The anatomical features of the leaf and stem were investigated by employing the usual light and scanning electron microtechniques, as a means of differentiating the taxa. The following anatomical characteristics can be considered to be diagnostic: the occurrence and type of stomata, midrib, stem and crystal shapes, and the presence of the petiole.

Introduction

Baccharis L., Asteraceae, includes about 354 species, which range from the USA to Argentina (90% occur in South America and about 178 species are found in Brazil) (Müller, 2013; BFG, 2015). However, further pharmacobotanical, genetic, chemical, biological, pharmacological and toxicological studies are required. Various ethnobotanical research studies have been carried out in South American communities that use these plants for the treatment of several diseases. These communities mostly make use of several species of *Baccharis* as analgesic, diuretic, spasmolytic, antidiabetic, anti-infective and stomachic medication (Zardini, 1984; Mentz et al., 1997; Abad and Bermejo, 2007). Many of these properties can be attributed to the presence of volatile oils that are found in *Baccharis* species (Lago et al., 2008; Budel et al., 2012; Onofre et al., 2013; Lage et al., 2015; Valarezo et al., 2015).

Previous data have revealed that *Baccharis pauciflosculosa* DC. and *B. microdonta* DC. have antimicrobial activities (Perez and Anesini, 1993, 1994) and the volatile oil of *Baccharis microdonta* shows a high concentration of oxygenated sesquiterpenes (49.91%), which are mainly caryophyllene oxide (24.06%), α-cadinole (8.44%) and viridiflorol (7.67%) (Lago et al., 2008). There is no pharmacobotanical, pharmacological or chemical characterization available for *B. brevifolia* DC. and *B. trilobata* A.S. Oliveira & Marchiori.

Vegetable raw materials are a means of initially determining the quality of a medicinal plant or an herbal drug. The herbal industry experiences serious problems due to the substitution and/or adulteration of plant species by alternatives that are similar. Such tampering impairs the effectiveness of herbal drugs and in some cases may cause intoxication (WHO, 2003). In the case of *Baccharis*, the problem is even worse because a lot of its species have a similar morphology.

An additional problem is the inappropriate use of popular names, which can cause mistakes in the identification of herbal drugs. The same species often has several folk names and furthermore, a singular common name may designate several species (Upton et al., 2011). *B. brevifolia*, *B. microdonta*, *B. pauciflosculosa* and *B. trilobata*, popularly known in Brazil as "vassouras" ("broom") occur in Southern Brazil and their morphology is similar. As a result, some confusion and/or mistakes can result from the popular use of this term.

For these reasons, the purpose of this paper was to study anatomical data regarding the leaves and stems of *B. brevifolia*, *B. microdonta*, *B. pauciflosculosa* and *B. trilobata* as a means of providing additional support for differentiating these species.

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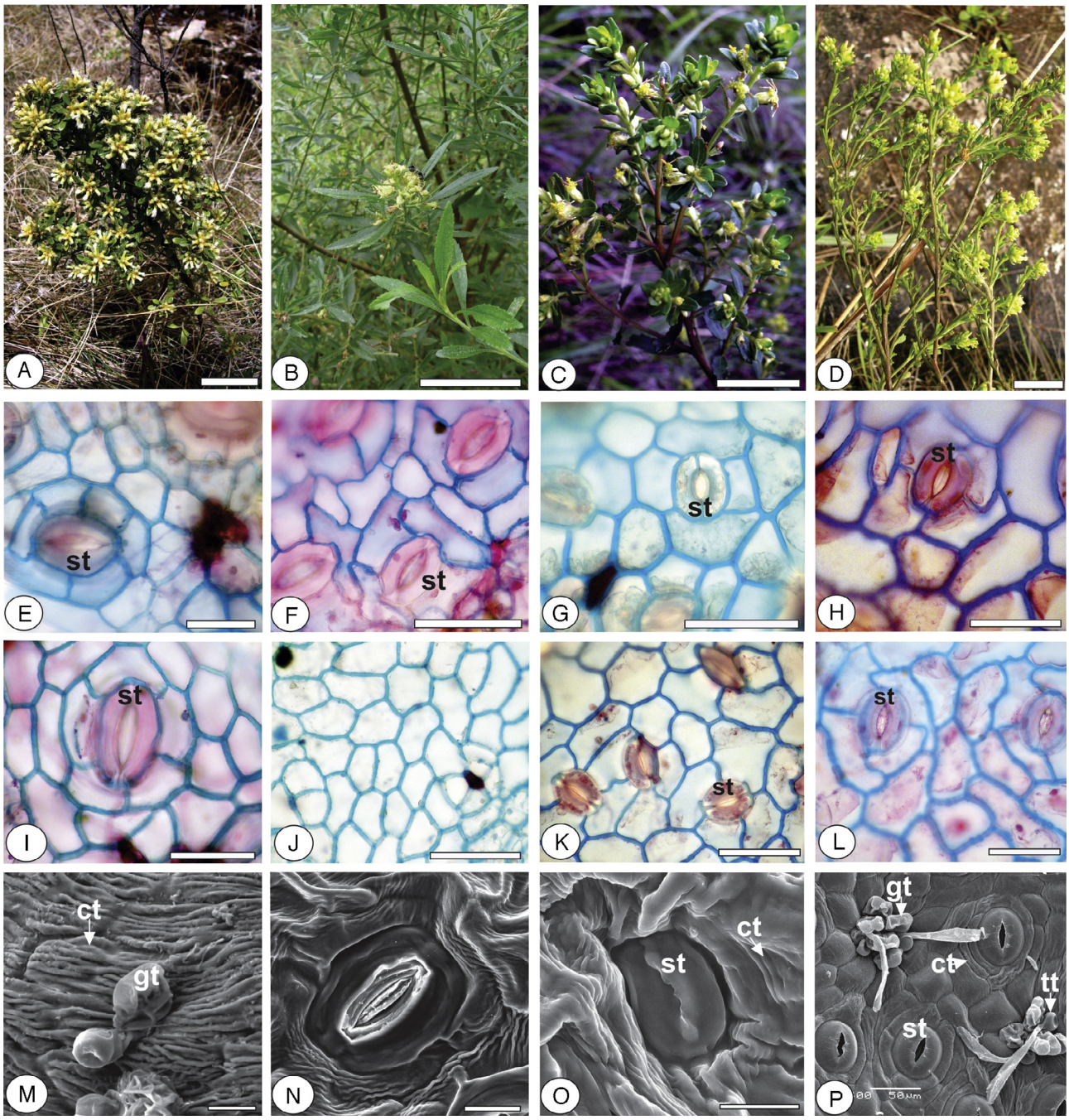

Fig. 1. (A, E, I, M) *Baccharis brevifolia* DC.; (B, F, J, N) *Baccharis microdonta* DC., (C, G, K, O) *Baccharis pauciflosculosa* DC., (D, H, L, P) *Baccharis trilobata* A.S. Oliveira & Marchiori; (A, B, C, D) vegetative and reproductive branch. (E, F, G, H) Abaxial side of leaf epidermis and (I, J, K, L) adaxial side of leaf epidermis, showing epidermal cell walls and stomatum (st). (M, N, O, P) View of the leaf surface, stomatum (st), striated cuticle (ct), non-glandular trichome (nt), and glandular trichome (gt) by SEM; Scale bar = 5 cm (A, B, C, D), 50 μm (E, F, G, H, I, J, K, L), 20 μm (M, N, O).

Materials and methods

Plant material

Aerial parts of at least four specimens of *Baccharis brevifolia* DC., *B. microdonta* DC., *B. pauciflosculosa* DC. and *B. trilobata* A.S. Oliveira & Marchiori, Asteraceae, were collected in the region of Campos Gerais, Ponta Grossa, Paraná, Southern Brazil (coordinates 25° 08′ S and 50° 27′ W) during the summer of 2013. A voucher was identified by taxonomists and registered under the registration numbers HUPG 20411 (*B. brevifolia*), HUPG 20406 (*B. microdonta*), HUPG 20413 (*B. trilobata*) and HUPG 20409 (*B. pauciflosculosa*) in the herbarium at the State University of Ponta Grossa.

Anatomical study

In order to determine the anatomy of the leaf and stem, leaf and stem fragments were fixed in FAA 70 (Johansen, 1940) and kept in 70% ethanol solution (Berlyn and Miksche, 1976). Transverse and longitudinal freehand sections were stained; either with toluidine blue (O'Brien et al., 1964) or basic fuchsin combined with astra blue (Roeser, 1972). Slides were then mounted in 50% glycerin

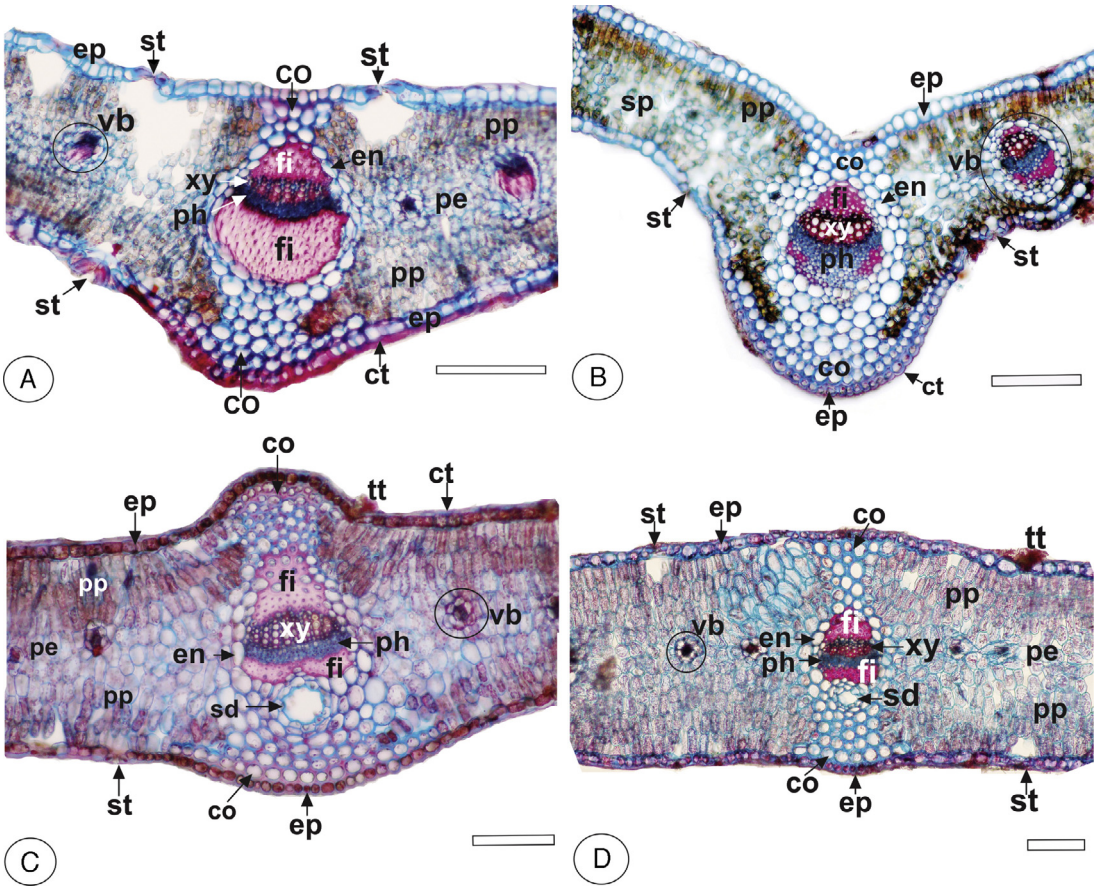

Fig. 2. (A) *Baccharis brevifolia* DC., (B) *Baccharis microdonta* DC., (C) *Baccharis pauciflosculosa* DC., (D) *Baccharis trilobata* A.S. Oliveira & Marchiori. Cross-section of the leaves, showing collenchyma (co), cuticle (ct), epidermis (ep), endodermis (en), fibers (fi), palisade parenchyma (pp), phloem (ph), spongy parenchyma (sp), secretory duct (sd), stomatum (st), trichomes in cluster (tc), vascular bundle (vb), and xylem (xy). Scale bar = 100 μm (B), 50 μm (A, C, D).

(Berlyn and Miksche, 1976). These materials were also dehydrated and embedded in glycol methacrylate (Leica historesin®) (Feder and O'brien, 1968). After inclusion, the blocks that were obtained were sectioned at 7–9 μm using a rotary microtome (Spencer 820) with a type-C steel knife. Longitudinal sections were placed on slides and stained with basic fuchsin combined with astra blue (Brito and AlquinI, 1996). The slides were mounted in synthetic resin (Entellan®).

The diaphanization of the leaves was performed by following the technique of Fuchs (1963), and that of Patel (1979) was used for the stomata classification. The results were illustrated with the aid of photos that were taken with an Olympus CX 31 optical microscope, which was attached to a C7070 digital camera. In conducting the ultra-structural analysis (Souza, 2007), the samples were fixed in FAA 70, dehydrated in a graded ethanol series through the critical point procedure (Balzers CPD 030), coated with gold (Balzers Sputtering SCD 030) and analyzed using a scanning electron microscope (Jeol JSM 6360 LV).

Results and discussion

Morphological characteristics are often used to identify species, although when the morphology is similar mistakes can occur during classification. In this case, anatomical features can help in the identification, especially when they are powdered (Nodari and Guerra, 2000). Similar morphological features tend to occur with *Mikania* spp. (Araújo et al., 2015), *Passiflora* spp. (Wosch et al., 2015), *Piper* spp. (Gogosz et al., 2012), among other genera.

In botanical products, adulterations involve the whole or partial replacement of one species for another, especially when they are similar, belong to the same genus, or are used for the same popular usage (Upton et al., 2011). In this context, *B. brevifolia* (Fig. 1A), *B. microdonta* (Fig. 1B), *B. pauciflosculosa* (Fig. 1C) and *B. trilobata* (Fig. 1D) showed a similar morphology to what has been observed in relation to other *Baccharis* species (Barroso and Bueno, 2002; Oliveira et al., 2011; Jasinski et al., 2014; Budel et al., 2015).

In several *Baccharis* species the anatomical shape of the anticlinal epidermal cell walls of the leaves varies from straight to wavy (Souza et al., 2011; Oliveira et al., 2011; Bobek et al., 2015; Barreto et al., 2015). According to the pattern, all the species analyzed in the present study show anticlinal epidermal cell walls that were straight to slightly wavy and with relatively thin anticlinal walls on both sides (Fig. 1E–L). The cuticle is striated (Fig. 1M) for the four species, especially around the stomata (Fig. 1N–P).

The presence of stomata, as well as the amount and type of stomata in leaf epidermis, are important features for characterizing and differentiating a species. Rodriguez et al. (2010) reported that the density of stomata can help to differentiate between *B. articulata* (Lam.) Pers. and *B. trimera* (Less.) DC. Both amphistomatic and hypostomatic leaves occur in *Baccharis* (Molares et al., 2009; Budel et al., 2013; Bobek et al., 2015; Barreto et al., 2015).

In the present study, only *B. microdonta* has hypostomatic leaves (Fig. 1F and J), the others are amphistomatic (Fig. 1E and G–L). Van Cotthem (1970) stated that the type of stomatum does not only have a diagnostic value but in many cases it can also be used as a marker of natural taxonomic affinity. Anomocytic and

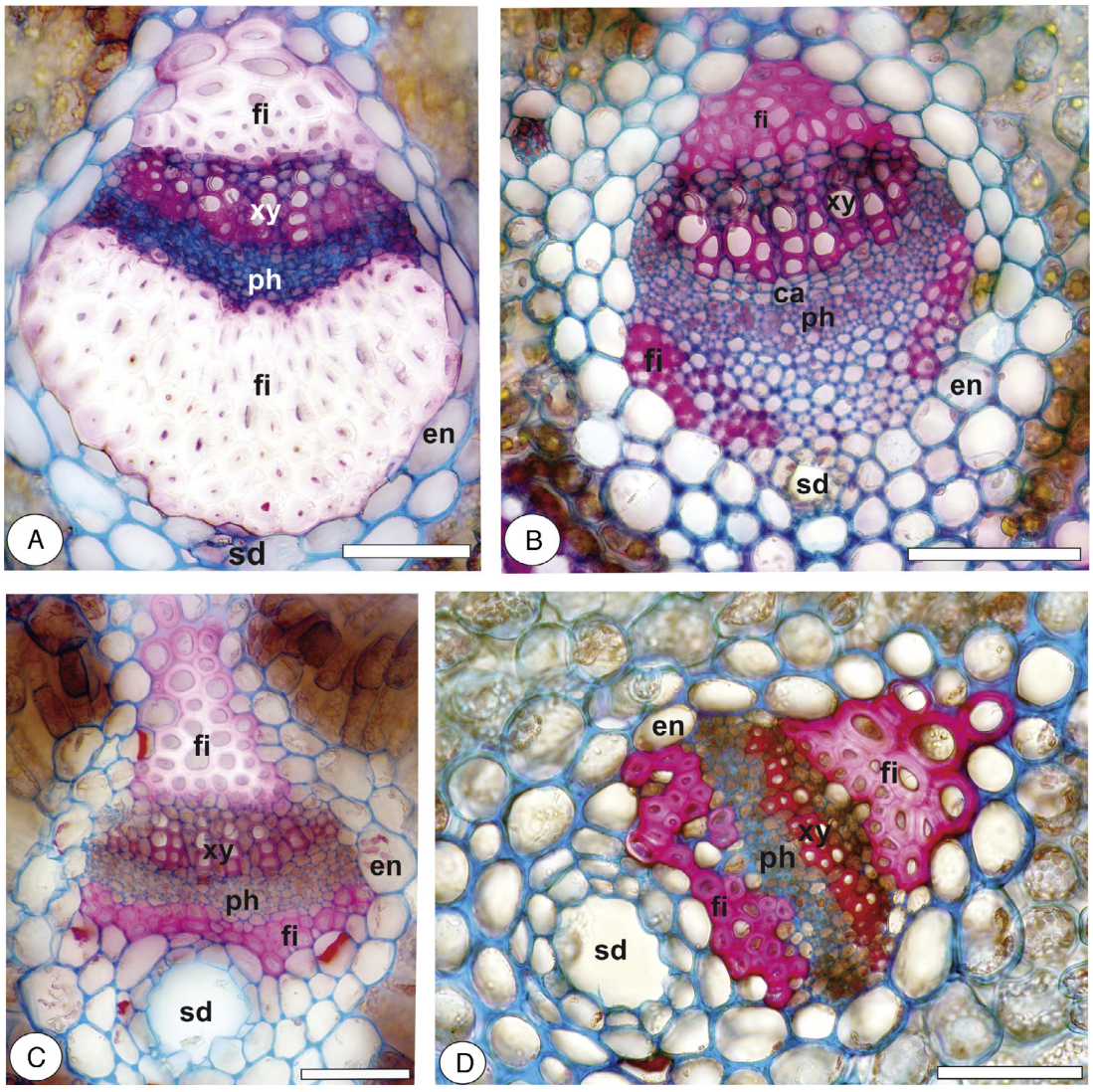

Fig. 3. (A) *Baccharis brevifolia* DC., (B) *Baccharis microdonta* DC., (C) *Baccharis paucifloscula* DC., (D) *Baccharis trilobata* A.S. Oliveira & Marchiori. Cross-section of the midribs, showing cambium (ca), collenchyma (co), endodermis (en), fibers (fi), phloem (ph), secretory duct (sd), stomatum (st), vascular bundle (vb), and xylem (xy). Scale bar = 50 μm.

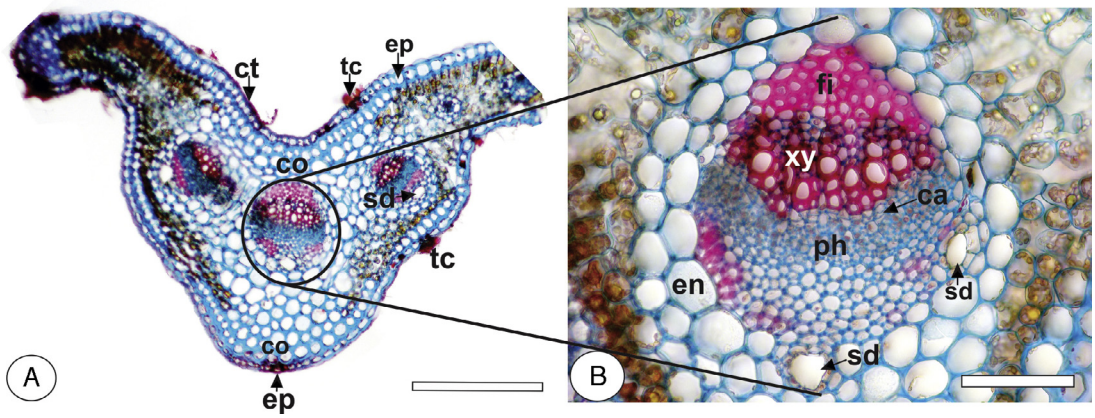

Fig. 4. *Baccharis microdonta* DC. Petiole in cross-section. (A) General appearance showing a vascular bundle (vb). (B) Detail of the vascular bundle, showing endodermis (en), fibers (fi), phloem (ph), secretory duct (sd), and xylem (xy). Scale bar = 200 μm (A), 50 μm (B).

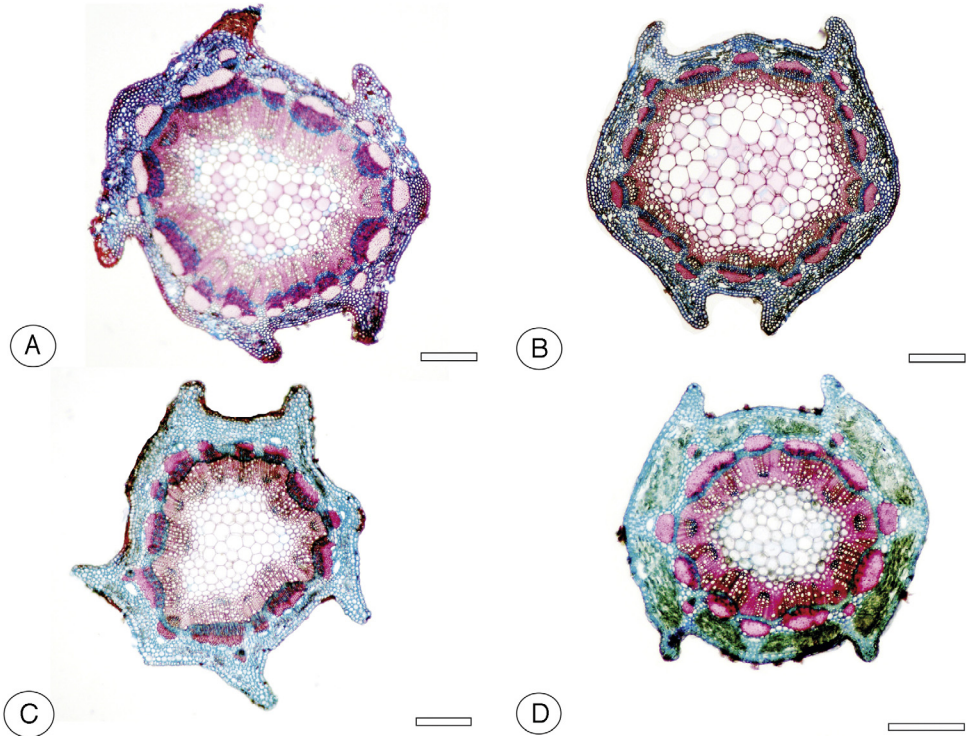

Fig. 5. (A) *Baccharis brevifolia* DC., (B) *Baccharis microdonta* DC., (C) *Baccharis pauciflosculosa* DC., (D) *Baccharis trilobata* A.S. Oliveira & Marchiori. General appearance of the stem in cross-section. Scale bar = 50 μm.

anisocytic stomata have been described for most species of *Baccharis* (Budel and Duarte, 2008a,b; Souza et al., 2011; Oliveira et al., 2011). However, other types of stomata have also been reported, such as cyclocytic (Budel et al., 2013), staurocytic, tetracytic (Freire et al., 2007) and actinocytic (Pereira et al., 2014).

In the present study, *B. brevifolia* shows hexacytic (Fig. 1E) and cyclocytic stomata (Fig. 1I), *B. microdonta* has signs of stauro-tetracytic and tetracytic stomata (Fig. 1F), *B. pauciflosculosa* shows tetracytic (Fig. 1G) and anomocytic stomata (Fig. 1K), and *B. trilobata* has stauro-tetracytic (Fig. 1H and L) and tetracytic stomata.

A single-layer epidermis in a cross-section covered by a thin cuticle, biseriate glandular trichome, flagelliform non-glandular trichome, isobilateral mesophyll, minor collateral vascular bundles surrounded by an endodermis, and secretory ducts, have been extensively described in *Baccharis* (Molares et al., 2009; Budel et al., 2012, 2015; Barreto et al., 2015; Bobek et al., 2015) and were found in all the studied species (Figs. 1M, P and Figs. 2A–D).

The midrib shape is a significant feature for differentiating species (Oliveira et al., 2011; Gogosz et al., 2012; Barreto et al., 2015; Wosch et al., 2015). In the present study this information was also used to differentiate *Baccharis* species. *B. brevifolia* has a flat-convex shape in the midrib (Fig. 2A), whereas *B. microdonta* has a concave–convex shape (Fig. 2B), *B. pauciflosculosa* has a biconvex shape (Fig. 2C) and in the case of *B. trilobata* the midrib is almost flat on both sides (Fig. 2D).

In the case of all the studied species, beneath the uniseriate epidermis there are 2–5 layers of angular collenchyma on both sides and there is a single collateral vascular bundle in the ground parenchyma tissue (Figs. 3A–D).

In this study, only the *B. microdonta* leaf has a petiole (Fig. 4A), which when viewed in cross-section, is concave-convex with three slight projections on the abaxial surface, the central part being more prominent. The epidermal coating has the same characteristics as those observed in the leaf. The collenchyma is an angular type and occurs in continuous strips of 1–2 sets of cells. There are three free collateral vascular bundles surrounded by the endodermis and in open arc organization (Fig. 4A) with secretory ducts in an external phloem position (Fig. 4B).

In transection, the stem of *B. brevifolia* (Fig. 5A) and *B. pauciflosculosa* (Fig. 5C) have an irregular shape with five conspicuous ribs. *B. microdonta* (Fig. 5B) and *B. trilobata* (Fig. 5D) have an almost hexagonal shape with four conspicuous ribs.

In this study, the stem epidermis is very similar to the leaf epidermis for these taxa, since it possesses stomata, a striated cuticle, and non-glandular or glandular trichomes (Fig. 6A and D) which can either appear isolated or in clusters. Angular collenchyma alternates with chlorenchyma beneath the epidermis occurs in all studied species (Fig. 6B–D) although a continuous stratum of collenchyma could be observed (Fig. 6A and B). This characteristic has been reported for other *Baccharis* species, such as *B. singularis* (Vell.) G.M. Barroso (Souza et al., 2011). One to five layers of angular collenchyma can be found for the four studied species, particularly in the ribs.

The endodermis bound the cortex internally with visible Casparian strips. Secretory ducts and perivascular fiber caps are found next to the phloem. Fiber is observed in the phloem. The xylem tracheary elements are arranged in rows in an orderly manner and separated by parenchyma cells and fibers (Fig. 6A–D). The pith consists of isodiametric thin-walled parenchymatic cells (Fig. 5A–D).

The type, presence or absence of crystals can be considered to be taxonomic characteristics (Meric, 2009). Calcium oxalate crystals are often found in the perimedullary region of the pith in *Baccharis* (Budel and Duarte, 2008b; Souza et al., 2011; Oliveira et al., 2011; Jasinski et al., 2014; Barreto et al., 2015; Bobek et al., 2015).

In this study, *B. brevifolia* shows crystal sand, styloids with pointed or square ends (Fig. 7A and B) and square dipyramids, *B. microdonta* has rare styloids with pointed ends (Fig. 7C), *B. pauciflosculosa* shows crystal sand and square dipyramids

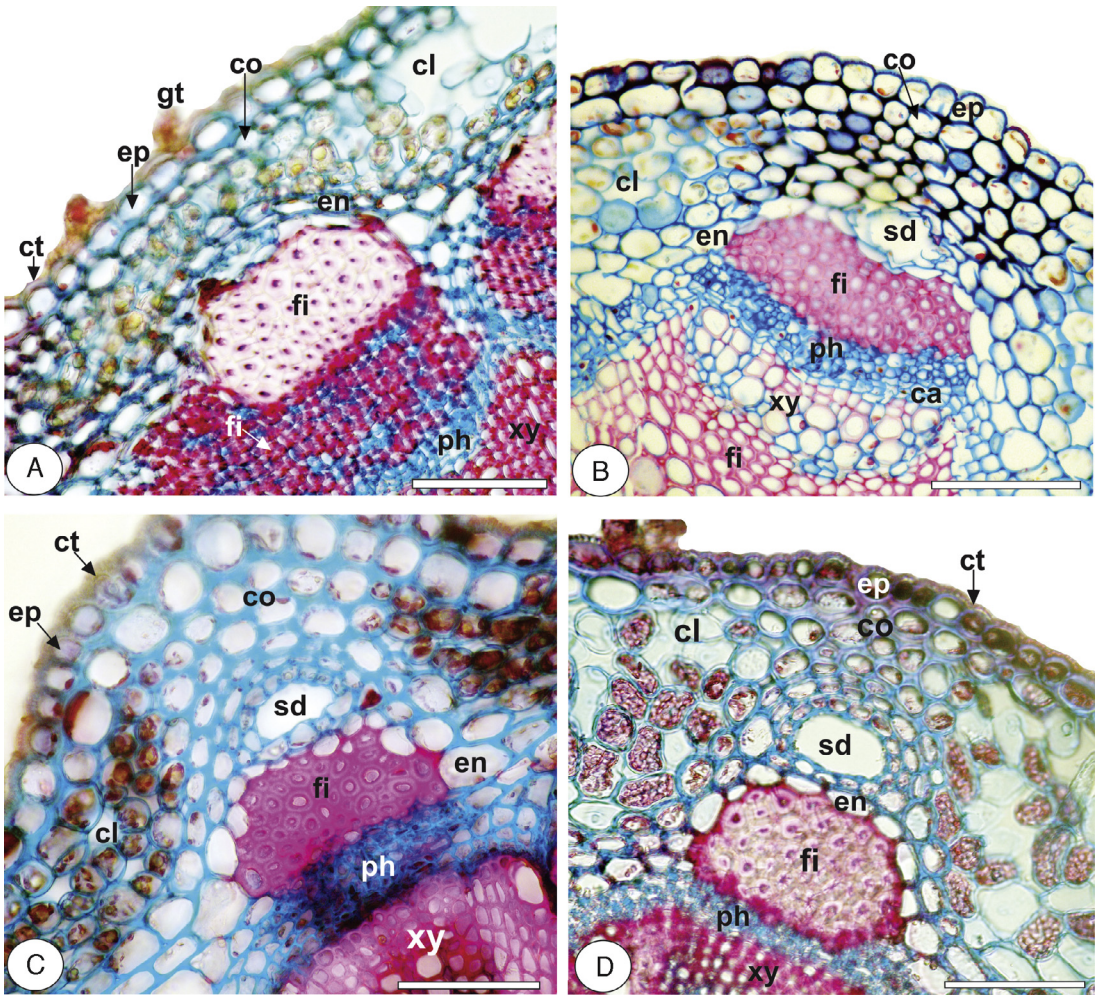

Fig. 6. (A) *Baccharis brevifolia* DC., (B) *Baccharis microdonta* DC., (C) *Baccharis paucifloscula* DC., (D) *Baccharis trilobata* A.S. Oliveira & Marchiori. Stem in cross-section, showing chlorenchyma (cl), collenchyma (co), cuticle (ct), epidermis (ep), endodermis (en), fibers (fi), glandular trichome (gt), phloem (ph), secretory duct (sd), and xylem (xy). Scale bar = 50 μm.

(Fig. 7D), and *B. trilobata* has signs of crystal sand, square dipyramids, elongated square dipyramids, styloids with pointed and square ends (Fig. 7E and F), as well as tabular crystals in piles that look like a tower (Fig. 7G). This type has not been mentioned before in the genus.

Anatomical analysis is an inherent procedure for nearly all pharmacopeias and is one of the main identification tests required for the herbal industry. Although the individual structural elements are relatively common within the same type of plant parts, the manner in which the elements are set gives a vegetable species its characteristic fingerprint (Upton et al., 2011).

In that sense, even though most of the anatomical features of the leaf and stem of *Baccharis* spp. are quite similar, several characteristics observed in this study support the need for a differentiation of the four studied species, as confirmed in Box 1. The occurrence and type of the stomata, midrib, stem and crystals shapes, and the presence of the petiole are recommended as good markers for a diagnosis of the species.

Box 1: Anatomical characteristics of *Baccharis brevifolia, B. microdonta, B. paucifloscula* and *B. trilobata*.

Anatomical characteristics	*B. brevifolia*	*B. microdonta*	*B. paucifloscula*	*B. trilobata*
Occurrence of stomata in the leaves	Amphistomatic	Hypostomatic	Amphistomatic	Amphistomatic
Types of stomata	Ciclocytic and hexacytic	Stauro-tetracytic and tetracytic	Anomocytic and tetracytic	Stauro-tetracytic and tetracytic
Shape of midrib shape	Flat-convex	Concave-convex	Biconvex	Flat on both sides
Presence of petiole	Absent	Present	Absent	Absent
Shape of stem	Irregular with six ribs	Hexagonal with four ribs	Irregular with six ribs	Hexagonal with four ribs
Types of crystals	Crystal sand, styloids with pointed or square ends and square dipyramids	Rare styloids with pointed ends	Crystal sand and square dipyramids	Square dipyramids, elongated square dipyramids, styloids with pointed and square ends, tabular crystals in piles

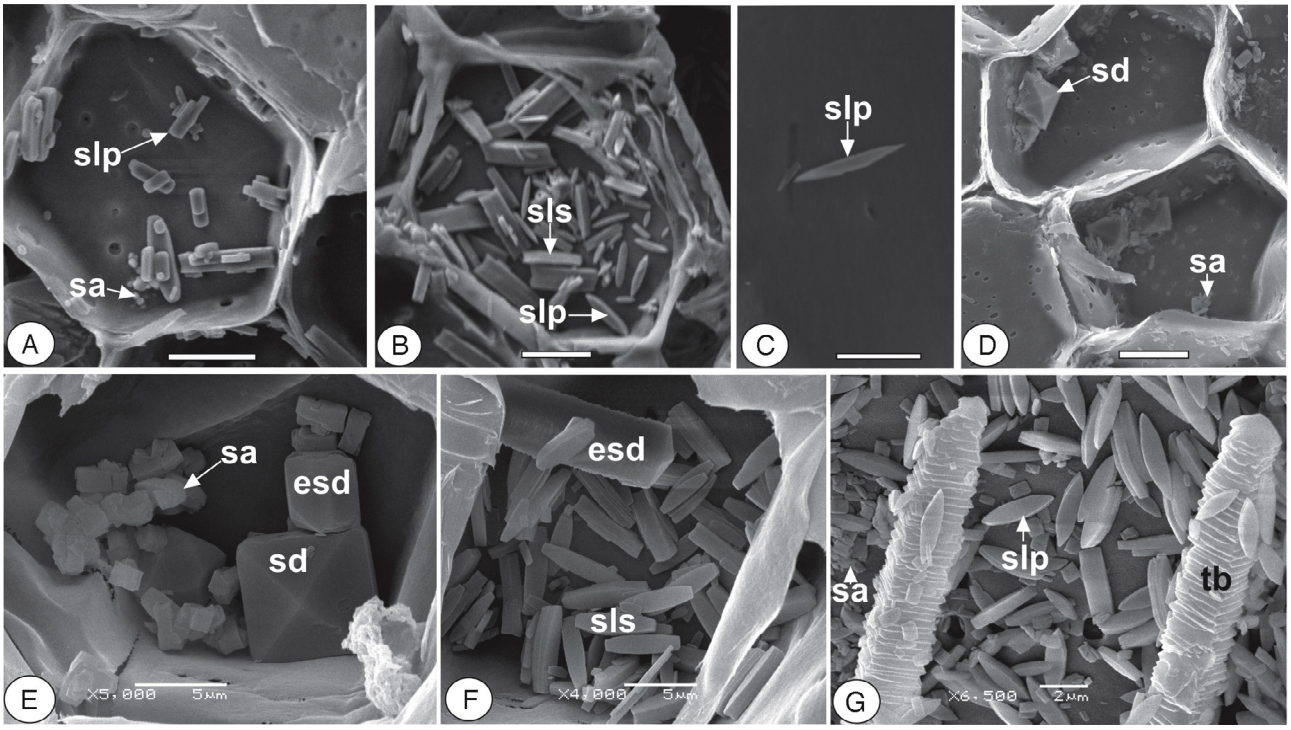

Fig. 7. (A, B) *Baccharis brevifolia* DC., (C) *Baccharis microdonta* DC., (D) *Baccharis paucifloscula* DC., (E, F, G) *Baccharis trilobata* A.S. Oliveira & Marchiori. Stem in cross-section, showing perimedullary region with crystals, sand (sa), stiloyds with pointed ends (slp), stiloyds with square ends (sls), square dipyramids (sd), elongated square dipyramids (esd), and tabular crystals in piles (tb). Scale bar = 10 µm (A, B, C, D).

Authors' contributions

VBB carried out the research. These results form a part of her MA course work. GH identified the plant samples and performed the voucher. VPA and CFO assisted by conducting the anatomical tests. JPP and PVF provided a critical reading of the manuscript. TN created the project and was responsible for arranging the scholarship. JMB created the project and supervised the laboratory work, as well as analyzing the data and providing a draft of the article. All the authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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