

Macro and Microscopic Characters of the Aerial Vegetative Organs of Carqueja: *Baccharis usterii* Heering

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ABSTRACT

This work aimed to study the macro and microscopic characters of the aerial vegetative organs of *Baccharis usterii* for quality control purposes. The plant was prepared for light and scanning electron microscopic analyses. The stem consisted of three-winged cladodes with small leaves. In the caulinar axis, there was a uniseriate epidermis, chlorenchyma alternating with angular collenchyma, perivascular fibre caps adjoining the phloem which was outside the xylem, and parenchymatic pith. The wings and the leaves were alike, presenting a uniseriate epidermis, palisade parenchyma next to the both sides of the epidermis and spongy parenchyma traversed by minor collateral vascular bundles in the central region. In the leaf midrib, a single collateral vascular bundle was embedded in the ground parenchyma. Secretory ducts and glandular trichomes occurred in the stem and leaf.

Key words: *Baccharis usterii*, Carqueja, Medicinal plant, Morpho-anatomy, Secretory ducts

INTRODUCTION

The genus *Baccharis* L. (Asteraceae) comprises about 500 species, distributed from the United States to Argentina, mainly in South America. Several members of the taxon are relevant as medicinal plants (Zardini, 1984; Verdi *et al.*, 2005), ornamentals (Carneiro & Fernandes, 1996) and source of essential oil for industry (Boldt, 1989; Agostini *et al.*, 2005). Some species which exhibit caulinar expansions forming cladodes are commonly known as carqueja and are considered diuretic and stomachic in Brazilian and Argentina folk medicine (Corrêa, 1984; Alonso, 1998; Castro & Ferreira, 2001). Pharmacological essays have demonstrated anti-ulcer (Baggio *et al.*, 2003), antispasmodic (Torres *et al.*, 2000; Weimann *et*

al., 2002), anti-inflammatory (Muschiatti *et al.*, 1998; Cifuentes *et al.*, 2001), antimicrobial (Cobos *et al.*, 2001; Feresin *et al.*, 2001; 2003) and anti-oxidant activities (Mongelli *et al.*, 1997; Heras *et al.*, 1998).

Baccharis trimera (Less.) DC. is claimed to be the most popular and investigated species, and it has been recently included in the Farmacopéia Brasileira IV (2003). It is a perennial shrub which may attain 1 m height and presents three-winged cladodes with no leaves or reduced ones, and is chemically evaluated in terms of flavonoid and essential oil contents. Similar in appearance, *Baccharis usterii* Heering (Fig. 1a) is a small shrub having three-winged cladodes, although it shows sparse leaves (Barroso, 1976; Barroso & Bueno, 2002; Farmacopéia Brasileira, 2003). It

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plays a relevant role in folk medicine, despite the lack of available information on this species.

Since *carquejas* are one of the most commercialized medicinal plants in Brazil (Correia-Júnior *et al.*, 1991) and their morpho-anatomical identification is required for quality control in phytotherapy industry, this work aimed to study the macro and microscopic characters of the aerial vegetative organs of *B. usterii*.

MATERIALS AND METHODS

The plant material was collected at the Fazenda São Maximiano, located in the region of Serra do Sudoeste, in the city of Guaíba, state of Rio Grande do Sul, Brazil (coordinates 30° 10' S and 51° 20' W, and 27 m high), in December 2003. The voucher was identified and registered as ICN53622 in the Herbarium at the Instituto de Ciências Naturais, Universidade Federal do Rio Grande do Sul.

Cladode fragments obtained 5 cm from the apex and adult leaves were collected from at least five specimens and fixed in FAA 70 (Johansen, 1940), which was replaced by 70 % ethanol (Berlyn & Miksche, 1976). The material was either sectioned by hand or dehydrated, embedded in glycol-methacrylate (Leica historesin[®]) and sectioned using the microtome Leica RM-2145. Transverse and longitudinal sections were stained with toluidine blue (O'Brien *et al.*, 1964) or astra blue and basic fuchsin combination (Roeser, 1972).

The following standard solutions were employed 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.*, 1991) and iodine-iodide for starch (Berlyn & Miksche, 1976). Photomicrographs were taken with the light microscope Olympus BX-40 equipped with the control unit PM-20.

For scanning electron analysis (SEM) (Souza, 1998), the fixed material was dehydrated in a graded ethanolic series and by CO₂ critical point apparatus Balzers CPD-030 and coated with gold by Balzers Sputtering SCD-030. Electron micrographs were taken with the scanning microscope Jeol JSM-6360LV.

RESULTS

The stem (Fig. 1a) achieved height of 1 m and consisted of cladodes with three expansions or wings. The axis was rigid and the wings were discontinuous and regularly distributed around it. In the cladode cross-section, the axis tended to be circular shaped and exhibited ribs between the wings (Figs. 1b, 2a).

In the wings, the epidermal cells were polygonal and had thick anticlinal walls with evident primary pits on surface view. The epidermis was uniseriate (Figs. 1c, 1d) and coated with a thin and slightly striate cuticle (Fig. 1e). Anomocytic stomata were located at the same level or nearly above the surrounding cells, and glandular trichomes were inserted in small epidermal depressions. They were non-capitate, multicellular and uniseriate (Figs. 1d, 1f) or capitate and biseriata (Fig. 1e). The chlorenchyma consisted of a palisade parenchyma, composed of approximately three strata of short cells below both sides of the epidermis, and a spongy parenchyma in the central region. Minor collateral vascular bundles encircled by a parenchymatic sheath traversed the spongy parenchyma (Fig. 1c).

Each bundle directed the xylem to the upper and lower side alternately. One or more secretory ducts, presenting uniseriate epithelium, dense cytoplasm, evident nucleus and lipophilic content, were found next to the parenchymatic sheath, in the phloem direction.

At the wing borders, there were several layers of angular collenchyma below the epidermis, a collateral vascular bundle with a perivascular fibre cap adjoining the phloem and secretory ducts (Fig. 1c). In the caulinar axis, the epidermis showed the same characteristics of the wings and, below it was the chlorenchyma alternate with angular collenchyma, which had about eight rows in the ribs (Fig. 2a). Secretory ducts, similar to the ones in the wings, was encountered next to the parenchymatic sheath that limited internally the cortex (Fig. 2b) and whose walls were impregnated with lipophilic compounds. The vascular cylinder presented cambia forming phloem outward and xylem inward (Fig. 2d), and perivascular fibre caps adjoined the phloem. The xylem was completely lignified.

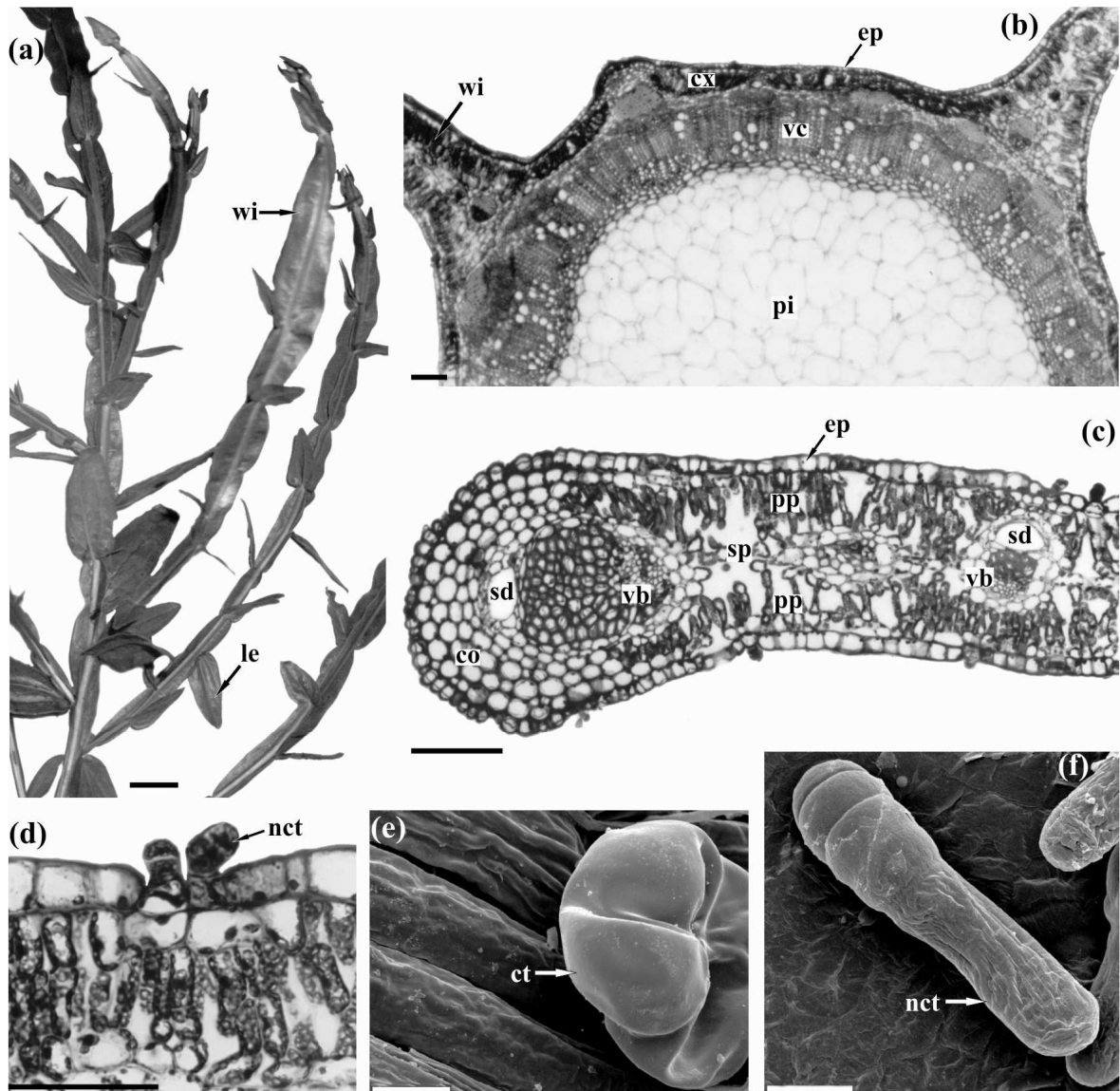


Figure 1 - *Baccharis usterii* Heering. (a) Aspect of aerial vegetative organs, showing cladodes and leaves; (b) cladode cross-section, exhibiting a rib between the wings; (c) wing cross-section near the border; (d) non-capitate glandular trichomes; (e) capitulate glandular trichome and striate cuticle (SEM); (f) non-capitate glandular trichome (SEM). co: collenchyma; ct: capitulate glandular trichome; cx: cortex; ep: epidermis; le: leaf; nct: non-capitate glandular trichome; pi: pith; pp: palisade parenchyma; sd: secretory duct; sp: spongy parenchyma; vb: vascular bundle; vc: vascular cylinder; wi: wing. Bar = 1 cm (a); 100 μ m (b), (c), (d); 10 μ m (e), (f).

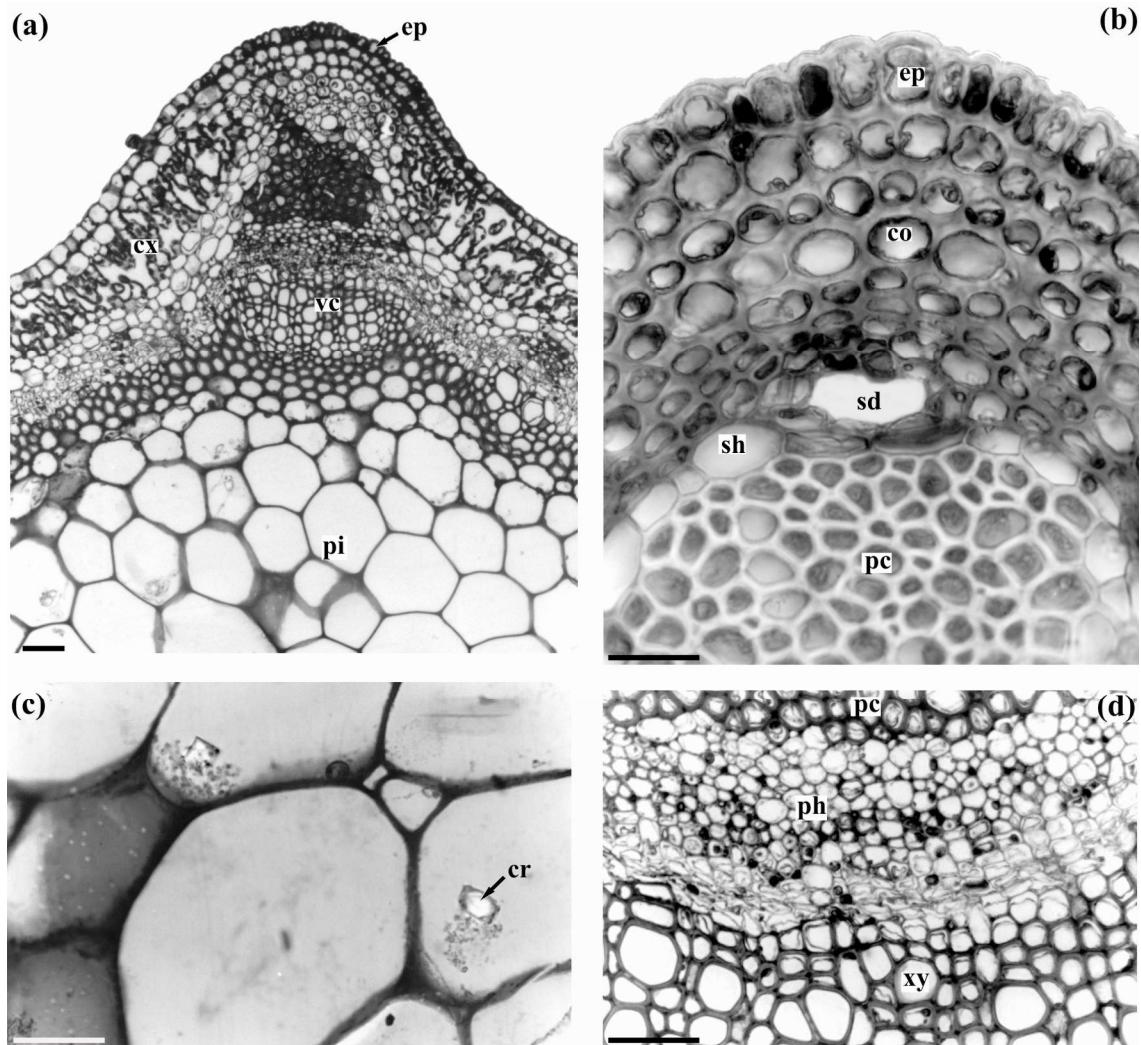


Figure 2 - Stem axis cross-section of *Baccharis usterii* Heering. (a) Organization of the rib region; (b) detail of the previous figure, showing angular collenchyma, secretory duct, parenchymatic sheath and perivascular fibre cap; (c) prisms of calcium oxalate in the pith; (d) phloem and xylem arrangement in the vascular cylinder. co: collenchyma; cr: crystal; cx: cortex; ep: epidermis; pc: perivascular fibre cap; ph: phloem; pi: pith; sd: secretory duct; sh: parenchymatic sheath; vc: vascular cylinder; xy: xylem. Bar = 50 μ m.

The pith occupied most part of the stem volume and was formed by relatively large parenchymatic cells with thin walls. Small prisms of calcium oxalate occurred in the perimedular region (Fig. 2c).

The leaf (Fig. 1a) had ovate shape, acute apex, cordate base and approximately 3 cm of length and 1.5 cm of width. It was sessile and exhibited three larger veins, being the midvein prominent on the abaxial side. The blade had a uniseriate epidermis (Fig. 3c), composed of polygonal-shaped cells

with moderately thick anticlinal walls (Figs. 3a, 3b), exhibiting primary pits on surface view. The dermal system was coated with a thin and slightly striate cuticle. Anomocytic stomata occurred on both sides (Figs. 3a, 3b) and they were even or slightly above the other epidermal cells (Fig. 3d). Glandular trichomes, similar to the cladode, were observed on both surfaces and placed in small depressions. The mesophyll was isobilateral, comprising a palisade parenchyma with three or four layers of relatively short cells below the

epidermis, and a spongy parenchyma with about two strata of cells forming small intercellular spaces in the central region (Fig. 3c). Collateral vascular bundles traversed the spongy parenchyma and were encircled by a parenchymatic sheath that was accompanied by secretory ducts in the phloem direction (Fig. 3d).

The midrib (Fig. 3e), in cross-section, was slightly convex on the adaxial side and convex on the opposite surface. The uniseriate epidermis was coated with a slightly striate cuticle and the

chlorenchyma was interrupted and replaced by various layers of angular collenchyma on both sides. A single vascular bundle, collateral and ovate shaped, was embedded in the ground parenchyma and had caps of perivascular fibres adjoining the xylem and phloem, at different stages of lignification. It was enclosed in a parenchymatic sheath and associated with secretory ducts nearby the phloem (Fig. 3f). These secretory structures showed an epithelium formed by four to ten cells arranged in one stratum.

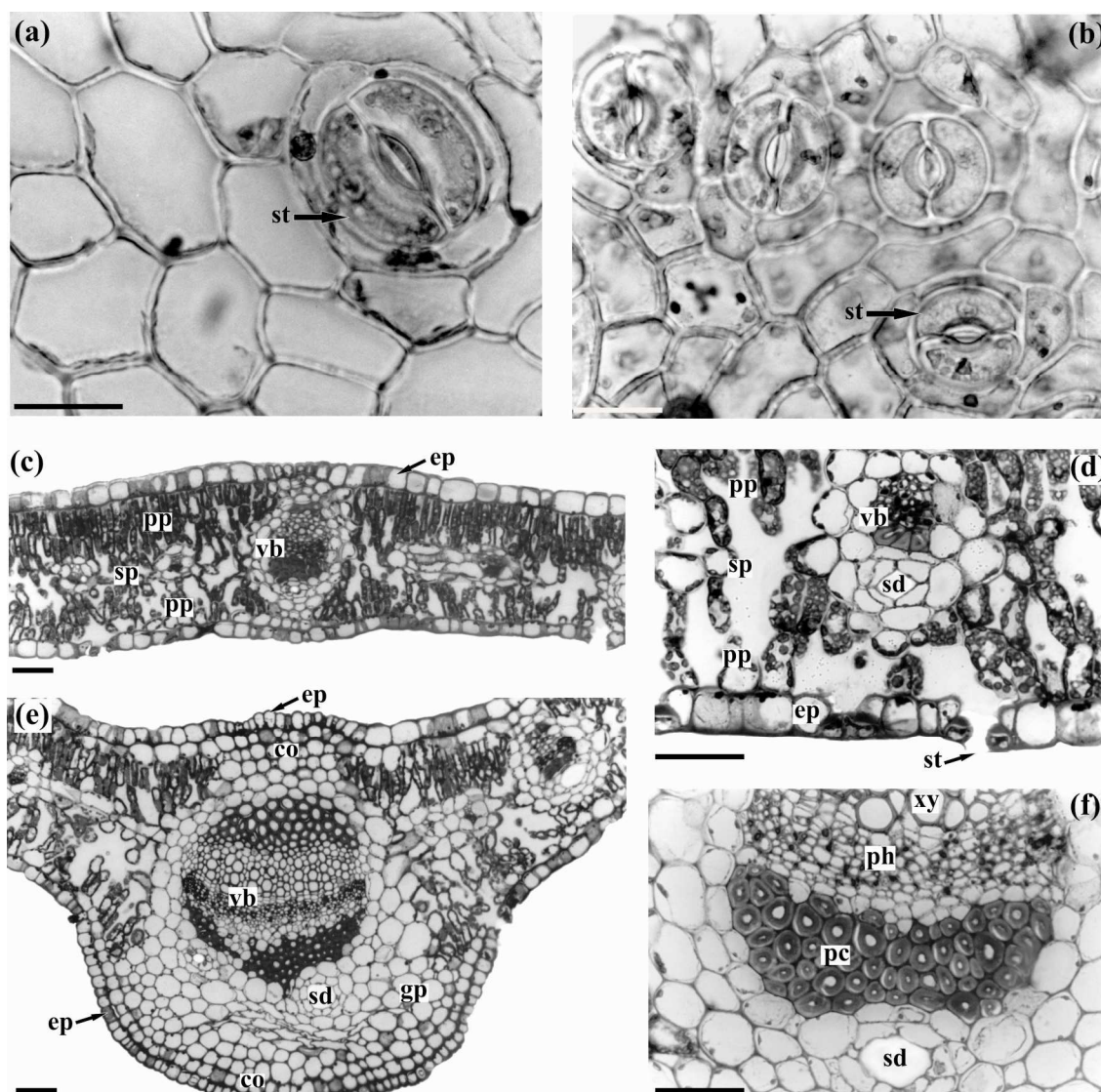


Figure 3 - Leaf cross-section of *Baccharis usterii* Heering. (a), (b) Adaxial and abaxial side of the epidermis, in surface view, respectively; (c), (d) blade organization, revealing isobilateral mesophyll; (e), (f) midrib traversed by a collateral vascular bundle associated with secretory ducts. co: collenchyma; ep: epidermis; gp: ground parenchyma; pc: perivascular fibre cap; ph: phloem; pp: palisade parenchyma; sd: secretory duct; sp: spongy parenchyma; st: stomatum; vb: vascular bundle; xy: xylem. Bar = 50µm.

DISCUSSION

Owing to the characteristic features of the cladodes, they are considered important for the diagnosis of vegetal drugs, such as carquejas (Oliveira *et al.*, 1991). The occurrence of these caulinar expansions has been mentioned in *Baccharis*, particularly in the *Trimeria* group which comprises shrubs with winged stems, whose expansions are continuous or discontinuous forming different sized segments (Barroso, 1991; Barroso & Bueno, 2002). Considering this group, *B. usterii* differs from *B. articulata* (Lam.) Pers. as the latter displays two-wings (Ariza-Espinar, 1973; Cortadi *et al.*, 1999; Ortins & Akisue, 2000), and from *B. stenocephala* Baker which presents both two and three expansions (Barroso & Bueno, 2002). Regarding the three-winged species *B. cylindrica* (Less.) DC. (Budel *et al.*, 2004a), *B. gaudichaudiana* DC. (Budel *et al.*, 2003a), *B. myriocephala* DC. (Sá & Neves, 1996) and *B. trimera* (Jorge *et al.*, 1991; Cortadi *et al.*, 1999; Farmacopéia Brasileira, 2003; Budel *et al.*, 2003b), *B. usterii* shows leaves that are lacking or inconspicuous in these species.

With reference to microscopic characters, the caulinar axis showing remaining epidermis, strands of chlorenchyma alternating with collenchyma, either endodermis or starch sheath limiting internally the cortex and vascular cambia forming phloem outward and xylem inward are common features of Asteraceae (Metcalf & Chalk, 1950) and described in *Baccharis* (Ariza-Espinar, 1973; Sá & Neves, 1996; Cortadi *et al.*, 1999; Ortins & Akisue, 2000). Additionally, included phloem may occur in the family (Metcalf & Chalk, 1950), as in *B. cylindrica* (Budel *et al.*, 2004a). These aspects were observed in this investigation, except for the phloem.

The occurrence of calcium oxalate crystals is frequent in plants, although their size and number are responsive to changes in the concentration of calcium in the environment (Nakata, 2003). These crystals have been reported in *B. articulata* (Ariza-Espinar, 1973; Cortadi *et al.*, 1999; Ortins & Akisue, 2000; Budel *et al.*, 2003b), *B. cylindrica* (Budel *et al.*, 2003b; Budel *et al.*, 2004a), *B. gaudichaudiana* (Budel *et al.*, 2003a; Budel *et al.*, 2003b), *B. myriocephala* (Sá & Neves, 1996) and *B. trimera* (Cortadi *et al.*, 1999; Farmacopéia Brasileira, 2003; Budel *et al.*, 2003b), as well as in *B. usterii*.

The cladode plays a photosynthetic role and the wings have microscopic characteristics similar to the leaf. For Metcalfe and Chalk (1988), the cuticle ornamentation may be relevant for diagnostic purposes. As found in the species studied, striate cuticle has been cited for the genus (Ariza-Espinar, 1973) and for *B. cylindrica* (Budel *et al.*, 2003b; Budel *et al.*, 2004a), *B. gaudichaudiana* (Budel *et al.*, 2003a; Budel *et al.*, 2003b), *B. retusa* (Silva & Grotta, 1971) and *B. trimera* (Cortadi *et al.*, 1999; Budel *et al.*, 2003b). In Asteraceae, the stomata may occur on both sides of the blade and they are anomocytic or anisocytic, being the former predominant (Metcalf & Chalk, 1950). These types have been cited for *Baccharis* (Ariza-Espinar, 1973) and in *B. articulata* (Ariza-Espinar, 1973; Cortadi *et al.*, 1999; Ortins & Akisue, 2000; Budel *et al.*, 2003b), *B. cylindrica* (Budel *et al.*, 2003b; Budel *et al.*, 2004a), *B. gaudichaudiana* (Budel *et al.*, 2003a; Budel *et al.*, 2003b), *B. retusa* (Silva & Grotta, 1971) and *B. trimera* (Budel *et al.*, 2003b; Alquini & Takemori, 2000). Concerning *B. myriocephala*, beside these two types, tetracytic stomata have also been described (Sá & Neves, 1996). Accordingly, *B. usterii* shows anomocytic stomata. Seen as epidermal appendages of microscopic diagnostic value, glandular and non-glandular trichomes are referred to *Baccharis* for Metcalfe & Chalk (1988) and Ariza-Espinar (1973). In this work, multicellular capitate and non-capitate glandular trichomes were encountered in the leaf and cladode. Similar trichomes have been reported in *B. articulata* (Ariza-Espinar, 1973; Cortadi *et al.*, 1999; Ortins & Akisue, 2000; Budel *et al.*, 2003b), *B. mesoneura* DC. (Barroso, 1976), *B. myriocephala* (Sá & Neves, 1996) and *B. trimera* (Cortadi *et al.*, 1999; Farmacopéia Brasileira, 2003; Budel *et al.*, 2003b). As regards as non-glandular trichomes, they are often reported in different species of *Baccharis*, e.g., *B. anomala* DC. (Barroso, 1976), *B. artemisioides* Hook. et Arn., *B. lilloi* Heering, *B. pulchella* Sch. Bip., *B. rupestris* Heering, *B. salicifolia* (Ruiz et Pav.) Pers. (Ariza-Espinar, 1973) and *B. trimera* (Farmacopéia Brasileira, 2003). These appendages have in common being multicellular (two to eight cells), uniseriate and with a relatively long to whip-like apical cell (Ariza-Espinar, 1973). Nevertheless, non-glandular trichomes are not observed in *B. usterii*.

In the family, the chlorenchyma organization

varies (Metcalf & Chalk, 1950) and the isobilateral mesophyll described in the blade of *B. usterii* by Barroso (1976) was confirmed in this investigation and also mentioned in *B. dracunculifolia* (Oliveira & Bastos, 1998; Budel *et al.*, 2004b) and *B. retusa* (Silva & Grotta, 1971). This organization has correspondence to the chlorenchyma arrangement in the wings of *B. articulata* (Ariza-Espinar, 1973; Cortadi *et al.*, 1999; Ortins & Akisue, 2000; Budel *et al.*, 2003b), *B. cylindrica* (Budel *et al.*, 2004a); Budel *et al.*, 2003b), *B. gaudichaudiana* (Budel *et al.*, 2003a; Budel *et al.*, 2003b) and *B. myriocephala* (Sá & Neves, 1996). However, contradictory information on *B. trimeria* reveals dorsiventral mesophyll (Farmacopéia Brasileira, 2003) or homogeneous mesophyll either with palisade parenchyma (Cortadi *et al.*, 1999) or spongy parenchyma (Jorge *et al.*, 1991; Chicourel *et al.*, 1997). As observed in this study and well registered in various species of *Baccharis*, the vascular bundles were collateral and there was an association with them and secretory ducts (Silva & Grotta, 1971; Ariza-Espinar, 1973; Sá & Neves, 1996; Oliveira & Bastos, 1998; Cortadi *et al.*, 1999; Ortins & Akisue, 2000; Budel *et al.*, 2003a; Budel *et al.*, 2004a). According to Pagni and Masini (1999), in many members of Asteraceae, the ducts were usually related to the endodermis, were next to the phloem, the secretory structures were associated with this conducting system.

CONCLUSIONS

As expected, the macro and microscopic aspects of the aerial vegetative organs of *B. usterii* agreed with the *Baccharis* genus. These characters should be evaluated as a whole to characterize the species, although the following structures might be emphasized as distinguishing features among the *Trimeria* group: three-winged cladode accompanied by leaves, striate cuticle, anomocytic stomata, multicellular capitate and non-capitate glandular trichomes, isobilateral mesophyll and secretory ducts in the phloem direction.

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RESUMO

Caracteres macro e microscópicos dos órgãos vegetativos aéreos de carqueja: *Baccharis usterii* Heering. Este trabalho objetivou estudar os caracteres macro e microscópicos dos órgãos vegetativos aéreos de *Baccharis usterii*, com aplicação no controle de qualidade. O material botânico foi preparado de acordo com técnicas para microscopia óptica e de varredura. O caule consistiu de cladódios triados, nos quais se inserem pequenas folhas. O eixo caulinar apresentou epiderme unisseriada, clorênquima em alternância com colênquima angular, calotas de fibras perivasculares apostas ao floema, o qual se forma externamente ao xilema, e medula parenquimática. As alas e as folhas mostraram-se semelhantes, apresentando epiderme unisseriada, parênquima paliçádico junto a ambas as faces epidérmicas e parênquima esponjoso na região central, atravessado por feixes vasculares de pequeno porte. Na nervura central da folha, um feixe vascular colateral encontrou-se mergulhado no parênquima fundamental. Dutos secretores e tricomas glandulares ocorreram no caule e na folha

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