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Leaf Anatomical Characters of *Stachys byzantina* C. Koch, Lamiaceae

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Abstract

Stachys byzantina C. Koch (Lamiaceae) is a perennial herb with elliptic lanceolate and densely pilose leaves. It is employed in Brazilian traditional medicine as analgesic, mainly to treat headaches. This work has analysed the foliar anatomical characters, aiming to contribute to the species identification. The botanical material was fixed, freehand sectioned or embedded in glycol methacrylate and sectioned by microtome, stained either with astra blue and basic fuchsine or toluidine blue. Histochemical tests and ultrastructure analysis were also conducted. The blade has uniseriate epidermis coated with a thin and slightly striate cuticle, anomocytic and diacytic stomata on both surfaces and dorsiventral mesophyll. The midrib and petiole are concave-convex and traversed by collateral vascular bundles. Numerous glandular and non-glandular trichomes are seen. The latter is long, pluricellular and uniseriate, with an acute apex. The glandular trichomes are peltate and capitate and may have uni- or pluricellular head and stalk.

Key words Anatomy, Lamiaceae, Leaf, *Stachys byzantina*, Trichomes

Introduction

The Lamiaceae family comprises 258 genera and 6970 species (Judd *et al.*, 1999), distributed worldwide, especially in the Mediterranean region and

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eastward into central Asia (Cronquist, 1981). Among the major genera, it is included *Stachys* L., which belongs to Lamiaceae and whose members are edible (Judd *et al.*, 1999) and ornamental (Lorenzi and Souza, 1995). Different studies on various species have evaluated the chemical composition of volatile oils (Maly, 1985; Skaltsa *et al.*, 2001) and their antimicrobial activity (Skaltsa *et al.*, 2003; Stamatis *et al.*, 2003). They have also reported diterpenoids (Fazio *et al.*, 1992; 1994), iridoid glycosides (Calis *et al.*, 1992) and flavonoids (El-Ansari *et al.*, 1991; Lenherr and Mabry, 1987; Meremeti *et al.*, 2004) and investigated anti-inflammatory (Maleki *et al.*, 2001) and anxiolytic effects (Rabbani *et al.*, 2003).

In Brazilian traditional medicine, the leaves of *Stachys byzantina* C. Koch (Fig. 1) are employed as analgesic, mainly to treat headaches. This species is a perennial herb, with elliptic-lanceolate and densely pilose leaves (Lorenzi and Souza, 1995). This work has aimed to analyse the anatomical characters of the leaf of *S. byzantina*, in order to contribute to the species identification for pharmacognostic purposes.

Materials and Methods

The botanical material was collected from cultivated species on the outskirts of the city of Curitiba, state of Paraná, in Brazil (approximately 25° 26'S and 49° 14'W, 900m height), in April 2004. The voucher was registered at the Museu Botânico Municipal de Curitiba - PR as MBM302668. Mature



Fig. 1. *Stachys byzantina* C. Koch, Lamiaceae-aspect of vegetative organs.

leaves were fixed in FAA 70 (Johansen, 1940) and kept in 70 % ethanol solution (Berlyn and Miksche, 1976). The lower third of the blade and the petiole were freehand sectioned or embedded in glycol methacrylate and sectioned by the microtome Spencer 820 and stained either with toluidine blue (O'Brien *et al.*, 1965) or astra blue and basic fuchsine (Roeser, 1962). Histochemical tests were applied with iodine-iodide to reveal starch (Berlyn and Miksche, 1976), Sudan IV to lipophilic substances (Foster, 1949), ferric chloride to phenolic compounds (Johansen, 1940) and hydrochloric phloroglucin to lignin (Sass, 1951). The results were registered by the light microscope Olympus BX40 attached to the control unit PM20.

For the ultrastructure study (Souza, 1998), the leaves fixed in FAA 70 were dehydrated in a graded ethanolic series and by the critical point procedure (Bal-Tec CPD-030), coated with gold (Balzers SCD-030) and analysed by the scanning electron microscope Jeol JSM-6360LV.

Results

The blade has a uniseriate epidermis (Fig. 2), whose cells show sinuous shape in surface view and are coated with a thin and slightly striate cuticle (Figs. 4, 5). Anomocytic and diacytic stomata occur on both sides and the guard-cells exhibit an outer ledge and are slightly raised (Fig. 6). Numerous glandular and non-glandular trichomes are seen. The latter (Fig. 3) is long, pluricellular, often comprising three to six cells aligned in one rank and ending in an acute apex. The glandular trichomes are distinctly varied (Figs. 2, 5-11), being capitate and peltate. The capitate ones may be short or long, with uni- or pluricellular stalk and uni- or pluricellular round head. The peltate trichomes present a short stalk and a pluricellular ovoid head. The mesophyll is dorsiventral, consisting of one or two layers of palisade parenchyma and four to six strata of spongy parenchyma, corresponding to 50 % of the chlorenchyma (Figs. 2, 11).

The midrib, in transection, is concave on the adaxial surface and prominently convex in acute angle on the opposite side (Figs. 12, 13). The single-layered epidermis has polygonal anticlinal cell walls and numerous glandular and non-glandular trichomes similarly to the blade. A strand of lamellar collenchyma occurs below the epidermis and one to four collateral vascular bundles are embedded in the ground parenchyma. They are distributed as an open arc and the central one is encircled by a starch sheath. The cambial zone is evident and the tracheary elements are aligned in rows and separated by parenchymatic cells (Fig. 14).

The petiole is concave-convex with two lateral projections or small wings. The epidermis has the same characteristics of the midrib and an angular collenchyma is seen in the wings. Approximately three collateral vascular bundles, forming an open arc, traverse the ground parenchyma. The central bundle is major and encircled by a starch sheath.

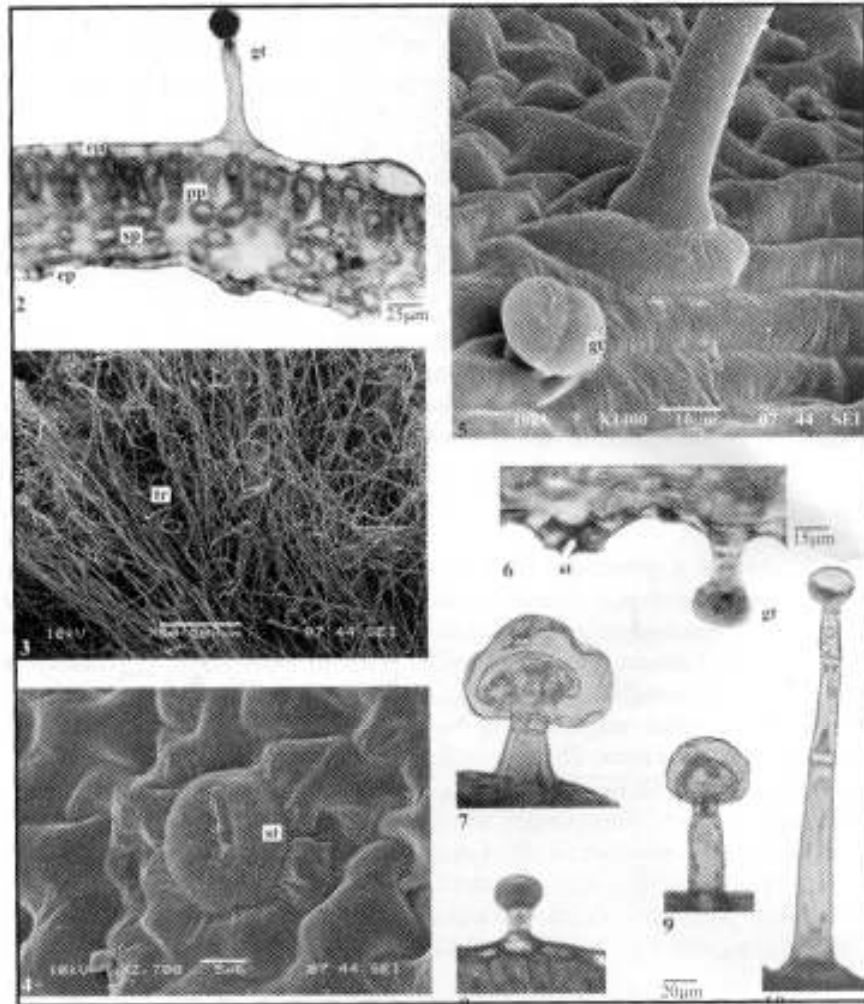


Fig. 2-10. *S. byzantina*-leaf, 2. transection of the blade, 3. non-glandular trichomes, 4. stomatum in surface view, 5. glandular trichome and striate cuticle, 6. stomatum in transection and glandular trichome, 7-10. glandular trichomes. ep-epidermis, gt-glandular trichome, pp-palisade parenchyma, sp-spongy parenchyma, st- stomatum, tr-non-glandular trichome.

Discussion

Several species of Lamiaceae are densely covered with trichomes and the occurrence together of diverse kinds of non-glandular and short-stalked glands with uni- or multicellular heads is characteristic of the whole family (Cronquist, 1981; Metcalfe and Chalk, 1950). These appendages are considered important for systematic comparisons (Metcalfe and Chalk, 1988)

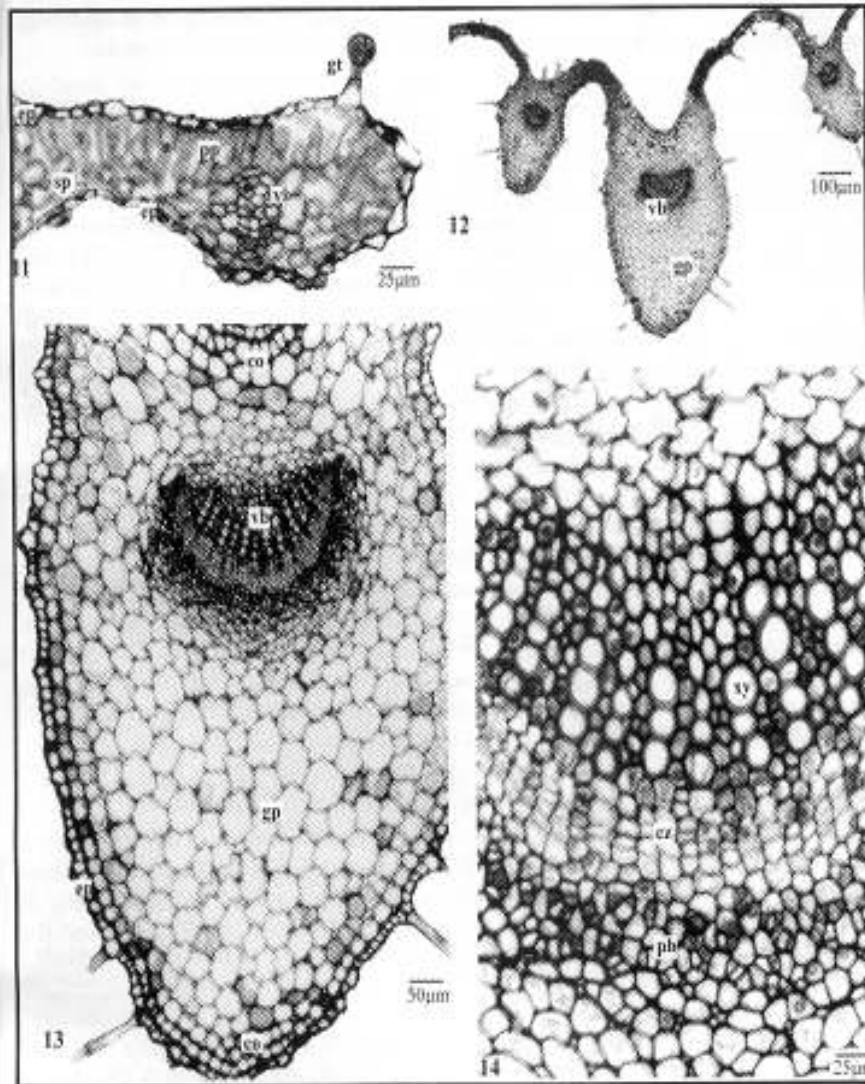


Fig. 11-14. *S. byzantina*-leaf: 11. transection of the margin, 12. midrib and lateral veins, in cross section, 13. midrib organization, 14. detail of the vascular bundle of the midrib. co-collenchyma, cz-cambial zone, ep-epidermis, gp-ground parenchyma, gt-glandular trichome, ph-phloem, pp-palisade parenchyma, sp-spongy parenchyma, vb-vascular bundle, xy-xylem.

and the ones described in this work have been reported for the genus *Stachys*, despite *S. byzantina* having not shown the branched multicellular trichomes mentioned in *Stachys affinis* Bunge (Metcalf and Chalk, 1950). Comparatively to other species, *Plectranthus madagascariensis* (Pers.)

Benth. (Ascensão *et al.*, 1998) and *Plectranthus ornatus* Codd (Ascensão *et al.*, 1999) have exhibited similar trichomes in vegetative organs.

From a functional point of view, glandular trichomes produce essential oils which attract pollinators in reproductive parts and protect against herbivores and pathogens (Werker, 1993). These compounds are released in large amounts as soon as the glandular trichomes are ruptured by herbivore feeding, movement on the plant surface or the growth of microorganisms (Wittstock and Gershenzon, 2002). Regarding the densely ornamented epidermis, beside acting as a mechanical barrier and interfering with the attachment to plants by phytophagous insects (Eigenbrode, 2004), non-glandular trichomes may contain relatively high concentrations of flavonoids and play a protective role against ultraviolet radiation (Hollósy, 2002).

In Lamiaceae members, the epidermal cell walls in surface view are generally sinuous, the stomata are often restricted to the lower epidermal side and are frequently diacytic, although the anomocytic type has been also reported (Metcalf and Chalk, 1950). These anatomical characters are coincident with the present findings, except for the stomata occurrence, since *S. byzantina* has shown amphistomatic leaves.

Isobilateral, centric and dorsiventral mesophyll have been usually cited in the family (Metcalf and Chalk, 1950; 1988) and the latter pattern has been observed in this species. Additionally, the midrib and petiole organization described herein have been reported for the genus *Stachys* and subsidiary vascular bundles in the petiolar wings are present in species of Lamiaceae (Metcalf and Chalk, 1950).

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