



# Stem and leaf anatomy of *Plectranthus neochilus* Schltr., Lamiaceae

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**RESUMO:** “Anatomia caulinar e foliar de *Plectranthus neochilus* Schltr., Lamiaceae”. *Plectranthus neochilus* Schltr. é uma erva aromática denominada boldo ou “boldo-gambá” e empregada no tratamento de insuficiência hepática e dispepsia na medicina popular. Este trabalho investigou a anatomia caulinar e foliar, com a finalidade de contribuir na identificação dessa planta medicinal. O material botânico foi preparado de acordo com microtécnicas usuais. O caule tem secção transversal quadrangular e, em crescimento secundário no nível analisado, exibe epiderme unisseriada e numerosos tricomas. Os tricomas glandulares são capitados e peltados. Os primeiros têm pedicelo curto e unicelular ou longo e pluricelular e cabeça uni ou bicelular. Os peltados apresentam pedicelo curto e cabeça ovóide com oito células. Os tricomas tectores são pluricelulares, unisseriados e recobertos com cutícula granulosa. Observam-se colênquima angular, câmbio vascular formando floema em direção centrífuga e xilema para o centro, e calotas de fibras perivasculares apostas ao floema. A lâmina foliar tem epiderme uniestratificada revestida por cutícula estriada, estômatos diaclíticos em ambas as superfícies, numerosos tricomas similares aos do caule e mesófilo homogêneo. A nervura central possui um ou dois feixes vasculares colaterais e o pecíolo apresenta vários feixes colaterais em arco aberto.

**Unitermos:** *Plectranthus neochilus*, Lamiaceae, anatomia, boldo-gambá, tricomas.

**ABSTRACT:** *Plectranthus neochilus* Schltr. is an aromatic herb named “boldo” or “boldo-gambá” and employed for treating hepatic insufficiency and dyspepsia in folk medicine. This paper has investigated its stem and leaf anatomy, in order to contribute for the medicinal plant identification. The botanical material was prepared according to standard microtechniques. The stem has quadrangular transection and, in secondary growth at the level analyzed, shows uniseriate epidermis and numerous trichomes. The glandular ones are capitate and peltate. The former has short unicellular or long multicellular stalk and uni- or bicellular head. The latter presents short stalk and eight-celled ovoid head. The non-glandular trichomes are multicellular, uniseriate and coated with granular cuticle. It is observed angular collenchyma, cambium forming phloem outward and xylem inward, and perivascular fiber caps next to the phloem. The blade has uniseriate epidermis coated with striate cuticle, diacytic stomata on both surfaces, numerous trichomes similar to the stem ones, and homogeneous mesophyll. The midrib shows one or two collateral bundles and the petiole has many of them distributed as an open arc.

**Keywords:** *Plectranthus neochilus*, Lamiaceae, anatomy, “boldo-gambá”, trichomes.

## INTRODUCTION

The family Lamiaceae Lindl. consists of 258 cosmopolitan genera (Judd et al., 1999), mostly distributed in the Mediterranean region and eastward into Central Asia (Cronquist, 1981). Considered one of the largest genera, *Plectranthus* L'Hér. includes ornamental (Judd et al., 1999), edible (Temple et al., 1991; Allemann; Hammes, 2003; Allemann et al., 2003) and medicinal species, e.g. *Plectranthus amboinicus* (Lour.) Spreng. (Ruiz et al., 1996) and *Plectranthus barbatus* Andrews (Matu; Van Staden, 2003; Pereira et al., 2004; Morais et al., 2005; Tôrres et al., 2005;

Vendruscolo et al., 2005; Silva et al., 2006; Oliveira et al., 2007a).

Among the medicinal species of the genus, *Plectranthus neochilus* Schltr. is an aromatic herb employed in folk medicine for treating hepatic insufficiency and dyspepsia and commonly called “boldo” or “boldo-gambá” in Brazil. Its fresh leaves have a distinct odour and are taken as infusion or aqueous extract for healing purposes (Lorenzi; Matos, 2002).

Various works have been carried out to identify the chemical constituents of the genus (Vera et al., 1993; Liu; Ruedi, 1996; Ngassoum et al., 2001; Abdel-Mogib

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et al., 2002; Kerntopf et al., 2002; Mendes et al., 2006), and to investigate their antioxidant (Burgi; Ruedi, 1993; Juch; Ruedi, 1997; Narukawa et al., 2001), antimicrobial (Rabe; Van Staden, 1998; Zollo et al., 1998; Mathpal et al., 2002; Rijo et al., 2002; Gaspar-Marques et al., 2006; Oliveira et al., 2007b) and pharmacological properties (Buznego; Perez-Saad, 1999; Cerqueira et al., 2004; Uawonggul et al., 2006). An extensive review of ethnobotanical uses of *Plectranthus* has been presented by Likhoba et al. (2006). Since few studies are devoted to the morphological characterization, this paper has investigated the stem and leaf anatomy of *P. neochilus* (Figure 1), in order to contribute for the medicinal plant identification and for distinguishing it from other species commonly named boldo, such as “boldo-brasileiro” (*P. barbatus* Andrews), “boldo-japonês” (*Vernonia condensata* Baker, Asteraceae) (Lolis; Milaneze-Gutierrez, 2003), and boldo-do-chile (*Peumus boldus* Molina, Monimiaceae).

## MATERIAL AND METHODS

The botanical material was collected at the Horto de Plantas Mediciniais, Departamento de Farmácia, Universidade Federal do Paraná, in Curitiba-PR (coordinates of 25°26'45" S and 49°14'25" W, altitude of 930 m), in March 2003. The voucher specimen was identified and registered as MBM 219584 in the herbarium at the Museu Botânico Municipal de Curitiba.

Stem fragments cut at 5 cm from the caulinar apex (approximately at the 6<sup>th</sup> node) and fully developed leaves were fixed in FAA 50 (Johansen, 1940) and kept in 50% ethanol solution (Berlyn; Miksche, 1976). The examined foliar region was the lower half of the blade, including the midrib, and the petiole. Transverse and longitudinal freehand sections were stained either with astra blue and basic fuchsin (Roeser, 1972) or toluidine blue (O'Brien et al., 1964). Microchemical reactions were applied with hydrochloric phloroglucin to reveal lignified elements (Sass, 1951), iodine-iodide to starch (Berlyn; Miksche, 1976), Sudan IV to lipophilic substances (Foster, 1949) and ferric chloride to phenolic compounds (Johansen, 1940). The photographs were taken by the light microscope Olympus BX40 attached to the control unit PM20.

## RESULTS

*Plectranthus neochilus* has a quadrangular stem in transection, and it reveals an incipient secondary growth at the level analyzed (Figure 2). The epidermis is uniseriate (Figure 3), consisting of polygonal cells in shape in front view, being wider tangentially than radially. It remains as the dermal system, although the phellogen has been established (Figure 3). Many glandular and non-glandular trichomes are seen (Figures 3, 5-8). The

former varies, being capitate and peltate. The capitate trichomes are numerous and show short unicellular or long multicellular stalk and uni- or bicellular head (Figures 3, 5). The peltate trichomes are scarce and slightly sunken in the caulinar epidermis, presenting short stalk and eight-celled ovoid head (Figure 6). The secretion of glandular trichomes is lipophilic and cuticle detachments can be seen on the head. The non-glandular trichomes (Figure 7) are single, multicellular and uniseriate, consisting of about three to ten cells, with an acute apex and eventually a stalk cell dehydrated. These trichomes are coated with a granular cuticle (Figure 8).

In the cortex, below the phellogen, a continuous strand of collenchyma is found (Figures 2, 3). It is angular and comprises three or four rows. The multilayered cortical parenchyma has chloroplasts and shows small intercellular spaces. The innermost boundary of the cortex is represented by a single layer of large parenchymatic cells, whose tangential and radial walls are impregnated with lipophilic substances. The vascular cambia form xylem inward and phloem outward, being active mainly in the fascicular region and toward the xylem, although collateral bundles can be distinguished (Figure 2). Caps of perivascular fibers are next to the phloem and all the secondary xylem cells are lignified (Figure 4). The pith consists of thin walled parenchymatic cells, comparatively greater, containing many amyloplasts and forming small intercellular spaces (Figure 2).

The blade, in surface view, has epidermal cells with sinuous contour, more evident on the abaxial surface (Figures 9, 10). In transection, the single-layered epidermis is covered with a thin and striate cuticle. There are glandular and non-glandular trichomes similarly to the stem ones on both surfaces. The epidermal cells which surround the trichome bases (two to eight cells) have slightly polygonal shape and detachment areas are distinguished. Diacytic stomata with unequally sized subsidiary cells occur on the lamina, predominantly on the abaxial side (Figures 9, 10), and they are slightly raised above the other epidermal cells (Figure 11). The inner and outer periclinal cell walls of the guard cells are thicker and the cuticle forms ledges on the outer side.

The chlorenchyma comprises about twelve layers of nearly isodiametric cells, progressively smaller to the abaxial side (Figure 12). Minor collateral bundles are encountered in the middle of the homogeneous mesophyll. They are collateral and encircled by a parenchymatic sheath.

The midrib transection shows a plain-convex shape (Figure 12). Below the uniseriate epidermis is the collenchyma which is angular and consists of about two rows. Embedded in the ground parenchyma, there are one or two collateral vascular bundles (Figures 12, 13). The tracheary elements are distributed orderly in ranks isolated by parenchymatic cells and the phloem is reduced (Figure 14).

The petiole (Figure 15) has nearly plain-convex contour in transection and the epidermis shows the same characteristics of the blade. A continuous strand of angular collenchyma, formed by two or three rows (Figure 16), encircles the ground parenchyma, in which various vascular bundles are embedded. They are collateral and distributed as an open arc, where the two major bundles are seen in the centre (Figures 15, 17).

## DISCUSSION

The caulinar secondary growth of *P. neochilus* is of the ordinary type, as observed in many herbaceous dicotyledons (Esau, 1977). The rectangular transection is frequently described for Lamiaceae (Metcalf; Chalk, 1950; Barroso, 1991), as well as the evident collenchyma in the four angles (Cronquist, 1981), which is considered of diagnostic value, according to Metcalf; Chalk (1950). In the studied species, the stem reveals quadrangular cross-section, however the arrangement of the collenchyma is not restricted to the angles.

The epidermis remaining as the dermal system, despite the formation of the phellogen below it, has been recorded for herbaceous species during early secondary growth (Esau, 1977) and the first phellogen often arises soon after the vascular cambium (Mauseth, 1988). Comparing with the related species *P. barbatus*, commonly called "boldo-brasileiro", the establishment of the phellogen occurs just below the epidermis and it becomes active, producing phellem outward (Scavone, 1965).

As regard as the innermost layer of the cortex whose walls are impregnated with lipophilic substances, Mauseth (1988) has stated that a thin layer of hydrophobic matrix can permeate all primary walls of the endodermis, and the presence of Casparian strips is more characteristic in roots than stems. Accordingly, in *P. barbatus* stem, the endodermis is not typical and it contains plenty of amyloplasts (Scavone, 1965).

The occurrence of wide interfascicular regions with distinguishable collateral bundles has been mentioned for herbaceous dicotyledons (Esau, 1977). The vascular system organization of *P. neochilus* resembles of *P. barbatus* (Scavone, 1965) and of the related genus *Coleus* Lour., whose xylem and phloem are predominantly formed in the fascicular regions (Esau, 1977).

Concerning the leaf anatomy, the family exhibits predominantly diacytic stomata on both surfaces (Metcalf; Chalk, 1950). *Plectranthus neochilus* shows this pattern, unlike the reported anomocytic stomata for *P. barbatus* (Table 1) (Scavone, 1965) or *Plectranthus australis* R.Br., in which an alternative type has been described. According to Metcalf and Chalk (1988), this complex is named diallelocytic, consisting of three or more C-shaped cells of graded sizes at the right angles of the guard cells.

Trichomes are considered relevant in comparative systematic investigations and morpho-diagnosis (Metcalf; Chalk, 1988). The occurrence together of diverse kinds of glandular and non-glandular trichomes is characteristic of Lamiaceae (Metcalf; Chalk, 1950). The peltate trichomes, which have a uniform morphology, as well as the other types of capitate glandular and non-glandular trichomes have been described for the family (Metcalf; Chalk, 1950; Werker, 1993) and *Plectranthus madagascariensis* (Pers.) Benth. (Ascensão et al., 1998), *Plectranthus ornatus* Codd (Ascensão et al., 1999) and *P. barbatus* (Scavone, 1965). Moreover, based on Ascensão et al. (1999), the peltate trichomes of *P. ornatus* are confined to the abaxial leaf surface, differing from *P. madagascariensis* (Ascensão et al., 1998) and from the present findings, since these species have exhibited them on both surfaces. As Werker (1993) mentioned, considering a functional viewpoint, glandular trichomes produce essential oils which apparently protect against herbivores and pathogens, as well as attract pollinators in floral parts. Similarly, the histochemical results on the secretion of *P. ornatus* indicate that peltate trichomes produce essential oils, resiniferous acids and flavonoid aglycones, and capitate trichomes also contain polysaccharides (Ascensão et al., 1999).

With reference to the chlorenchyma organization, isobilateral, dorsiventral or centric mesophyll have been frequently reported for Lamiaceae (Metcalf; Chalk, 1950), as the dorsiventral type mentioned in *P. barbatus* (Scavone, 1965). Nevertheless, homogeneous mesophyll occurs in *P. neochilus* (Table 1).

In comparison with other species called boldo which belong to different botanical families (Table 1), there are monographs of boldo-do-chile (*Peumus boldus* Molina, Monimiaceae) in different pharmacopoeias which describes its leaves as showing deciduous non-glandular trichomes, unicellular and united at a prominent base forming a star-shaped or stellate type, anomocytic stomata found exclusively on the abaxial surface, nearly polygonal epidermal cells in surface view, hypoderm next to the adaxial side, secretory cells distributed in the dorsiventral mesophyll and concave-convex midrib in cross-section (Ph. Helv. VII, 1991; BHP, 1996; F. Bras. IV, 1996). In addition, *Vernonia condensata* Baker, Asteraceae, popularly known as "boldo-japonês", has leaves with tetracytic or anomocytic stomata on both epidermal surfaces, two-armed non-glandular trichomes consisting of various cells in one rank and a T-shaped long apical one, capitate glandular trichomes, multicellular and inserted in an evident depression, prisms and druses of calcium oxalate, dorsiventral mesophyll and a biconvex midrib (Barreto et al., 1994).

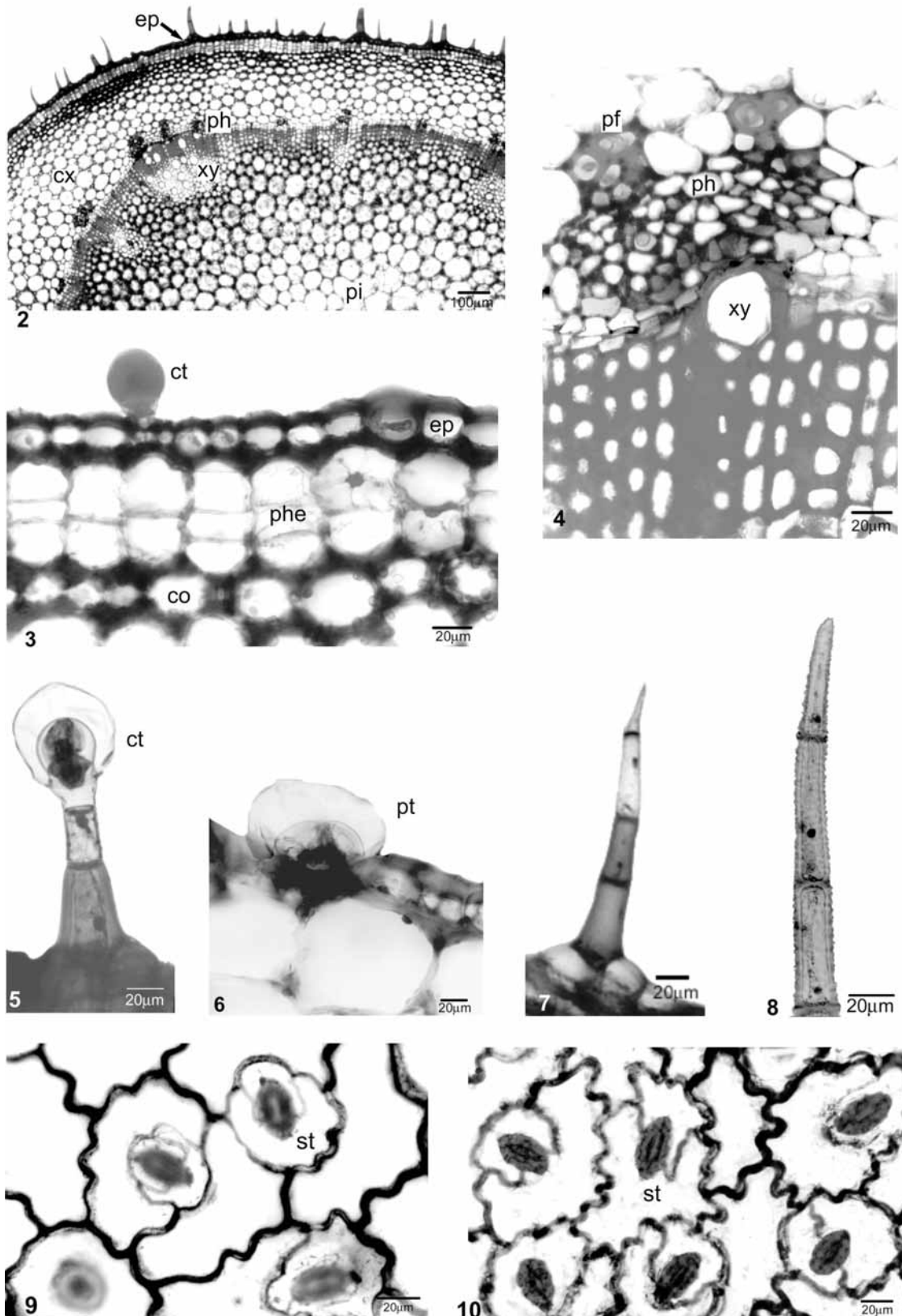
**Table 1.** Comparative anatomical characters of leaves of species named “boldo”: *Plectranthus neochilus*, *Plectranthus barbatus*, *Peumus boldus* and *Vernonia condensata*.

Leaf anatomical characters	<i>P. neochilus</i>	<i>P. barbatus</i> (Scavone, 1965)	<i>P. boldus</i> (F. Bras IV)	<i>V. condensata</i> (Barreto et al., 1994)
Epidermal cell shape (fv)	sinuous	polygonal	polygonal	polygonal <sup>ad</sup> sinuous <sup>ab</sup>
Stomatum type	diacytic	anomocytic	anomocytic	anomocytic tetracytic
Stomatum occurrence	ad / ab	ad / ab	ab	ad / ab
Trichome type	capitate gt peltate gt ngt	capitate gt peltate gt ngt	stellate ngt	capitate gt T-shaped ngt
Mesophyll	homogeneous	dorsiventral	dorsiventral hypoderm <sup>ad</sup> secretory cells	dorsiventral
Midrib	plain-convex 1-2 vb	biconvex various vb in ring	concave-convex 1 vb in open arc	biconvex 3-5 vb in open arc
Calcium oxalate crystals	-	rare druses	rare raphides	druses prisms

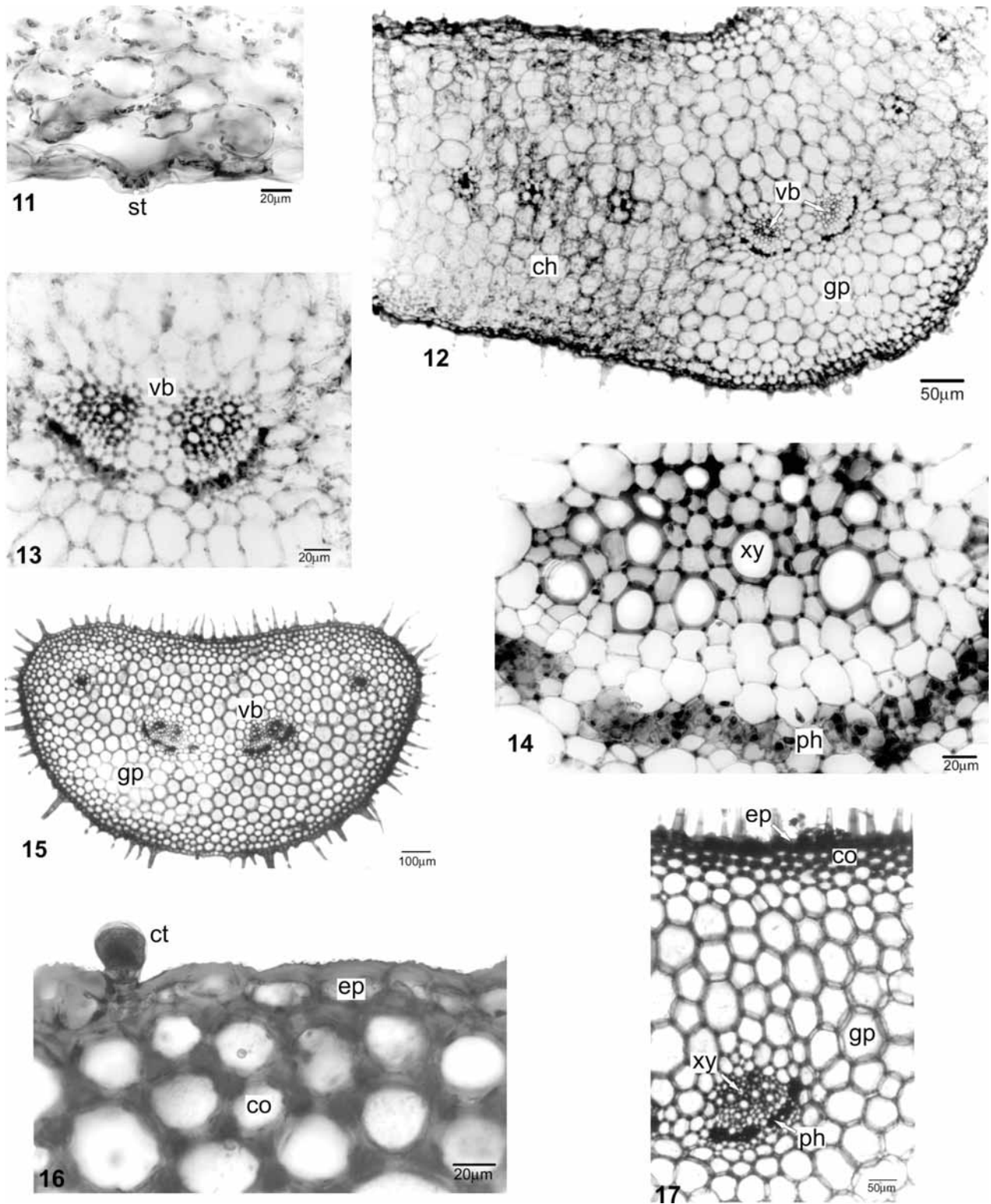
ab – abaxial surface of the epidermis, ad – adaxial surface of the epidermis, fv – front view, gt – glandular trichomes, ngt – non-glandular trichomes, vb – vascular bundle.



**Figure 1.** *Plectranthus neochilus* Schltr., Lamiaceae - aspect of a vegetative branch.



**Figures 2-10.** *P. neochilus* - 2-8 - Stem: 2 - transection of the stem in secondary growth; 3 - detail of the caulinar dermal system and collenchyma; 4 - phloem and xylem; 5 - capitulate trichome; 6 - peltate trichome; 7 - non-glandular trichome; 8 - granular cuticle covering the non-glandular trichome; 9-10 - Blade - surface view of the adaxial and abaxial epidermal cells, respectively. co - collenchyma, cp - capitulate trichome, cx - cortex, ep - epidermis, pf - perivascular fibers, ph - phloem, phe - phellogen, pi - pith, pt - peltate trichome, st - stomatum, xy - xylem.



**Figures 11-17.** *P. neochilus* - Leaf: 11 - stomatum slightly above the other epidermal cells; 12 - blade and midrib in transection; 13-14 - details of the collateral bundles in the midrib; 15 - petiole transection; 16 - epidermis and collenchyma of the petiole; 17 - collateral bundle embedded in the ground parenchyma. ch - chlorenchyma, co - collenchyma, ct - capitate trichome, ep - epidermis, gp - ground parenchyma, ph - phloem, st - stomatum, vb - vascular bundle, xy - xylem.

## CONCLUSION

This investigation shows that the assembled anatomical characters of *P. neochilus* contribute for this medicinal species identification. Although the stem organization of *P. neochilus* is similar to *P. barbatus*, the leaf characters are distinctive. To sum up, *P. neochilus* and *P. barbatus* can be distinguished since the latter has leaves with polygonal epidermal cells in surface view, anomocytic stomata, comparatively more trichomes and dorsiventral mesophyll. Besides, *P. boldus* differs from *P. neochilus* for exhibiting hypostomatic leaf, stellate trichomes, hypoderm, secretory cells and dorsiventral mesophyll. This chlorenchyma organization also occurs in *V. condensata* leaf, as well as tetracytic or anomocytic stomata, T-shaped non-glandular trichomes and calcium oxalate crystals.

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