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A N I N T E R N A T I O N A L J O U R N A L

Anatomical Characters of Leave and Stem of *Calea serrata* Less., Asteraceae

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ABSTRACT

The genus Calea *belongs to the tribe Heliantheae and presents about 125 species.* Calea serrata*, popularly known as erva-de-cobra, chá-amargo and quebra-tudo, is an endemic species found in southern Brazil and is used in traditional medicine to treat ulcers and livers problems. The present work aimed to study the pharmacobotanical characters of leaves and stems of* C. serrata *for quality control purposes. The plant material was processed according to standard methods of light and scanning electron microscopy. Glandular capitate-stalked and capitate-sessile, uniseriate multicellular non-glandular trichome with tapered apical cell, conical non-glandular trichome, isobilateral mesophyll, secretory ducts near the endoderm and circular shape with six ribs in the stem were important characters, which contributed to the identification of the species.*

Key words: *Calea serrata*, anatomical study, quality control

INTRODUCTION

The family Asteraceae, also known as Compositae, is considered one of the largest among the Angiosperms. It presents about 1100 genera and approximately 25000 species, which have been highlighted by great therapeutic potential (Bremer 1994). The genus *Calea* L. belongs to the tribe Heliantheae and presents about 125 species that inhabit in tropical and subtropical regions of the New World, and 85 of them are found in Brazil, mainly in the states of Distrito Federal, Bahia, Minas Gerais, Rio de Janeiro, São Paulo and Paraná. Representatives of *Calea* are often mistaken with the species of *Aspilia* and *Wedelia* (Roque and Carvalho 2011).

Calea species has been chemically studied for the isolation and characterization of compounds, such as *Calea uniflora* Less. (Nascimento et al. 2002; Nascimento et al. 2004), *C. clematidea* Baker (Flach et al. 2002), *C. urticifolia* (Mill.) DC. (Yamada et al. 2004) and *C. zacatechichi* Schltdl. (Wu et al. 2011). Pharmacological activities have been demonstrated for the species of the genus, such as antispasmodic (Kohler et al. 2002), antifungal (Flach et al. 2002), anti-hypertensive (Guerrero et al. 2002), trypanocidal (Nascimento et al. 2002; Nascimento et al. 2004), cytotoxic (Nakagawa et al. 2005) and leishmanicidal (Wu et al. 2011).

Calea serrata, popularly known as erva-de-cobra, chá-amargo and quebra-tudo, is an endemic species found in Southern Brazil (Ribeiro et al.

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2008). It is a woody vine that presents simple, opposite, and serrated margin leaves and ligulate and yellowish flowers. It is used in traditional medicine to treat the ulcers and liver problems. Pharmacological studies have shown acaricidal activities (Ribeiro et al. 2008; Ribeiro et al. 2011) and inhibition of acetylcholinesterase (Ribeiro et al. 2012).

Morpho-anatomical studies were conducted with species of *Calea*. However, no work has been carried out to investigate the anatomical data of *C. serrata* in order to provide additional features for differentiating this species from other *Calea*. Thus, the present work aimed to study the anatomical characters of leaves and stems of *C. serrata* for quality control purposes.

MATERIAL AND METHODS

Botanical material

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 30º 10´ S and 51º 20´ W, and 27 m high) in December 2010. The voucher was identified and its equivalent was registered under number ICN 51057 at the Herbário do Instituto de Ciências Naturais from Universidade Federal do Rio Grande do Sul.

Morpho-anatomical Study

Stems and leaves 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 and 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. 1965) or astra blue and basic fuchsine combination (Roeser 1962).

Micro-chemical Tests

The following standard solutions were employed for micro-chemical tests: hydrochloric phloroglucin to reveal lignin (Sass 1951), Sudan III for lipophilic compounds (Foster 1949), ferric chloride for phenolic substances (Johansen 1940), and iodine-iodide for starch (Berlyn and Miksche 1976). Photomicrographs were taken with the light microscope Olympus CX 31 coupled to the camera C7070.

Scanning Electron Analysis (SEM)

The fixed material was dehydrated in a graded ethanol series and by CO_2 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 AND DISCUSSION

The foliar blade of *C. serrata* Less. (Fig. 1A), in face view, showed sinuous shape in the epidermal cells and relatively thin anticlinal cell walls on both the sides (Figs. 1B, C). In cross-section, the epidermis was uniseriate and coated by a thin and smooth cuticle (Fig. 2B) on both the surfaces. The leaves of *C. serrata* were hypostomatic. The stomata were mainly anomocytic (Fig. 1B) or anisocytic and were located even or slightly above regarding the other epidermal cells.

Both non-glandular and glandular trichomes could occur singly and often were located in small epidermal depressions. There were two types of the glandular trichomes. One of them was capitate-stalked with uniseriate stalk, which consisted of 3-4 cells. The other one was capitate-sessile (Fig. 1E). They were covered by a cuticle with dense cytoplasm. Considering micro-chemical tests the glandular trichomes reacted with Sudam III. There were two types of non-glandular trichomes. One of them was conical (Fig. 1D) and consisted of 6-8 cells. The other one was multicellular (Figs. 2A, C) uniseriate and composed by 5-6 cells at the base and tapered apical cell.

The leaves of *C. serrata* presented dorsiventral mesophyll and comprised palisade parenchyma composed by a layer beneath the epidermis. The spongy parenchyma showed 4-5 layers with some small intercellular spaces (Figs. 2C, D). Collateral vascular bundles were embedded in the chlorenchyma and were surrounded by the endodermal cells with visible Casparian strips. Near the endoderm, secretory ducts could occur, lined with a uniseriate epithelium whose 4-6 cells released a lipophilic product. The midrib transection had biconvex shape. Below the uniseriate epidermis, there was the angular collenchyma composed by 1-2 rows. A single collateral vascular bundle occurred in the ground parenchyma. Secretory ducts had the same characteristics previously described.

In cross-section, the petiole was concave adaxially and convex abaxially. The angular collenchyma comprised 1-2 layers. Vascular system was formed by three free vascular bundles in open arc with lateral rib traces (Fig 2E). Secretory ducts as reported for leaf blade were observed next to the endoderm (Fig. 2G). Starch grains were observed in the fundamental parenchyma (Fig. 2F).

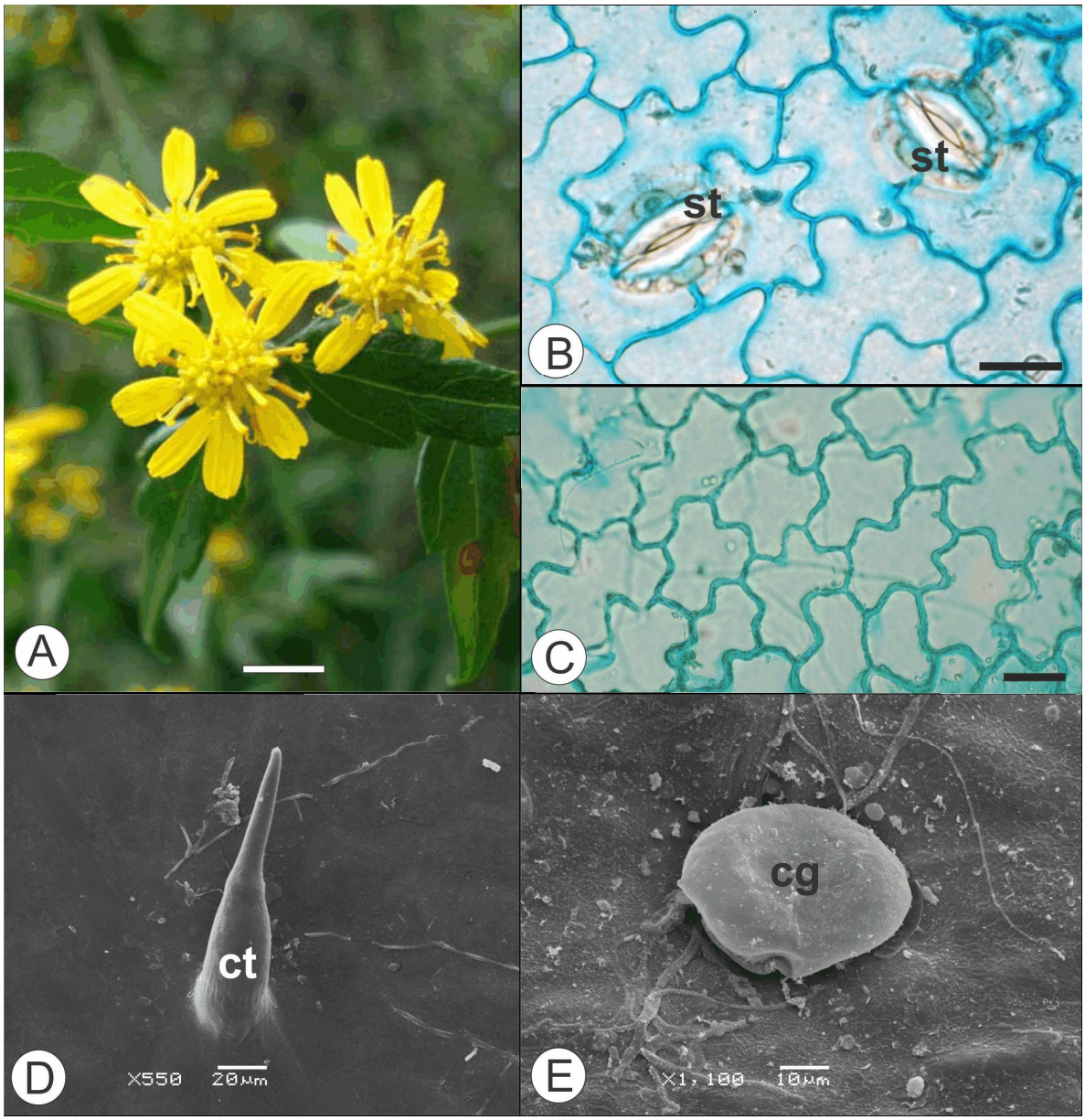

Figure 1 - *Calea serrata* Less., Asteraceae. A. Aspect of aerial vegetative and reproductive organs. B. Abaxial side of the epidermis, in surface view, revealing anticlinal epidermal cell walls and stomata (st). C. Adaxial side of the epidermis, in surface view, showing anticlinal epidermal cell walls. D. Conical non-glandular trichome (ct), in surface view. E. Capitate-sessile glandular trichome (ct). Bar = 2 cm (A); 20 µm (B, C).

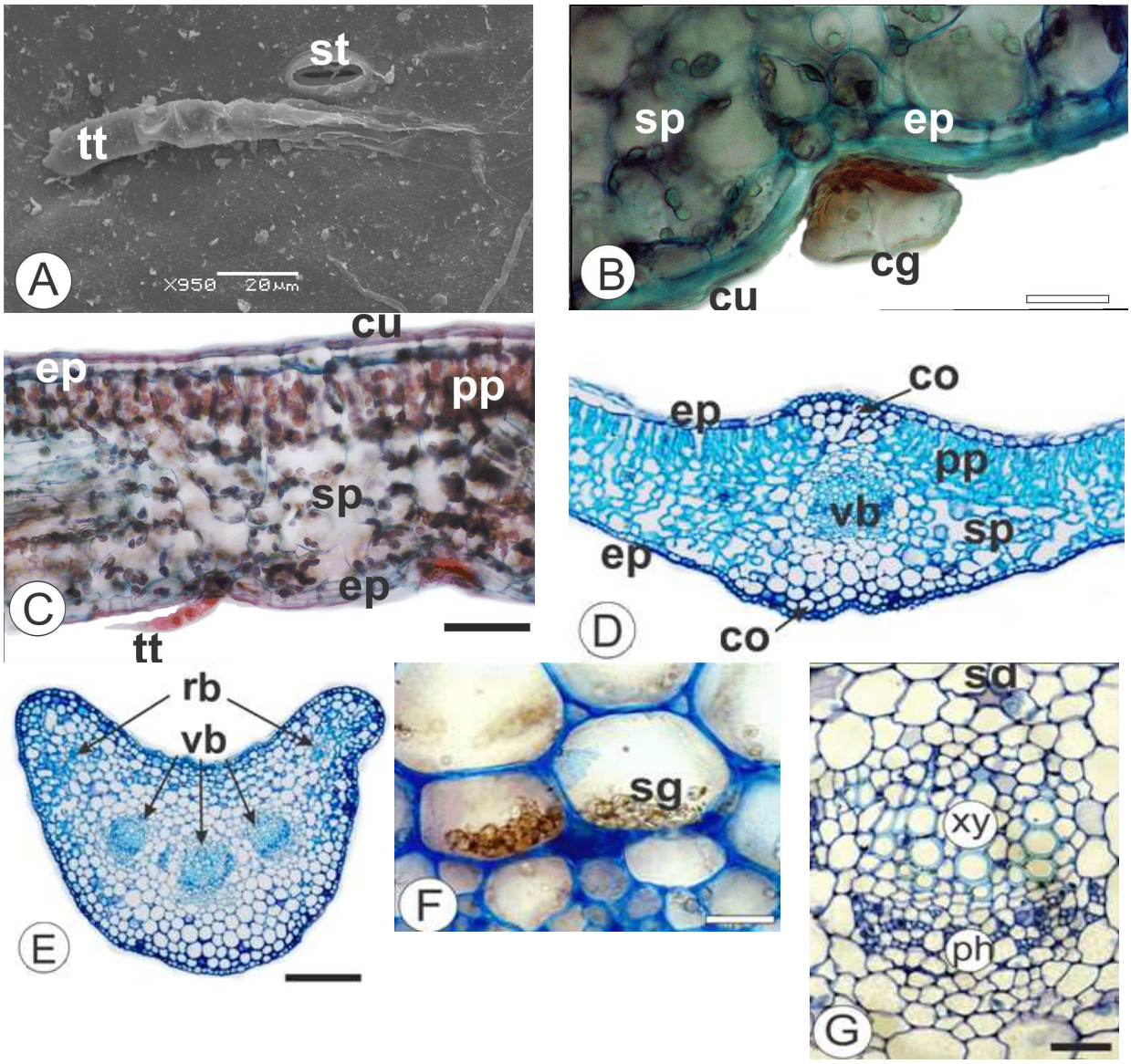

Figure 2 - *Calea serrata* Less., Asteraceae - Leaves. A. Abaxial epidermis in surface view, exhibiting stomata (st) and non-glandular trichome with tapered apical cell (tt). B. Cross-section of the blade showing capitate-sessile glandular trichome (cg) and cuticle (cu). C. Blade organization, in transection, revealing dorsiventral mesophyll, uniseriate epidermis (ep), cuticle, non-glandular trichome with tapered apical cell, palisade parenchyma (pp), and spongy parenchyma (sp). D. Midrib, showing vascular bundle (vb) in the center, epidermis, collenchyma (co), palisade and spongy parenchyma. E. Petiole, in cross-section, indicating three free vascular bundles in open arc with rib bundles (rb). F. Detail of the starch grains (sg) in the fundamental parenchyma of the petiole. G. Detail of the collateral vascular bundle, in the petiole, indicating phloem (ph), xylem (xy) and secretory duct (sd). Bar = 20 µm (B, G, F), 100 µm (C), 200 µm (D, E).

The stem had circular shape with six ribs in transection (Fig. 3A). The caulinar epidermis was uniseriate and the cuticle was thin and smooth (Fig. 3C) and reacted positively to lipophilic compounds. Below the epidermis, the chlorenchyma was alternate with angular collenchyma, which had about 6-7 rows (Figs. 3B, C). Perivascular fiber caps were observed (Figs. 3B, D). The secretory ducts had the same features as previously described for the foliar blade. The

endoderm (Fig. 3B) showed starch grains and encircled the cortex internally. The central region comprehended relatively large parenchymatic cells (Fig. 3B). The vascular cylinder presented cambia forming phloem outward and xylem inward (Figs. 3B, D, E).

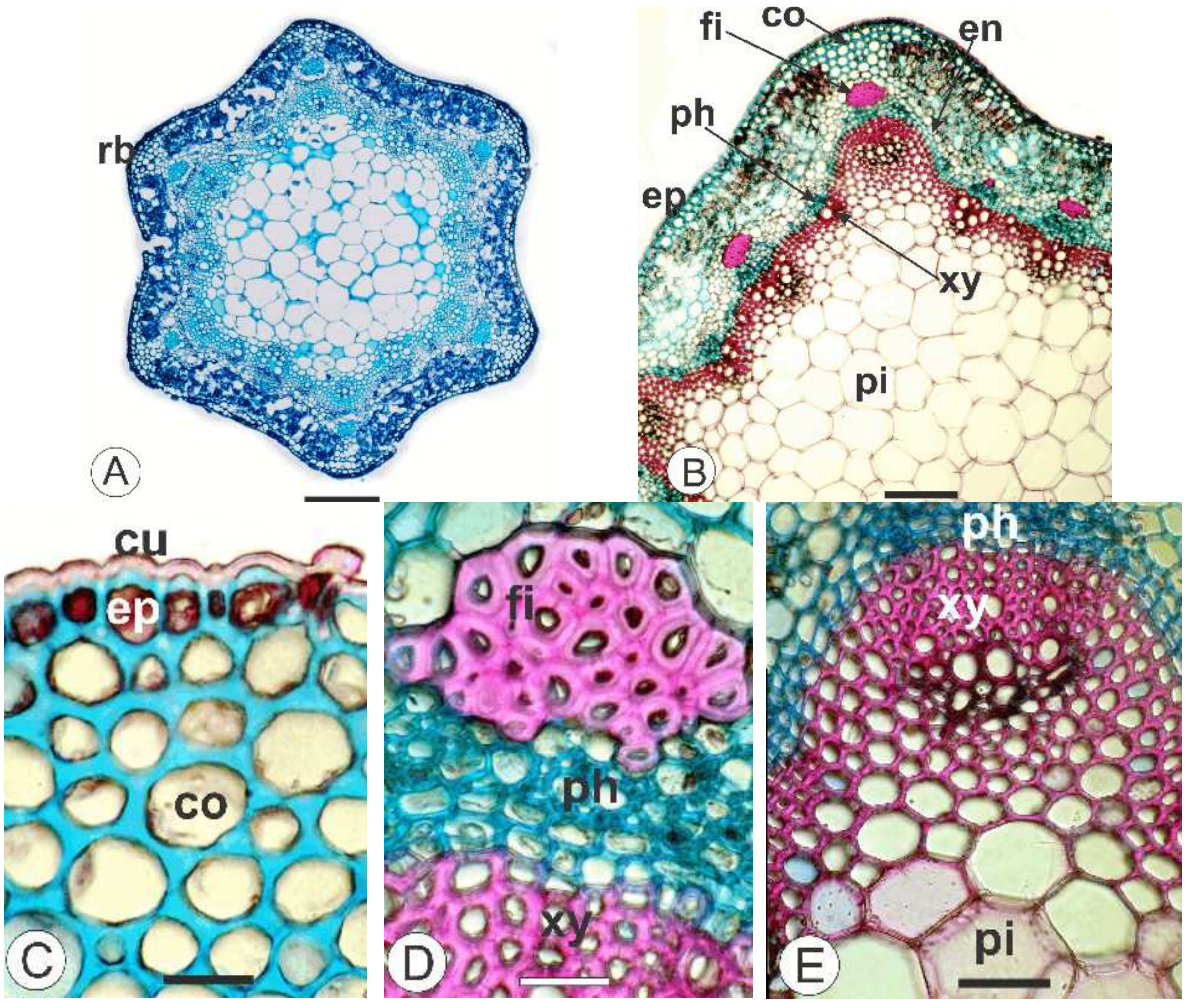

Figure 3 - *Calea serrata* Less., Asteraceae – Stem in cross-section – A. An overview of the stem exhibiting six ribs (rb). B. Stem, indicating fiber (fi), epidermis (ep), collenchyma (co), endoderm (en), phloem (ph), xylem (xy) and pith (pi). C. Detail of the epidermis, cuticle and collenchyma. D. Detail of the vascular cylinder and perivascular fiber cap. E. Detail of the vascular cylinder and pith. Bar = 200 µm (A), 100 µm (B), 20 µm (C, D, E).

DISCUSSION

According to Metcalfe and Chalk (1950), the presence of stomata in frontal view has diagnostic value in Asteraceae. Several genera of Asteraceae can present anomocytic (predominantly) and anisocytic stomata as observed in *Gochnatia* (Youssef et al. 2013), *Lucilia* (Duarte et al. 2011), *Baccharis* (Oliveira et al. 2011; Souza et al. 2011), *Mikania*, *Porophyllum* and *Vernonia* (Milan et al. 2006). The studied species revealed both the anomocytic and anisocytic stomata only in the abaxial surface.

Cavities, idioblasts, ducts and glandular trichomes can be observed in leaves of many members of Asteraceae (Milan et al. 2006; Duarte et al. 2011; Budel et al. 2012; Budel et al. 2013). Trichome types are useful features in the quality control of herbal drug, mainly as powder or dried fragments. In this study, *C. serrata* showed glandular trichomes and secretory ducts. According to Werker (2000), there was no wholly appropriate

classification for the trichomes. In this work, morphology was used as classification criteria. Glandular trichomes have been described for Asteraceae family (Milan et al. 2006; Souza et al. 2011; Youssef et al. 2013; Pereira et al. 2014). *C. serrata* showed two types of glandular trichomes, capitate-stalked and capitate-sessile.

C. serrata showed two types of non-glandular trichomes. One of them was multicellular uniseriate and composed by 5-6 cells at the base and tapered apical cell. The other one was conical and consisted of 6-8 cells. This type was common in Asteraceae family (Krak1 and Mráz 2008; Tavares et al. 2012).

Asteraceae typically exhibits a mesophyll differentiated into palisade and spongy parenchyma (Oliveira et al. 2011; Souza et al. 2011; Duarte et al. 2011; Souza et al. 2013). *Calea serrata* have shown an isobilateral arrangement for chlorenchyma.

Secretory ducts are frequently related to the endoderm or vascular system for different members of Asteraceae (Pagni and Masini 1999; Budel and Duarte 2010; Souza et al. 2011; Budel et al. 2012). The evaluated species exhibited secretory ducts near to the endoderm. The occurrence of glandular trichomes and secretory ducts supports additional studies to be undertaken correlated to the isolation and characterization of chemical compounds and further microbiological, pharmacological and toxicological studies for *Calea* species.

With reference to the caulinar microscopic characters, the stem showed remaining epidermis, strands of chlorenchyma alternating with collenchyma, endoderm limiting internally the cortex and vascular cambia forming phloem outward and xylem inward common features of *Calea*. Concerning the caulinar shape, in cross-section, *C. serrata* had circular shape with six ribs.

CONCLUSIONS

As expected, the features of leaves and stems of *C. serrata* agreed with the *Calea* genus. These characters should be evaluated as a whole to describe the species, even though the subsequent structures might be emphasized as distinguishing structures among the *Calea* genus: glandular capitate-stalked and capitate-sessile, uniseriate multicellular non-glandular trichome with tapered apical cell, conical non-glandular trichome, dorsiventral mesophyll, secretory ducts near the endoderm and circular shape with six ribs in the steam.

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