

Anatomical characters of the medicinal leaf and stem of *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. (Asteraceae)

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Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp. (Asteraceae), better known by its former name *Vernonia amygdalina* Delile, is a small shrub used in folk medicine as an antipyretic, laxative, antimalarial and anthelmintic. Studies have demonstrated that different vegetal extracts possess antioxidant, antimicrobial and antiparasitic activities. Among the bioactive metabolites, there are sesquiterpene lactones, saponins, polyphenols and flavonoids. This study investigated the leaf and stem microscopic characters of *G. amygdalinum*, aiming to expand the knowledge on this medicinal species and indicate anatomical structures. Plant material was fixed and sectioned by freehand and using a microtome. The sections were either stained or underwent standard histochemical tests. Scanning electron microscopy was performed to investigate epidermal relief. The leaf is amphistomatic with anomocytic stomata. There are striate cuticle, glandular and non-glandular trichomes and dorsiventral mesophyll. In transverse section, the midrib and the petiole have a plano-convex shape. Both show several collateral vascular bundles and few crystals of calcium oxalate. In the stem, the epidermis persists and the phellogen has a peripheral origin. It presents typical endodermis and sclerenchymatic caps adjoining the phloem. The aspects that contribute to characterizing the species are stomata on both leaf surfaces, midrib and petiole features, the endodermis and sclerenchymatic caps in the stem, as well as the different types of trichome on both aerial organs.

Uniterms: *Gymnanthemum amygdalinum*/pharmacognosy. *Gymnanthemum amygdalinum*/microscopic characterization. *Vernonia amygdalina*/pharmacognosy. *Vernonia amygdalina*/microscopic characterization. Asteraceae/pharmacognosy. Medicinal plants.

Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp. (Asteraceae), espécie mais conhecida pela sinonímia *Vernonia amygdalina* Delile, é um pequeno arbusto empregado na medicina popular como antipirético, laxativo, antimalárico e anti-helmíntico. Estudos demonstraram que diferentes extratos do vegetal possuem atividades antioxidante, antimicrobiana e antiparasitária. Entre os metabólitos bioativos presentes, citam-se lactonas sesquiterpenoides, saponinas, polifenóis e flavonoides. Este estudo investigou os caracteres microscópicos de folha e caule de *G. amygdalinum*, a fim de ampliar o conhecimento acerca dessa espécie medicinal e apontar estruturas anatômicas características. O material foi fixado e seccionado à mão livre e em micrótomo. Os cortes foram corados ou submetidos aos testes histoquímicos clássicos. Empregou-se microscopia eletrônica de varredura para investigar a ultraestrutura da superfície epidérmica. A folha é anfistomática com estômatos anomocíticos. Há cutícula estriada, tricomas glandular e tector e mesófilo dorsiventral. Em secção transversal, a nervura central e o pecíolo têm contorno plano-convexo. Ambos mostram vários feixes vasculares colaterais e poucos cristais de oxalato de cálcio. No caule, a epiderme permanece e o felogênio tem instalação periférica. São observadas endoderme típica e calotas esclerenquimáticas apostas ao floema. Os aspectos que fornecem elementos para a identificação da espécie são a ocorrência de estômatos em ambas as superfícies foliares, a organização da nervura central e do pecíolo, a endoderme e as calotas esclerenquimáticas no caule, além dos diferentes tipos de tricoma nos órgãos aéreos estudados.

Unitermos: *Gymnanthemum amygdalinum*/farmacognosia. *Gymnanthemum amygdalinum*/caracterização microscópica. *Vernonia amygdalina*/farmacognosia. *Vernonia amygdalina*/caracterização microscópica. Asteraceae/farmacognosia. Plantas medicinais.

INTRODUCTION

Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp. (Asteraceae), formerly known as *Vernonia amygdalina* Delile, is a small shrub commonly called bitterleaf due to its peculiar taste (Momoh *et al.*, 2012). In Brazil, this species is referred to as alumã, boldo or alcachofra (Agra *et al.*, 2008; Coelho-Ferreira, 2009; Almeida *et al.*, 2012). It has an erect stem covered with white hairs, ovate-lanceolate to elliptic-lanceolate leaves with short petiole, and creamy-white inflorescences (Achigan-Dako *et al.*, 2009). The plant may grow up to 3 m high in tropical regions and is reputed to have several health benefits (Farombi, Owioye, 2011).

In folk medicine, the aerial organs of *G. amygdalinum* are mainly used as an antipyretic, laxative, antimalarial and anthelmintic (Igile *et al.*, 1994; Vigneron *et al.*, 2005; Agra *et al.*, 2008; Georgewill, Georgewill, 2010). Studies of different extracts have shown antioxidant (Igile *et al.*, 1994; Farombi, Owioye, 2011), antimicrobial (Erasto, Grierson, Afolayan, 2006; Okigbo, Mmeka, 2008), antiparasitic (Tadesse *et al.*, 1993; Ademola, Eloff, 2011; Adiukwu, Amon, Nambatya, 2011), antidiabetic (Akinola *et al.*, 2011; Ong *et al.*, 2011) and antihypertensive (Ajibola, Eleyinmi, Aluko, 2011; Saliu *et al.*, 2011) properties.

Chemical analysis of the leaf and stem found different groups of metabolites, such as sesquiterpene lactones (Babalola, Anetor, Adeniyi, 2001; Erasto, Grierson, Afolayan, 2006; Luo *et al.*, 2011), saponins (Adiukwu, Amon, Nambatya, 2011), polyphenols (Ong *et al.*, 2011; Saliu *et al.*, 2011) and flavonoids (Igile *et al.*, 1994; Atangwho *et al.*, 2009).

Although there are various reports on bioactive compounds and pharmacological activities of the species, there is a paucity of information on its anatomical features. Therefore, this study was designed to investigate the leaf and stem microscopic characters of *G. amygdalinum* and contribute to the knowledge on this medicinal plant indicating anatomical structures for its identification.

MATERIAL AND METHODS

Plant material

Specimen samples of *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. (Asteraceae) were collected from the spontaneous flora in Palotina, state of Paraná, Brazil (coordinates 24°17'S and 53°40'W, and altitude of 290m), in February 2005. The species was identified by the herbarium curator Osmar dos Santos Ribas at the

Museu Botânico Municipal de Curitiba where a voucher is registered as MBM 301457.

Methods

Fully-developed leaves and stems, obtained 5-30 cm from the shoot, were chemically fixed in FAA70 (Johansen, 1940) and preserved in 70% (v/v) ethanol solution (Berlyn, Miksche, 1976).

For anatomical investigation, the leaves (lower third part) and young stems were prepared according to the standard techniques for light microscopy, including histochemical tests, and scanning electron examination. Semipermanent slides were prepared with the freehand transverse and longitudinal sections of the fixed material. These sections were stained with astra blue and basic fuchsin (Roeser, 1972). For permanent slides, the material was embedded in glycol-methacrylate, sectioned using a rotary microtome and stained with toluidine blue (O'Brien, Feder, McCully, 1964).

In order to reveal the cell content and cell wall impregnation, histochemical tests were done with Sudan III for lipophilic substances (Sass, 1951), ferric chloride for phenolic compounds (Johansen, 1940), lugol for starch grains (Berlyn, Miksche, 1976), hydrochloric phloroglucin for lignin (Foster, 1949), and a diluted solution of sulphuric acid for calcium crystals (Oliveira, Akisue, 1997).

For scanning electron microscopy, the fixed fragments were dehydrated in a graded ethanol series and by carbon dioxide critical point drying, gold coated and observed in a high vacuum (Souza, 1998).

RESULTS

The leaf presents glandular and non-glandular trichomes on both epidermal surfaces. The glandular trichomes (Figures 1A, 1B, 1E, 2C, 3B) are capitate, with a bicellular head and short pedicel, located in a small epidermal depression. The non-glandular trichomes (Figures 1A, 1D) are comparatively more abundant. They are long, multicellular and uniseriate, having about 5-15 cells and an elongated apical cell. An evident striate cuticle coats the epidermis (Figures 1B, 1C). In face view, the epidermal cells are wavy on the adaxial side (Figure 1F) and sinuous on the abaxial surface (Figure 1G). There are anomocytic stomata on both sides (Figures 1F, 1G).

In transverse section, the epidermis is uniseriate and the stomata are inserted at the same level as, or slightly above, the surrounding cells, and show external cuticular ledges (Figure 2A). The mesophyll is dorsiventral, usually encompassing one layer of palisade parenchyma and five

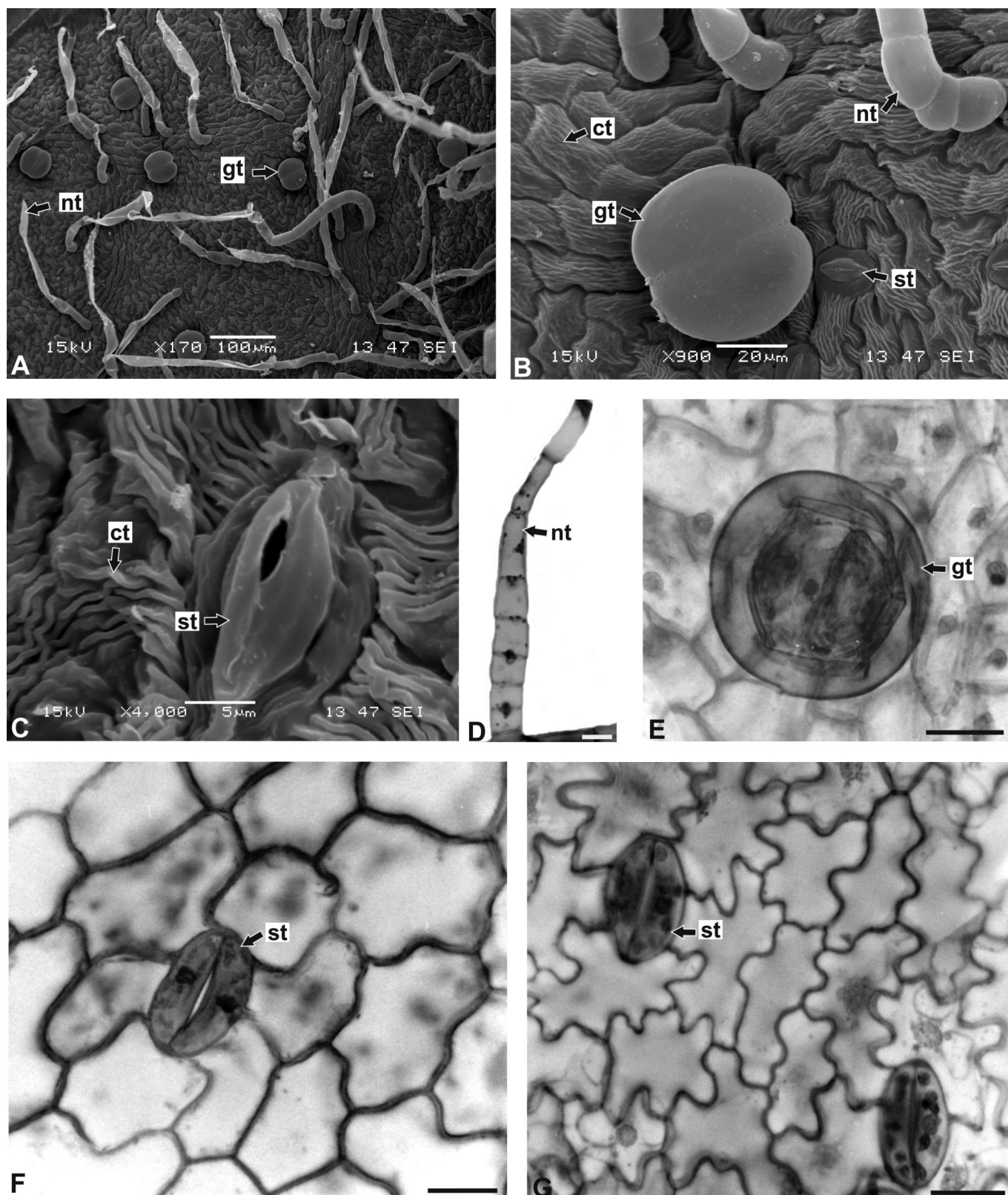


FIGURE 1 - A-G: *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. – leaf: A-C. Detail of non-glandular and glandular trichomes, stomata and striate cuticle under SEM; D. Medium and basal portion of a non-glandular trichome; E. Capitulate glandular trichome; F, G. Adaxial and abaxial epidermal surfaces, respectively. Abbreviations: ct – cuticle, gt – glandular trichome, nt – non-glandular trichome, SEM – scanning electron microscopy, st – stomatum. Bar = 20 μ m (D-G).

to seven rows of spongy parenchyma. Minor collateral bundles are distributed in the chlorenchyma and encircled by a parenchymatic sheath (Figure 2A).

The midrib and petiole are alike. Both have a plano-convex shape, in transverse section (Figures 2B,

3A). Below the uniseriate epidermis, a strand of angular collenchyma is evident (Figures 2C, 2E, 3B) and, traversing the ground parenchyma, several collateral vascular bundles with cambial zone (Figures 2E, 3C) are arranged in an open arc (Figures 2B, 3A). A few crystals

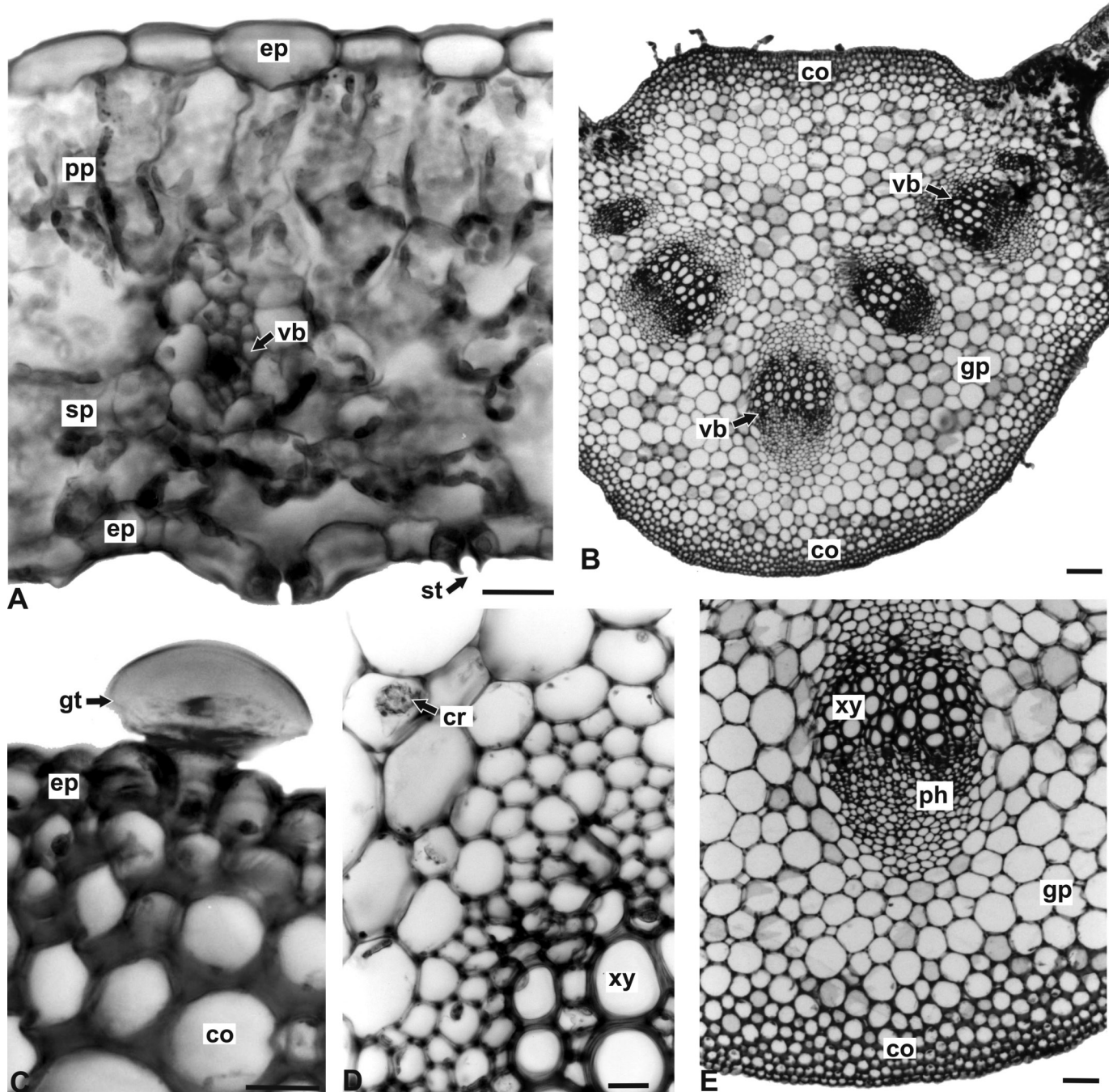


FIGURE 2 - A-E: *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. – leaf, transverse section: A. Interveinal region, showing dorsiventral mesophyll and a minor collateral vascular bundle; B. Midrib organization; C. Detail of a glandular trichome and angular collenchyma of the midrib; D. Calcium oxalate crystal near a vascular bundle; E. Collateral vascular bundle of the midrib. Abbreviations: co – collenchyma, cr – crystal of calcium oxalate, ep – epidermis, gp – ground parenchyma, gt – glandular trichome, ph – phloem, pp – palisade parenchyma, sp – spongy parenchyma, st – stomatum, vb – vascular bundle, xy - xylem. Bar = 100 μ m (B), 50 μ m (E), 20 μ m (A, C, D).

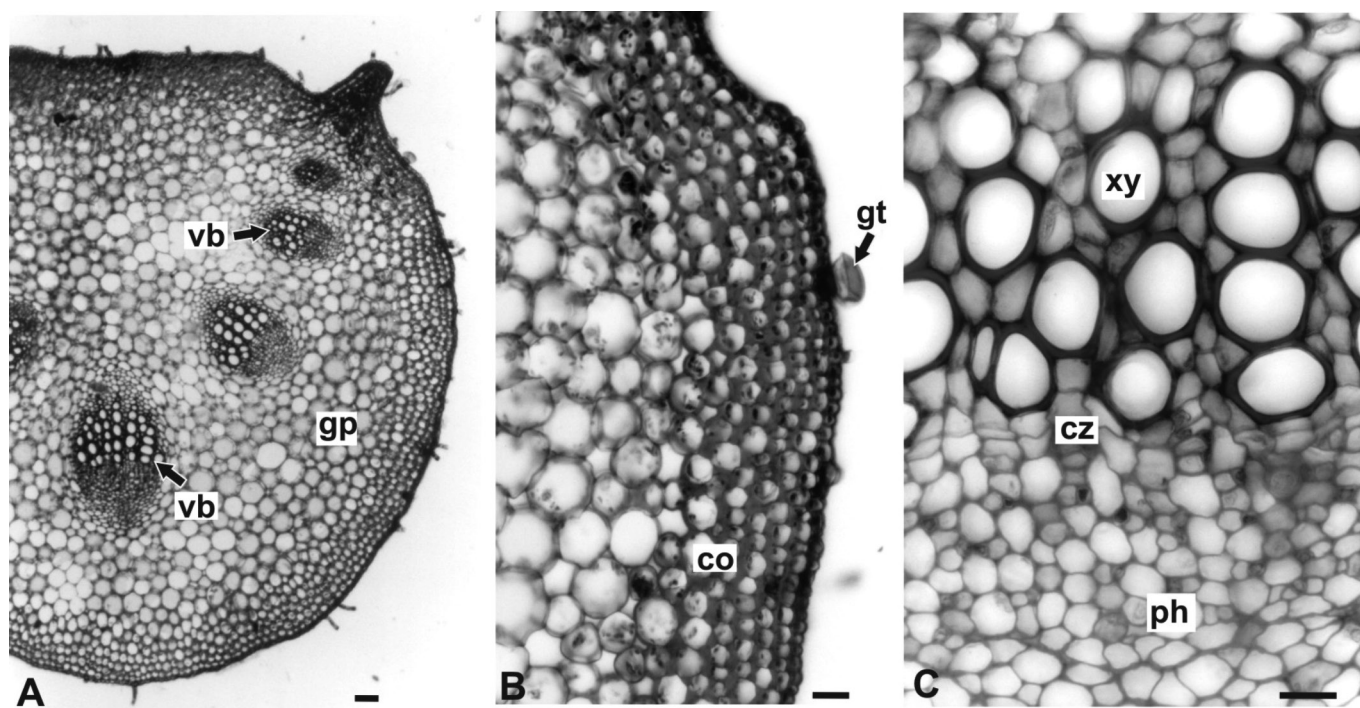


FIGURE 3 - A-C: *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. – petiole, transverse section: A. Overall feature of the petiole; B. Glandular trichome and collenchyma; C. Detail of a collateral vascular bundle. Abbreviations: co – collenchyma, cz – cambial zone, gp – ground parenchyma, gt – glandular trichome, ph – phloem, vb – vascular bundle, xy - xylem. Bar = 100 μ m (A), 20 μ m (B, C).

of calcium oxalate, resembling rough druses, are located near some vascular bundles (Figure 2D).

The stem is circular in cross-section. In incipient secondary development, the epidermis is uniseriate and persists, and the phellogen originates just below it (Figure 4C). Glandular and non-glandular trichomes are present as described previously. In the cortex, there are some layers of tangential-angular collenchyma and multiseriate cortical parenchyma (Figures 4A, 4C). The inner boundary consists of a typical endodermis (Figures 4B, 4D). The vascular system is represented by a cylinder of phloem external to the xylem (Figure 4A) and both show straight rays. The xylem is totally lignified and has solitary or small groups of tracheary elements (Figures 4A, 4B). There are sclerenchymatic caps adjoining the phloem (Figures 4A, 4B, 4D) and the pith is parenchymatic (Figure 4A).

DISCUSSION

The Asteraceae family is very large and well-represented in a wide range of latitudes and, as would be expected, the anatomical structure of its members shows considerable diversity in correlation with habitat differences, whilst ecological specializations also occur (Metcalf, Chalk, 1950). In this study, *Gymnanthemum*

amygdalinum displayed features in common to many medicinal species of this family, such as leaf with uniseriate epidermis, dorsiventral mesophyll, simple arc of collateral vascular bundles in the midrib and petiole, as well as stem with peripheral development of the phellogen, outer cortex consisting of collenchyma, well defined endodermis and crescent-shaped strands of sclerenchyma at the outer boundary of the phloem.

However, some aspects concerning trichomes, cuticle characters, patterns and occurrence of stomata on the leaf surface are of considerable taxonomic value and may help to distinguish one species from another (Metcalf, Chalk, 1950; 1988).

Due to lack of anatomical data on the genus *Gymnanthemum*, comparisons should be made with related taxa. In this case, with species of *Vernonia*, since *G. amygdalinum* was formerly circumscribed to this taxon. According to Adedeji and Jewoola (2008), *Vernonia* spp. have T-shaped or flabelliform trichomes as a delimiting character. These types of appendage were not seen in this investigation and contribute to support the taxonomic repositioning of *G. amygdalinum*.

Comparatively with close species, the presence of flagelliform trichomes and biconvex midrib mentioned in *Vernonia scorpioides* (Lam.) Pers. by Toigo *et al.* (2004)

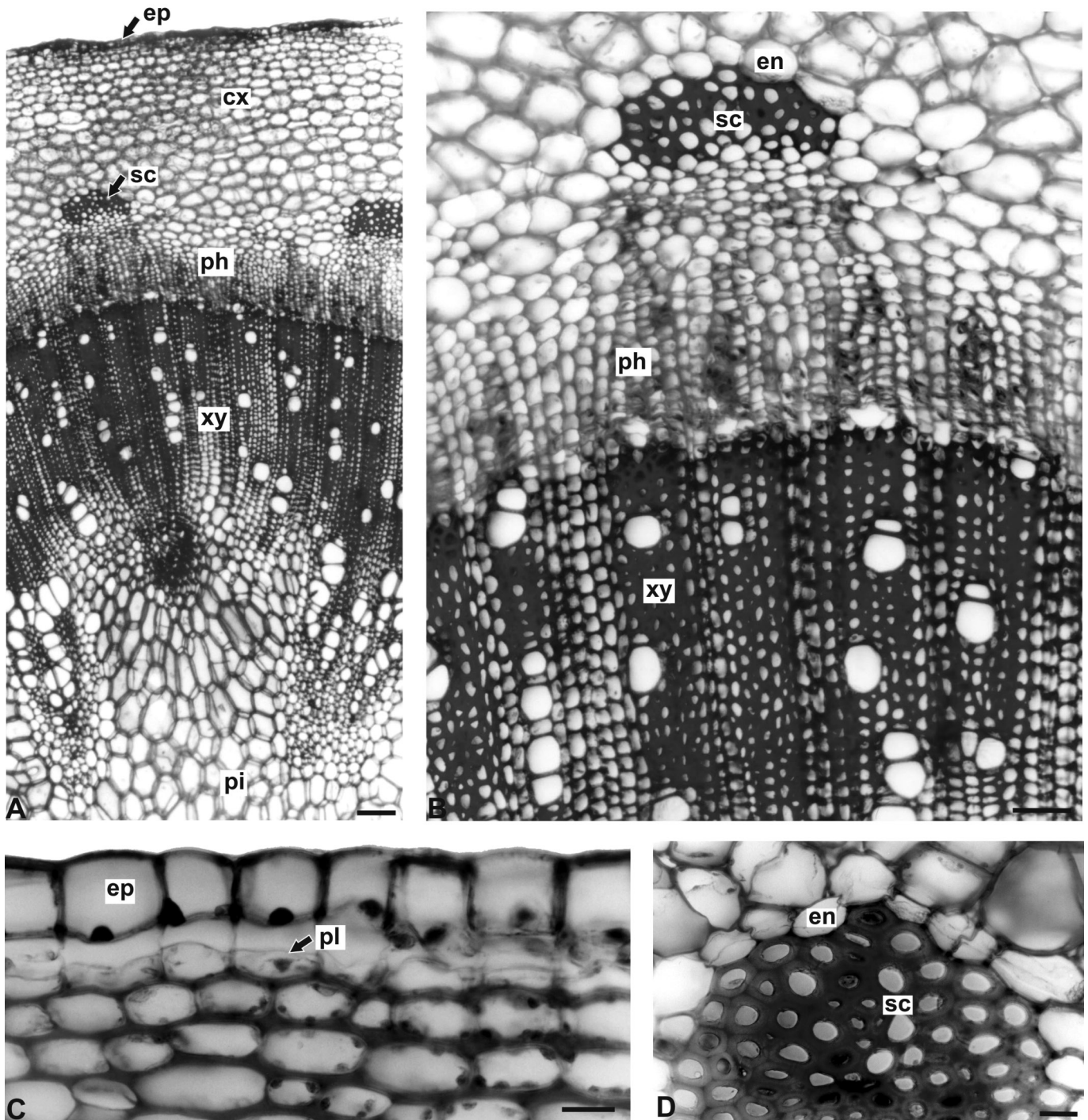


FIGURE 4 - A-D: *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. – stem, transverse section: A. Overall organization; B. Detail of the vascular cylinder; C. Phellogen development; D. Detail of the endodermis and a sclerenchymatic cap. Abbreviations: cx – cortex, en – endodermis, ep – epidermis, ph – phloem, pi – pith, pl – phellogen, sc – sclerenchymatic cap, xy - xylem. Bar = 100 μ m (A), 50 μ m (B), 20 μ m (C, D).

can be considered leaf structures that are distinct from the species examined herein. Concerning the stem anatomy, both species are alike.

With reference to the work that focused exclusively on leaf epidermal features (Oladele, 1990), *Vernonia*

cinerea (L.) Less. exhibited regular T-shaped trichomes, with a long apical cell attached centrally to a uniseriate stalk, which were neither observed in *G. amygdalinum* by Oladele (1990) nor in this survey.

Vernonia condensata Baker differs from *G.*

amygdalinum, as the former has hypostomatic leaf and biconvex midrib in cross-section as claimed by Lolis and Milaneze-Gutierrez (2003). However, controversial data was reported by Milan, Hayashi and Apezato-da-Glória (2006), authors who stated that the leaf of *V. condensata* is amphistomatic.

Relying on the investigation by Filizola *et al.* (2003), *Vernonia brasiliensis* (L.) Druce exhibited hypostomatic leaf, T-shaped trichomes, biconvex midrib, circular petiole and stem with notable sinuous contour in cross-section. None of these aspects or the previous ones referring to *Vernonia* species were found in this research and therefore may be viewed as diagnostic markers among the compared species.

Nevertheless, as is well known, some structures may be modified as response to environmental influences. Corroborating this statement, Eltahir and AbuEREish (2011) concluded that *G. amygdalinum* grown in relatively dry places in Africa showed thick cuticle and sunken stomata on both epidermal surfaces as adaptations to minimize the loss of water. These features were not found in this study and should not be taken into account for identification of the species.

Thus, on the basis of these comparisons, the main aspects that characterize *G. amygdalinum* are stomata on both leaf surfaces, midrib and petiole features, typical endodermis and sclerenchymatic caps in the stem, as well as the trichome peculiarities on both aerial organs. These anatomical characters, considered together, are relevant for the correct identification of this medicinal species for pharmacognostic purposes.

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