

## Comparative Leaf Morphology and Anatomy of Three Asteraceae Species

**Patricia Milan, Adriana Hissae Hayashi and Beatriz Appezzato-da-Glória\***

*Departamento de Ciências Biológicas; Escola Superior de Agricultura "Luiz de Queiroz"; Universidade de São Paulo; bagloria@esalq.usp.br; C. P. 9; 13418-900; Piracicaba - SP - Brasil*

### ABSTRACT

*The objective of this paper was to describe and compare the morphology and anatomy of mature leaves of Mikania glomerata Spreng., Porophyllum ruderales Cass. and Vernonia condensata Baker (Asteraceae) species that have different habits emphasizing their secretory structures. Longitudinal and transversal sections of mature leaf blades of the three species were analyzed at the apex, base, and medium third part of the midvein of the leaf blade and of the margin. M. glomerata had uniseriate glandular trichomes and secretory ducts; P. ruderales had hydathodes and secretory cavities; and V. condensata had idioblasts and uni- and biseriata glandular trichomes.*

**Key words:** *Mikania glomerata, Porophyllum ruderales, Vernonia condensata, secretory structures, medicinal plants*

### INTRODUCTION

The Asteraceae family consists of approximately 25000 species (Barroso, 1986) included in over 1100 genera. These species frequently present herbaceous habits, although arboreous and voluble herbaceous habits also occur (Cronquist, 1981). Because of this variety of habits, the family presents various anatomical structures and in some cases ecological specialization may occur (Metcalf and Chalk, 1950). Secretory structures are of great taxonomical interest and their restricted distribution has an important diagnosis value (Metcalf and Chalk, 1950; Fahn, 1979). Frequently, in Asteraceae, they occupy distinct positions in different organs of the plant occurring in all, some or in only one organ (Solereeder, 1908).

Castro et al. (1997) reviewed the types of secretory structures in the leaves of seventy two representatives of the Asteraceae family from the

cerrado vegetation in the Reserva Biológica de Mogi Guaçu, São Paulo, Brazil. When analyzed together these secretory structures presented diagnosis value at genus level. Eight types of leaf secretory structures were mentioned: ducts, cavities, idioblasts, laticifers, hydathodes, extrafloral nectaries, trichomes and glandular appendixes. According to Metcalfe and Chalk (1950), anatomical diversity is commonly observed in the structure of leaves of species belonging to the Asteraceae. Among the features that vary are: (a) stomata distribution on leaf surfaces; (b) guard-cell positioning in relation to ordinary epidermal cells; (c) hypoderm development on the upper side of the leaf surface; (d) mesophyll and fibrovascular system differentiation; and (e) wax secretion on leaf surface (Solereeder, 1908).

The anatomical features that can be observed in Asteraceae are: (a) presence of various types of glandular or covering trichomes; (b) papillae on

\* Author for correspondence

the abaxial epidermis; (c) anomocytic, anisocytic and rarely heliocytic stomata; (d) presence of hydathodes; (e) presence of hypoderm; (f) homogeneous or heterogeneous mesophyll; and (g) vascular bundles with parenchymatic sheath composed by large cells (Metcalf and Chalk, 1979). This family presents species of great importance in the fields of nutrition, cosmetics and pharmacy due to the production of essential oils. Among the species used popularly as medicinal plants are: *Mikania glomerata* Spreng., *Porophyllum ruderale* Cass. and *Vernonia condensata* Baker. Regarding the importance of these species as medicinal plants there are few information about their leaf (organ used for medicinal purposes) morphology and anatomy. Therefore, the objective of this paper was to describe the morphology and anatomy of mature leaves of these three species with different habits emphasizing their secretory structures.

## MATERIALS AND METHODS

The mature leaves of *Mikania glomerata* Spreng. (voluble herbaceous habit), *Porophyllum ruderale* Cass. (herbaceous habit), and *Vernonia condensata* Baker (arboreous habit) were collected at "Horto de Plantas Medicinaiis" which belongs to the Departamento de Ciências Biológicas of Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo. The identification of the botanical material was done by a specialist and the vouchers were deposited at ESA Herbarium: *M. glomerata* Spreng. (76651), *P. ruderale* Cass. (76658), and *V. condensata* Baker (76654).

The collection of the leaves was standardized regarding their location on the plants and their habits. Analyses were conducted on mature leaves of these three species. In *P. ruderale* the leaf primordium was also analyzed.

The leaf blades of *M. glomerata*, *P. ruderale* and *V. condensata* were analyzed at the apex, base, and medium third part of the midvein of the leaf blade and of the margin. Slides of the leaf primordia of *P. ruderale* were also prepared in order to confirm the presence of hydathodes and to observe the ontogenesis of the secretory cavities. The samples were fixed in Karnovsky solution (Karnovsky, 1965), dehydrated through an ethanol series and embedded with glycol methacrylate resin. Longitudinal and transversal sections with

5µm were stained with toluidine blue (Sakai, 1973) and mounted in synthetic resin "Entellan".

Histochemical tests were done using freehand sections of non-fixed samples. The presence of phenolic compounds were analyzed using ferric chloride (Johansen, 1940); the starch was visualized using iodide zinc chloride (Strasburger, 1913); the lipidic substances using Sudan IV (Jensen, 1962) and the polysaccharides using ruthenium red (Johansen, 1940). The epidermis analysis in frontal view was conducted using leaf fragments dissociated with hydroalcoholic saphranine (Foster, 1949). The study of the venation pattern was conducted using fresh material which was cleared in 5% NaOH and in 50% sodium hypochlorite, then stained with hydroalcoholic saphranine and mounted in synthetic resin (Foster, 1949). It was described following the terminology of Hickey (1973).

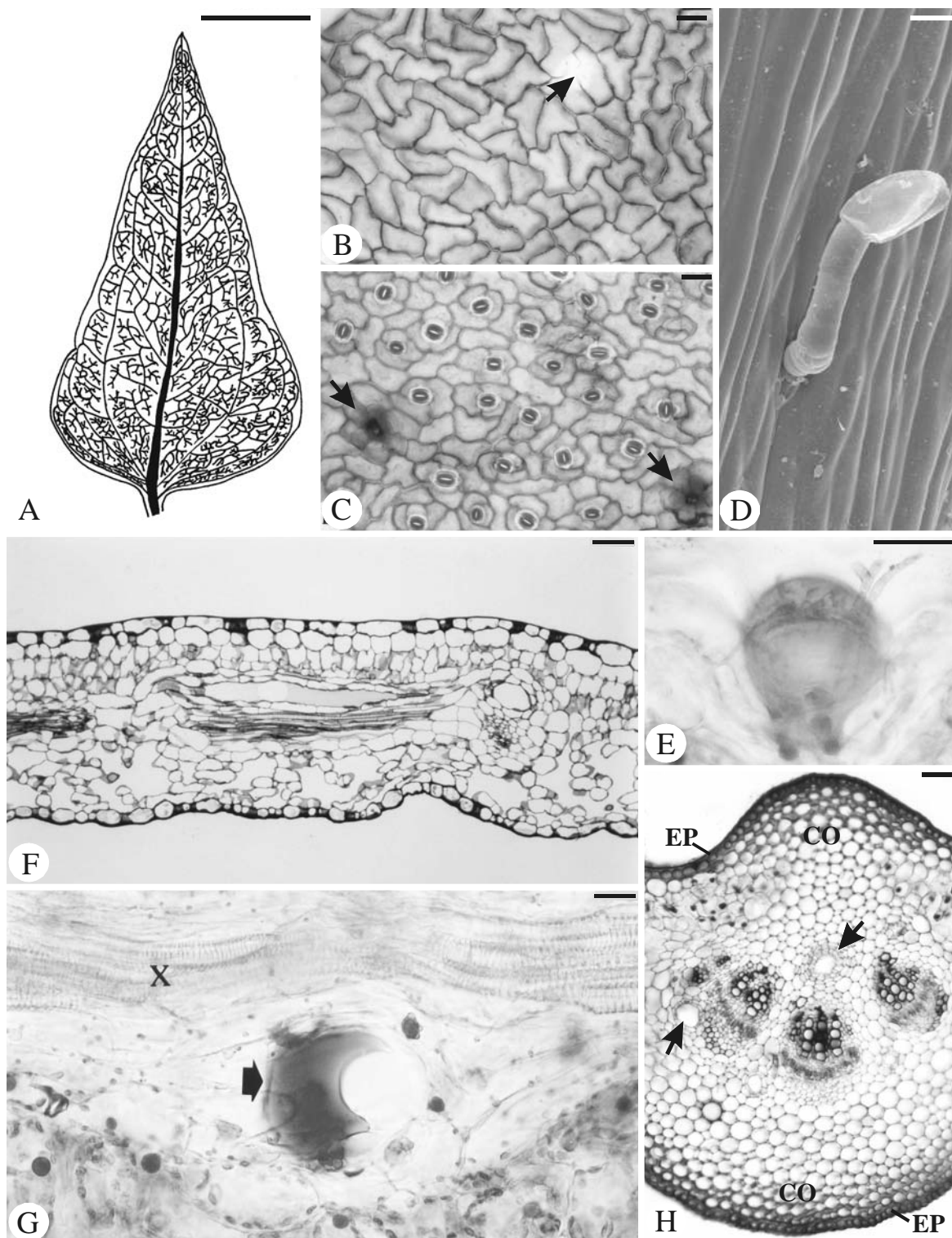
The photomicrographs of the prepared material in slides were taken using a Nikon Labophot microscope with micrometric scales photographed and amplified in the same optical conditions used. The analysis of the epidermis in frontal view on the scanning electron microscope was done using samples fixed in Karnovsky solution (Karnovsky, 1965), dehydrated in ethylic series and critical point dried through liquid CO<sub>2</sub>. The samples were then mounted on stubs and sputter coated with gold.

## RESULTS

### *Mikania glomerata* Spreng

**Morphology:** The leaves were simple (Fig. 1A) and inserted in an opposite decussate pattern. There were no leaf appendixes. The blade was asymmetrical with a cordiform to deltoid shape. The apex was acuminate to acute, the base was cordate to obtuse and the margin was slightly undulate. The venation pattern was camptodromous with brochidromous ramifications (Fig. 1A).

**Leaf blade anatomy:** In a frontal view the epidermal cells had slightly sinuous walls. This sinuosity was more evident on the abaxial epidermis (Fig. 1C) surface on which the cells were smaller when compared to those on the adaxial epidermis (Fig. 1B).



**Figure 1** - *Mikania glomerata* Spreng. **A.** Leaf venation pattern (Bar = 1 cm). **B-C.** Frontal view sections of upper and lower epidermis, respectively. Arrow in B indicates subepidermal cells with thick and silicified walls. In C the arrows indicate cells arranged radially around the base of the trichome and the stomata are anomocytic (Bars = 37  $\mu\text{m}$ ). **D-E.** Uniseriate and peltate glandular trichomes, respectively (Bars = 20  $\mu\text{m}$ ). **F-G.** Transversal sections of the medium third part of the leaf blade. Arrow in G indicates the lipidic content of the secretory duct (Bars = 74 and 19  $\mu\text{m}$ , respectively). **H.** Transversal section of the midvein showing secretory ducts (arrows) (Bar = 74  $\mu\text{m}$ ). CO = colenchyma, EP = epidermis, X = xylem.

The anomocytic stomata occurred more frequently on the lower epidermis and were usually surrounded by three ordinary epidermal cells (Fig. 1C). The glandular trichomes were usually positioned in a depression due to invaginations of the adjacent epidermal cells. The most observed type was uniseriate, filamentous, with variable number of stalk cells and a terminal cell that varied from a spherical to a spatulate shape (Fig. 1D). The other type was peltate (Fig. 1E). Both types of glandular trichomes secreted lipidic substances and in their surroundings, the presence of fungus (Fig. 1E) was common.

The organization of the mesophyll varied according to the different regions of the leaf blade. At the apex, the mesophyll was heterogeneous dorsiventral. There was one layer of palisade parenchyma composed by short and wide cells. Other features were similar to the mesophyll in the leaf blade medium third part. At this late region, turned towards the upper side of the leaf, there was a uniseriate and continuous layer of hypoderm with cells larger than the adjacent epidermal cells. The palisade parenchyma was composed by two layers of cells, which were smaller and more elongated at the inner layer. In some sectors it was difficult to distinct. The spongy parenchyma had seven layers of cells with various shapes loosely-arranged (Fig. 1F). At the leaf base, the uniseriate epidermis had few trichomes and stomata. Underneath, there were two to four continuous layers of collenchyma. At the margin, the mesophyll had isodiametric cells compactly-arranged. The walls of the cells on the first and second layers were thickened and the hypoderm was continuous.

The midvein at the medium third part of the leaf blade had a uniseriate epidermis provided with trichomes (Fig. 1H). There were three to four layers of angular collenchyma on both surfaces. Three collateral vascular bundles were immersed in a fundamental parenchyma (Fig. 1H). In the proximity of these bundles, there were secretory ducts which could be close to the phloem or xylem. The lumen was delimited by a uniseriate epithelium. Two concentric cell layers were around some ducts (Fig. 1H). The lateral veins had bundle sheaths composed by two layers of parenchymatic cells that presented extensions towards only one or both surfaces (Fig. 1F). Secretory ducts containing lipidic substances were associated with some or all veins close to the xylem (Fig. 1G).

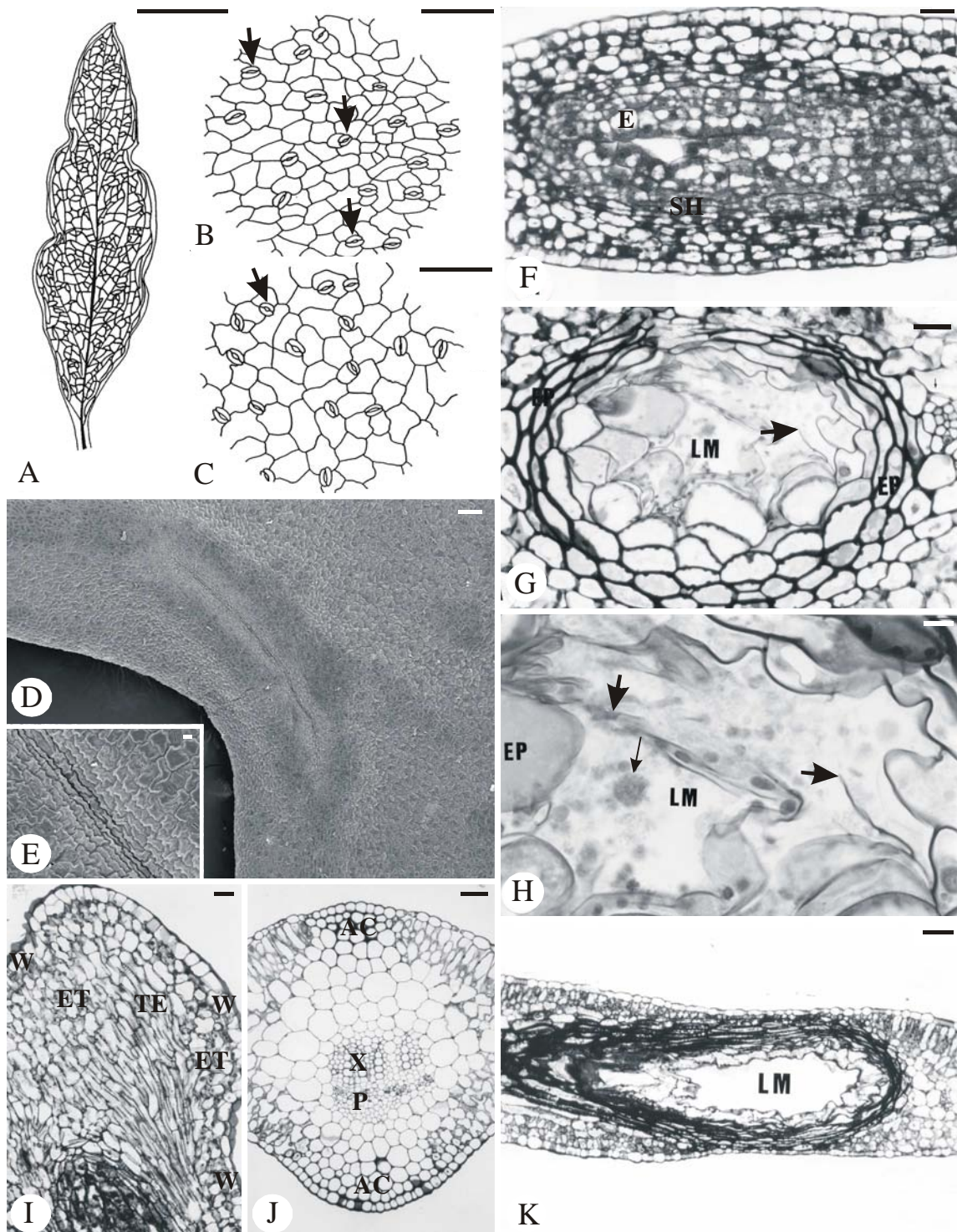
### **Porophyllum ruderale Cass.**

**Morphology:** The leaves were simple (Fig. 2A) and inserted in an opposite decussate pattern. The blade was a whole lamina, glabrous, membranaceous, with a spatulate shape and undulate margin. The apex was mucronate and the base was cuneate to obtuse. The venation pattern was pinnate, camptodromous, eucamptodromous (Fig. 2A). Translucent and elongated structures could be observed on fresh leaves along the margin (Fig. 2D) and, in some cases, near the apex.

**Leaf blade anatomy:** In frontal view, the abaxial and adaxial epidermis had cells with sinuous walls and ornamented cuticle. There were no trichomes and the leaf was amphistomatic, with mainly anomocytic stomata; among them there were few anisocytic (Figs. 2B and 2C). The epidermal cells above the secretory cavities were more elongated on the longitudinal plan of the leaf blade, being narrower than other epidermal cells (Figs. 2D and 2E). In transversal section, the epidermis was uniseriate and covered by a thin cuticle. On both surfaces, the external periclinal walls of the epidermal cells were thick and rich in pectic substances. Parietal thickening on anticlinal and periclinal walls was also observed on cells of the margin and midvein.

At the apex, the dorsiventral mesophyll was composed by one layer of palisade parenchyma with elongated and juxtaposed cells. The spongy parenchyma had four to five layers of loosely-arranged cells with various shapes. At the medium third part and leaf base, the dorsiventral mesophyll had an additional layer of spongy parenchyma. At the margin, the cells were rounded and there was no distinction between palisade and spongy parenchyma.

Hydathodes were observed at the apex and at the teeth of the leaf margin (Fig. 2I). Their structure presented spiral tracheary elements surrounded by an epithem. The epithem cells were rounded, had a conspicuous nucleus and some were chlorophyllous. On the epidermis there were water pores (Fig. 2I). The cleared and stained leaf showed a secretion, which was not identified inside the translucent structures mentioned earlier. These structures were secretory cavities formed prematurely at the medium third part, nearby the procambium at the mesophyll of the leaf primordia (Fig. 2F).



**Figure 2** - *Porophyllum ruderale* Cass. **A.** Leaf venation pattern (Bar = 1 cm). **B-C.** Upper and lower epidermis, respectively. The stomata are mainly anomocytic; among them there are few anisocytic (arrows) (Bars = 0.1 mm). **D.** Secretory cavity near the leaf margin (Bar = 100  $\mu$ m). **E.** Detail of epidermal cells above secretory cavity (Bar = 10  $\mu$ m). **F.** Premature formation of the secretory cavity (Bar = 19  $\mu$ m). **G-H.** Transversal sections of the secretory cavity. Larger arrows show cell lysis and smaller arrow shows a free nucleus in the cavity lumen (Bars = 19 and 7.4  $\mu$ m, respectively). **I.** Hydathode (Bar = 37  $\mu$ m). **J.** Midvein at the medium third part of the leaf blade (Bar = 74  $\mu$ m). **K.** Longitudinal section of the secretory cavity (Bar = 19  $\mu$ m). AC = angular collenchyma, E = future epithelium cells of the secretory cavity, EP = epithelium, ET = epithem, LM = lumen, P = phloem, SH = future cells of the cavity sheath, TE = tracheary elements, W = water pore, X = xylem.

The mesophyll cells that originated the cavity differed from ordinary mesophyll cells due to a dense cytoplasm and evident nucleus and nucleolus. The future epithelium cells were larger than the cells that formed the cavity sheath. The future sheath cells were longer and narrower and arranged in strata (Fig. 2F). In longitudinal sections of mature leaves, the epidermal cells above the secretory cavities were smaller and positioned in a lower level than other epidermal cells (Fig. 2K). In transversal section, the cavity lumen was wide and originated by cell lysis (Figs. 2G and 2H). The epithelium was irregular and composed by large and vacuolated cells with thin walls. It was also surrounded by a cavity sheath with small and rectangular shaped parenchymatic cells with dense cytoplasm and thick walls. The regular arrangement of these cells conferred a meristematic aspect to the sheath. The cavity was always close to the vascular system (Fig. 2G).

The midvein at the medium third part of the leaf blade had a uniseriate epidermis and three to four layers of angular collenchyma on both surfaces. The fundamental parenchyma involved the only vascular bundle (Fig. 2J). Immersed in the mesophyll there were fine vascular collateral branches surrounded by a sheath composed by one or two layers of parenchymatic cells without chloroplasts.

#### *Vernonia condensata* Baker

**Morphology:** The leaf blade was simple and had a whole lamina with an elliptic shape, an acute to acuminate apex, a cuneate base and dentate margin (Fig. 3A). Its consistence was membranaceous and the surface was hairy. The venation pattern was pinnate, craspedodromous, and semicraspedodromous (Fig. 3A). The leaves were inserted in a spiral pattern.

**Leaf blade anatomy:** The leaf was amphihypostomatic. In frontal view, the uniseriate epidermis had anomocytic stomata and trichomes, particularly on the abaxial surface, where the cuticle was ornamented throughout the leaf blade (Fig. 3B). On the adaxial surface the cuticular pattern occurred as striae and it was seen only on cells adjacent to guard-cells (Fig. 3C) or to a trichome base.

There were different types of glandular trichomes. Usually they were uniseriate with a variable number of stalk-cells and the terminal cell had a semi-spherical to elongated shape (Figs. 3B, 3E

and 3F). There were also short trichomes with a uni- or biseriate stalk and a spherical biseriate head (Figs. 3B, 3D and 3G). These trichomes might or might not be inserted in a depression due to invaginations of the adjacent epidermal cells (Figs. 3D, 3E, 3F and 3G). At the apex, the mesophyll was dorsiventral and had two to three layers of palisade parenchyma with slightly elongated juxtaposed cells. The spongy parenchyma was composed by four to five layers of loosely-arranged cells with various shapes. At the medium third part the mesophyll was as described at the apex, although the spongy parenchyma cells were more spaced out (Fig. 3E). Some chlorophyllous parenchyma cells had small druses (Fig. 3F). At the margin, the palisade and spongy parenchyma had less number of cell layers. Below the epidermis, there was a layer of collenchyma. At the leaf base, there were three to five layers of angular collenchyma adjacent to the epidermis and among the fundamental parenchyma cells, which presented druses, there were idioblasts.

The midvein at the medium third part of the leaf blade had five vascular collateral bundles, one large, three medium and one small size, organized in an open arch (Fig. 3H). The lateral veins of the vascular system were also collateral and might or might not present sheath extensions towards the surfaces. Both sheath cells and cells of the sheath extensions had chloroplasts.

Table 1 summarizes the leaf features of the three studied species, some of which are considered to have diagnosis value to the Asteraceae by Solereder (1908).

## DISCUSSION

The studied species showed variations in their morphological and anatomical features, such as venation patterns, stomata types, dorsiventral mesophyll with differentiated structures, presence or absence of parenchymatic bundle sheath extensions surrounding the lateral veins of the vascular system and the presence or absence of secretory structures. All the three species had stomata on both surfaces, particularly on the abaxial epidermis. Sajo and Menezes (1994) observed that the Asteraceae species *Vernonia psilophylla* and *V. sessilifolia* had stomata on both surfaces whilst *V. linearis* had stomata only on the

abaxial epidermis.

**Table 1** - Comparative anatomical features of *M. glomerata*, *P. ruderale* and *V. condensata*.

Species	<i>M. glomerata</i>	<i>P. ruderale</i>	<i>V. condensata</i>
Anatomical Features / Habit	Voluble herbaceous	Herbaceous	Arboreous
Stomata type	Anomocytic	Anomocytic and anisocytic	Anomocytic
Glandular trichomes	Present (uniseriate)	Absent	Present (uni and biseriate)
Hydathodes	Absent	Present	Absent
Secretory cavities	Absent	Present	Absent
Secretory ducts	Present	Absent	Absent
Idioblasts	Absent	Absent	Present
Bundle sheath extensions of the fine branches	Present	Absent	Present

*P. ruderale* and *V. condensata* had ornamented cuticle covering the epidermis. Cilliers and Kruger (1993) observed that although the cuticles were mainly smooth, as found in this work for *M. glomerata*, the adaxial cuticle of most of *Brachylaena* (Asteraceae) species might possess long continuous striae which could be branched and extend over several cells.

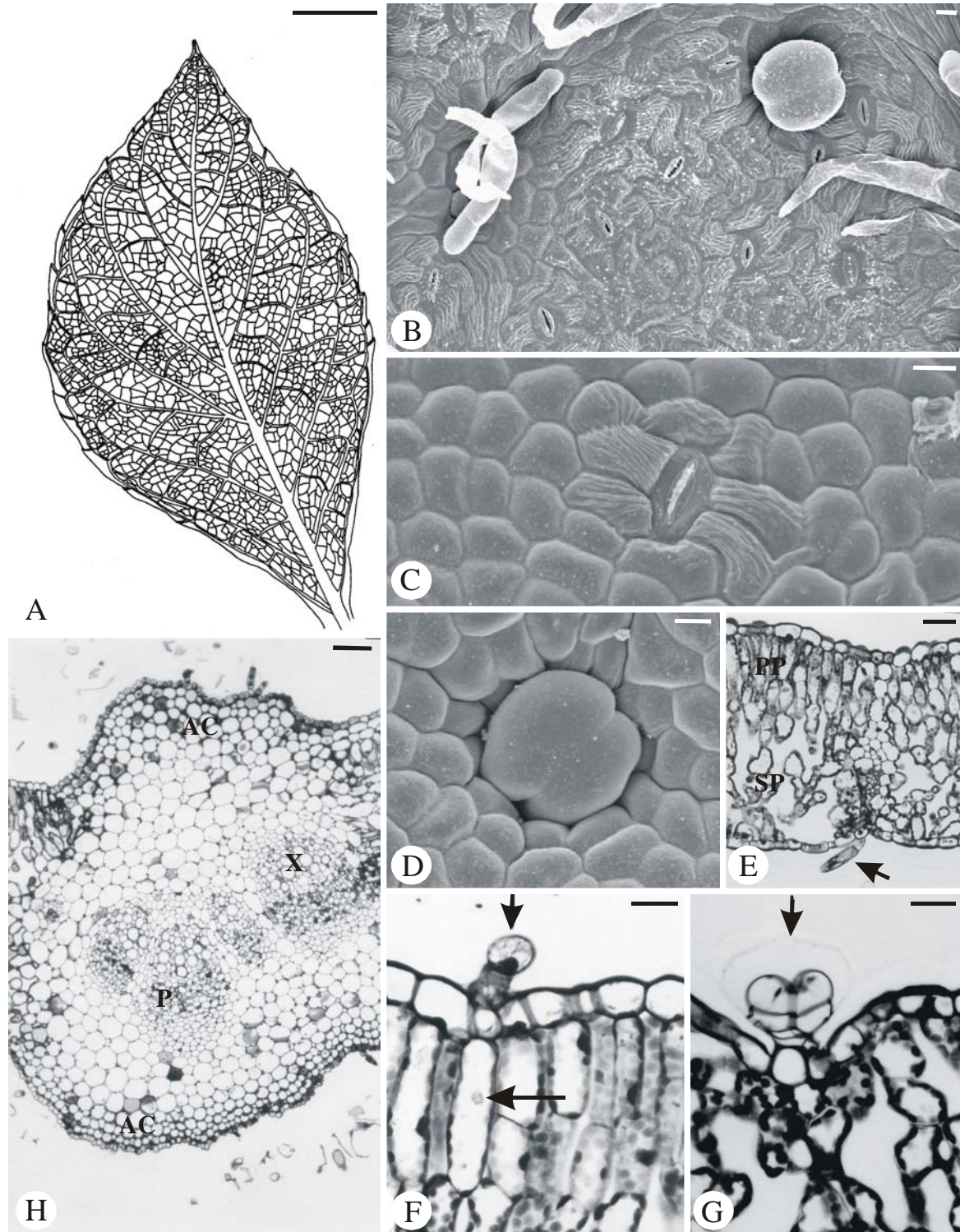
The mesophyll of the studied species varied according to the number of cell layers composing the palisade and spongy parenchyma. Sajo and Menezes (1994) observed that in *V. sessilifolia* and *V. linearis* the dorsiventral mesophyll had a palisade parenchyma close to the adaxial epidermis and three to four layers of spongy parenchyma. In *V. psilophylla* the isobilateral mesophyll had palisade parenchyma close to both epidermis and spongy parenchyma in the middle (Sajo and Menezes, 1994).

The secretory structures also varied among the three studied species. *M. glomerata* had secretory ducts and glandular trichomes; *P. ruderale* had secretory cavities and hydathodes; and *V. condensata* had idioblasts and glandular trichomes. Castro et al. (1997) used the secretory structures as parameters for identification of genera of Asteraceae from a Cerrado vegetation. The authors proposed a classification key which could be applied on *M. glomerata* due to the presence on the leaves of secretory ducts close to the xylem of the vascular bundles and also of glandular trichomes (types II and IV). They also described the characteristics of the secretory ducts

present in *M. officinalis* and *M. sessilifolia* which matched with the description found in this work for *M. glomerata*. These secretory ducts corresponded to the oleiferous reservoirs described by Lersten and Curtis (1985). The absence of these structures in *V. condensata* reinforced the observation made by Castro et al. (1997) that this feature was homogeneous in the tribe Vernonieae.

The analytical key proposed by Castro et al. (1997) did not match with the description of *V. condensata* because this species had no hydathodes, whilst these authors observed this structure confined in the apex of *V. brevifolia*. The different habits of the species could be related to this fact. *V. condensata* is arboreous while *V. brevifolia* is herbaceous. Metcalfe and Chalk (1950) reported that due to the diversity of habits, Asteraceae species show various anatomical structures and some present ecological specialization.

*Porophyllum* have secretory cavities (translucent glands) along the leaf margin or, less frequently, distributed throughout the leaf blade. Fragmented cells and a free nucleus in the lumen characterize its lysigenous origin nearby the procambium in the leaf primordia (Guillet et al., 1998). In *P. ruderale*, the secretory cavities were enveloped by lateral veins of the vascular system (Solereder, 1908). These cavities had also been identified in *P. lanceolatum* (Monteiro et al., 1995) and *P. gracile* (Guillet et al., 1998).



**Figure 3 - *Vernonia condensata* Baker.** **A.** Leaf venation pattern (Bar = 1 cm). **B.** Lower epidermis showing an ornamented cuticle throughout the leaf blade (Bar = 10  $\mu$ m). **C.** Upper epidermis showing striae on cells adjacent to guard-cells (Bar = 10  $\mu$ m). **D- G.** The small arrows indicate glandular trichomes. In E observe the mesophyll at the medium third part of the leaf blade and in F the large arrow shows a small druse in the chlorophyllous parenchyma (Bars = 10, 37, 19 and 19  $\mu$ m, respectively). **H.** Midvein at the medium third part of the leaf blade (Bar = 74  $\mu$ m). AC = angular collenchyma, P = phloem, PP = palisade parenchyma, SP = spongy parenchyma, X = xylem.



The structural features and the cavity's location at the leaf primordia on *P. ruderale* confirmed the description made by Solereder (1908). Guillet et al. (1998) also identified secretory cavities in the leaf of this species. According to these authors, the secretion was volatile; therefore, in this study it was not detected during the histochemical tests in which freehand sections of fresh material were used. Monteiro et al. (1995) found that the contents in the epithelial cells of secretory cavities in *P. lanceolatum* reacted differently to the histochemical tests according to the gland's development stage. The volatile compounds in *P. ruderale* have repellent properties giving the plant resistance against herbivorous insects (Guillet et al., 1998). When damaged, the glands in *P. lanceolatum* released a characteristic smell due to the presence of essential oils and resin in the volatile substance (Monteiro et al., 1995).

The glandular trichomes in *M. glomerata* and *V. condensata* also help the diagnosis of Asteraceae species. Castro et al. (1997) presented a description of some types of trichomes occurring in some genera of Asteraceae and used them to elaborate an identification key. Besides its taxonomical importance, glandular trichomes are useful in attracting the pollinators, defense against herbivorous insects and pathogens attacks (Wagner, 1991).

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## RESUMO

Este trabalho teve por objetivo descrever e comparar a morfo-anatomia das folhas adultas de *Mikania glomerata* Spreng., *Porophyllum ruderale* Cass. e *Vernonia condensata* Baker (Asteraceae), que possuem diferentes hábitos, enfatizando suas estruturas secretoras. Seções longitudinais e transversais dos limbos foliares foram analisadas nas regiões do ápice, da base e do terço médio na altura da nervura central, do limbo foliar e da margem. *M. glomerata* apresentou tricomas

glandulares unisseriados e ductos secretores; *P. ruderale* tinha hidatódios e cavidades secretoras; e *V. condensata* apresentou idioblastos e tricomas glandulares uni- ou bisseriados.

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