

Morpho-anatomical characters of the leaf and stem of *Mandevilla coccinea* (Hook. et Arn.) Woodson, Apocynaceae

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Mandevilla coccinea (Hook. et Arn.) Woodson, Apocynaceae is a herb native to South America employed in folk medicine as an analgesic, anti-inflammatory and to inhibit snake venom effects. This work was carried out to study the morpho-anatomical characters of the leaf and stem in order to advance knowledge on this medicinal plant and on pharmacognostic quality control. The plant material was fixed and prepared according to light and scanning electron microtechniques. Its leaves are simple, alternate and ovate-obovate. The epidermis is uniseriate and coated with a thick and striate cuticle. The stomata are paracytic and occur on both foliar surfaces. The dorsiventral-like mesophyll has a sub-epidermal parenchymatic layer containing phenolic substances and is traversed by minor collateral vascular bundles. The midrib is biconvex and the petiole is concave-convex, both presenting bicollateral vascular bundles in an open arc. The stem is circular in transverse section and the epidermis remains in incipient secondary growth. A sub-epidermal parenchymatic layer containing phenolic substances, a discontinuous sclerenchymatic sheath of non-lignified fibres and cylinders of external phloem, xylem and internal phloem occur. Numerous branched laticifers and idioblasts with phenolic substances are present in the leaf and stem.

Uniterms: *Mandevilla coccinea* (Hook. et Arn.) Woodson/pharmacognosy. *Mandevilla coccinea* (Hook. et Arn.) Woodson/morpho-anatomical characters. Apocynaceae/pharmacognosy. Medicinal plants/pharmacognostic quality control. Phenolic compounds.

Mandevilla coccinea (Hook. et Arn.) Woodson, Apocynaceae, é uma espécie herbácea nativa da América do Sul e empregada na medicina popular como analgésico, antiinflamatório e para inibir os efeitos de veneno de cobra. Este trabalho objetivou estudar os caracteres morfoanatômicos de folha e caule, a fim de fornecer conhecimento dessa espécie medicinal e para o controle de qualidade farmacognóstico. O material foi fixado e preparado de acordo com técnicas usuais de microscopia de luz e eletrônica de varredura. As folhas são simples, alternas e ovado-obovadas. A epiderme é unisseriada e recoberta por uma cutícula espessada e estriada. Os estômatos são paracíticos e ocorrem em ambas as superfícies foliares. O mesofilo tende a ser dorsiventral, apresenta uma camada subepidérmica parenquimática contendo compostos fenólicos e é percorrido por feixes vasculares colaterais de pequeno porte. A nervura central é biconvexa e o pecíolo é côncavo-convexo, ambos apresentando feixes vasculares bicolaterais em arco aberto no parênquima fundamental. O caule tem seção transversal circular e, em estrutura secundária incipiente, a epiderme permanece. Na sequência, encontram-se camada subepidérmica parenquimática contendo compostos fenólicos, bainha esclerenquimática descontínua composta de fibras não lignificadas, além de cilindros de floema externo, xilema e floema interno. Numerosos laticíferos ramificados e idioblastos com substâncias fenólicas estão presentes na folha e no caule.

Unitermos: *Mandevilla coccinea* (Hook. et Arn.) Woodson/farmacognosia. *Mandevilla coccinea* (Hook. et Arn.) Woodson/caracteres morfoanatômicos Apocynaceae/farmacognosia. Plantas medicinais/controle de qualidade farmacognóstico. Compostos fenólicos.

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INTRODUCTION

The genus *Mandevilla* Lindl. belongs to the Apocynaceae family and consists of approximately 110 species which are widely distributed in American tropical regions (Ezcurra, 1981). A few studies have been carried out to identify the chemical constituents of the taxon including cardiac glycosides (Lufrano, Starita, Baldini, 1982; Cabrera, Seldes, Gros, 1993), steroids and triterpenes (Cabrera *et al.*, 1991; Mahlberg, 1993) in *Mandevilla pentlandiana* (A. DC.) Woodson, oleic and linoleic acid in *M. pentlandiana* and *M. laxa* (Ruiz et Pav.) Woodson (Rodrigues, Gusman, 1995), acetylillustrol in *M. illustris* (Vell.) Woodson (Niero *et al.*, 1999), and pregnane glycosides (Calixto, Yunes, 1991b), velutinoside A (Bento *et al.*, 2003) and velutinol A (Yunes *et al.*, 1993; Bento *et al.*, 1996) in *M. velutina* K. Schum. The latter triterpenoid and its glycosylated derivatives were covered by a patent in Canada for their effect of antagonizing the hypotensive activity of bradykinin (Maraschin *et al.*, 2002).

Pharmacological essays conducted in animals have demonstrated antiedematogenic (Calixto *et al.*, 1989; Henriques *et al.*, 1991; Zanini-Jr. *et al.*, 1992; Niero *et al.*, 2002; Mattos *et al.*, 2006), antinociceptive (Santos *et al.*, 2003), anti-inflammatory (Calixto, Yunes, 1991a) and spasmolytic effects (Calixto, Nicolau, Yunes, 1985; Calixto *et al.*, 1988; Calixto, Yunes, 1990; 1991b) of *M. velutina* and *M. illustris* extracts. A significant linear correlation was found between phenol content and anti-oxidant capacity in *M. pentlandiana* (Borneo *et al.*, 2009). In addition, the inhibition of the enzymatic activity of snake toxins by *M. velutina* substances was confirmed (Biondo *et al.*, 2003; 2004) and associated with bioactive triterpenoids, which have also been obtained through cultured cells (Maraschin *et al.*, 2002).

Similar in appearance to *M. velutina*, *M. coccinea* (Hook. et Arn.) Woodson (Figure 1A) is a herb native to South America found in Argentina, Paraguay, Uruguay and Brazil, particularly in tropical savanna called Cerrado (Cervi *et al.*, 2007). It measures about 30 cm high, has an erect stem, alternate leaves and showy red flowers, being cultivated as an ornamental plant (Ezcurra, 1981). Popularly, it is known as jalapa-silvestre-encarnada in Portuguese, and has been employed in folk medicine as an analgesic, anti-inflammatory and to inhibit snake venom effects. Recently, it has been rated as an endangered species because of destructive exploitation (Markgraf, 1968; Takeda, Farago, 2001).

Based on the increasing attention that *Mandevilla* species have been receiving and the lack of available pharmacognostic data involving the taxa, the aim of this work

was to study the morpho-anatomical characters of the leaf and stem of the *M. coccinea*, in order to advance knowledge on this medicinal plant and to provide information for quality control analysis of this potential vegetal drug.

MATERIAL AND METHODS

Plant material

Mandevilla coccinea was collected from the spontaneous flora on the outskirts of the city of Guaíba, state of Rio Grande do Sul, Brazil (30° 10' S and 51° 20' W, altitude of 27 m), in December 2003. A voucher was registered as ICN 90862 in the Herbarium, at the Instituto de Biociências, Universidade Federal do Rio Grande do Sul.

Methods

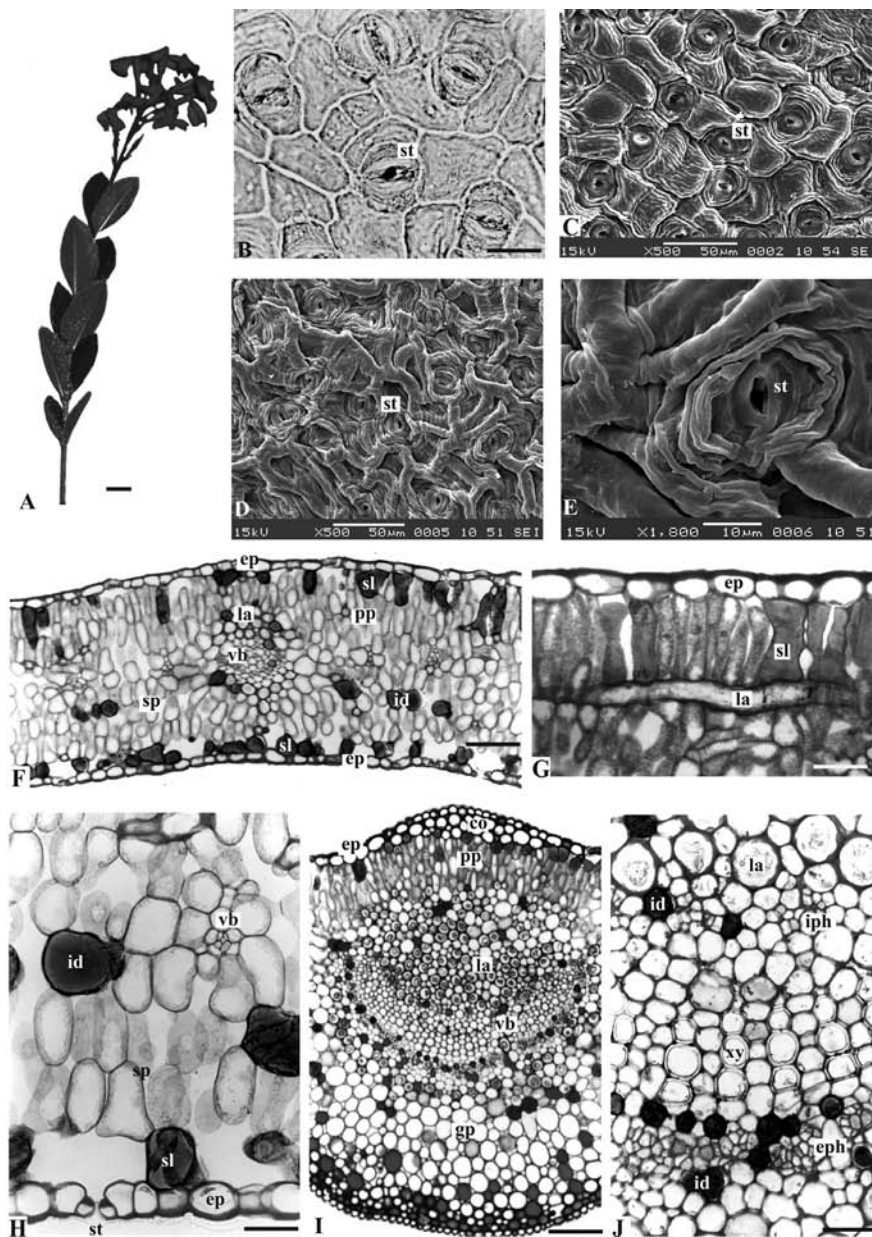
Mature leaves and stem fragments obtained 5–20 cm from the apex were fixed in FAA 70 (Johansen, 1940) and kept in 70 % EtOH solution (Berlyn, Miksche, 1976). The leaf region of the petiole and the lower third of the blade were examined. The fixed material was either freehand sectioned or embedded in glycol-methacrylate (Kraus, Arduin, 1997) and sectioned by microtome transversely and longitudinally. The sections were stained with toluidine blue (O'Brien, Feder, McCully, 1964) or with astra blue and basic fuchsin (Roeser, 1972). Microchemical tests were applied with hydrochloric phloroglucin to reveal lignin (Foster, 1949), Sudan III for lipophilic substances (Sass, 1951), ferric chloride for phenolic substances (Johansen, 1940) and iodine-iodide for starch (Berlyn, Miksche, 1976). The results were registered by photos taken using an Olympus BX40 light microscope.

For scanning electron analysis - SEM (Souza, 1998), leaves fixed in FAA 70 were dehydrated in a graded ethanolic series and by the CO₂ critical point procedure (Bal-Tec CPD-030), gold coated (Balzers SCD-030) and examined by a Jeol JSM-6360LV scanning electron microscope.

RESULTS

The leaves of *M. coccinea* (Figure 1A) are simple, alternate and membranaceous, measuring about 5 cm long and 2.5 cm wide. They are symmetric, ovate-obovate, with acute-acuminate apex, acute-decurrent base, entire margin and short petiole.

The blade has a single-layered epidermis (Figures 1F, 1G) coated with a thick and striate cuticle (Figures 1B-1E). The epidermal cells show wavy contours in



FIGURES 1 - A-J: *Mandevilla coccinea* (Hook. et Arn.) Woodson: A. Reproductive apical branch; B, C. Adaxial epidermal cells, in surface view; D, E. Abaxial epidermal cells, in surface view; F. Transverse section of the blade; G. Laticifer in the mesophyll; H. Minor vascular bundle and idioblasts containing phenolic substances in the blade; I. Midrib in transverse section; J. Detail of the bicollateral vascular bundle of the midrib. Abbreviations: co – collenchyma, ep – epidermis, eph - external phloem, gp - ground parenchyma, id - idioblast with phenolic content, iph - internal phloem, la – laticifer, pp - palisade parenchyma, sl - sub-epidermal layer, sp - spongy parenchyma, st – stomatum, vb - vascular bundle, xy - xylem. Bar = 25 μ m (B,G,H,J), 50 μ m (F,I), 2cm (A).

surface views and paracytic stomata (Figure 1B) occur on both sides. They have an outer ledge and thicker inner periclinal cell wall, and are located on the same level as the surrounding cells (Figure 1H). The mesophyll exhibits a sub-epidermal parenchymatic layer which contains phenolic substances (Figures 1F-1H). On the upper side, the sub-epidermal cells are sparse and have a palisade shape (Figures 1F, 1G), while on the lower side, the layer

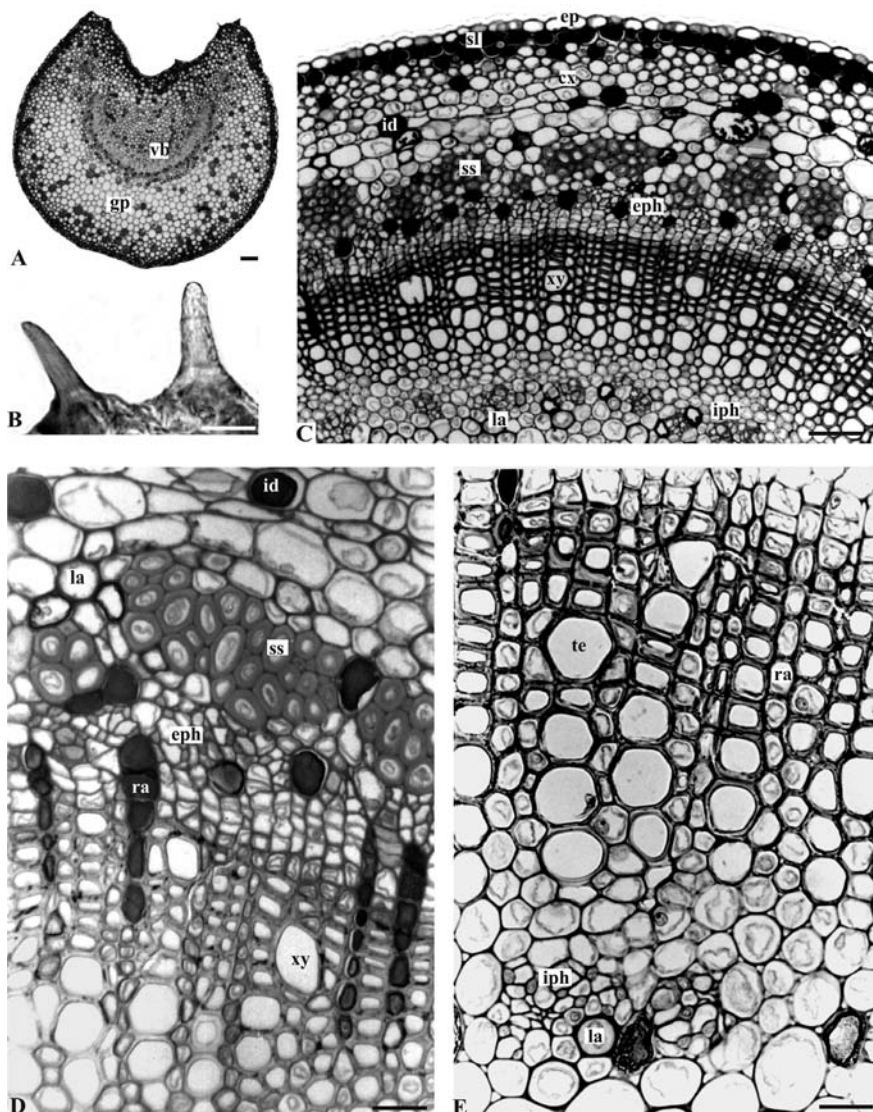
is continuous, except in the substomatal chamber region, and the cells are lobed (Figures 1F, 1H). The mesophyll is dorsiventral-like (Figure 1F), consisting of two or three rows of palisade parenchyma and three to six strata of spongy parenchyma, which represents 50-60% of the chlorenchyma height. The spongy parenchyma is not typically distinctive since its lobed cells tend to be progressively elongated perpendicular to the surface of the blade.

Minor collateral vascular bundles traverse the mesophyll (Figure 1H).

In transverse section, the midrib (Figure 1I) is biconvex, being more prominent on the abaxial side. The epidermis is uniseriate, coated with a thick and striate cuticle, and shows lenticular cells in surface views. Next to the upper epidermal cells, one to three layers of an annular to angular collenchyma occur. Its inner row has cells bearing phenolic substances similarly to the sub-epidermal layer in the blade. The palisade parenchyma is not interrupted and a bicollateral vascular bundle arranged in an open arc is embedded in the ground parenchyma. The cambial zone is evident and the xylem is composed of tracheary elements

in rows separated by parenchymatic cells. The external phloem forms a narrow and continuous strand, while the internal phloem is distributed in small groups (Figure 1J). Some fibres are visible next to the external phloem. On the lower surface, a sub-epidermal parenchymatic layer occurs with phenolic substances and two or three layers of collenchyma.

The petiole has a concave-convex shape in transverse section, with two lateral projections (Figure 2A). These small wings contain few simple and unicellular, non-glandular trichomes with acute or round apex coated with a granular cuticle (Figure 2B). The epidermis is similar to the midrib and next to it a nearly continuous



FIGURES 2 - A-E: *Mandevilla coccinea* (Hook. et Arn.) Woodson: A. Petiole in transverse section; B. Unicellular non-glandular trichomes of the petiole; C. Transverse section of the stem; D, E. Detail of the vascular cylinder. Abbreviations: cx – cortex, ep – epidermis, eph - external phloem, gp - ground parenchyma, id - idioblast with phenolic content, iph - internal phloem, la – laticifer, ra - parenchymatic ray, ss - sclerenchymatic sheath, sl - sub-epidermal layer, te - tracheary element, vb - vascular bundle, xy - xylem. Bar = 25 µm (B,D,E), 50 µm (A,C).

sub-epidermal parenchymatic layer and one to three rows of annular collenchyma are present. A major bicollateral vascular bundle in an open arc (Figure 2A) and about four minor collateral bundles traverse the ground parenchyma.

Several branched laticifers having lipophilic and phenolic substances, dense cytoplasm and thick cell walls (Figures 1G, 1J), while idioblasts with phenolic content (Figures 1F, 1H, 1J) are located in the mesophyll, as well as in the ground parenchyma and phloem of the midrib and petiole.

The stem, in incipient secondary growth (Figure 2C), exhibits circular transverse section. It has a uniseriate epidermis coated with a thick and striate cuticle. The cortex is formed by a sub-epidermal layer whose content is similar to the leaf, plus several rows of thin-walled parenchymatic cells forming small intercellular spaces and an endodermis containing relatively large amyloplasts (Figures 2C, 2D).

In the vascular cylinder, a discontinuous sclerenchymatic sheath composed of fibres with thick and non-lignified cell walls (Figures 2C, 2D) encircles the vascular system. The cambial zone is evident, and the external phloem forms a narrow and continuous strand (Figure 2D), while the internal phloem consists of small groups side by side (Figure 2E). The tracheary elements are distributed orderly in rows, separated by narrow parenchymatic rays which may contain phenolic substances and extend to the external phloem (Figure 2D). The pith occupies a large volume of the stem and comprises isodiametrical thin-walled parenchymatic cells which contain amyloplasts.

Numerous branched laticifers, having polygonal or circular transverse section, thick cell walls and dense cytoplasm, containing lipophilic and phenolic substances (Figures 2D, 2E) are found in the cortex, phloem and pith. Several idioblasts (Figure 2D) also occur and contain phenolic substances.

DISCUSSION

The leaf morphology of *M. coccinea* corresponds to the description for the same species by Markgraf (1968), Ezcurra (1981) and Takeda and Farago (2001), and is similar to the report on *M. illustris* and *M. velutina* by Appezzato-da-Glória (1993).

With regard to anatomical aspects, compared to other species of the family, the occurrence of uniseriate epidermis with wavy anticlinal cell walls in surface view and coated with thick and striate cuticle were also registered in *Mandevilla tenuifolia* (J.C. Mikan) Woodson, *Himatanthus lancifolius* (Müll. Arg.) Woodson (Barros, 1986/88), *H. sucuuba* (Spruce ex Müll. Arg.) Woodson

(Larrosa, Duarte, 2005) and *Plumeria rubra* L. (Araújo, Silva, Gil, 1984; Barreto *et al.*, 2001). By contrast, the epidermis of *M. illustris*, *M. velutina* (Appezzato-da-Glória, 1993), *M. dardanoi* M.F. Sales, Kin.-Gouv. et A.O. Simões and *M. scabra* (Hoffmanns. ex Roem. et Schult.) K. Schum. (Martins, Alves, 2008) exhibit polygonal contours, although this aspect may be influenced by the environment. In the midrib, the epidermal cells of *M. coccinea* have lenticular outer periclinal cell walls, which is equivalent to the papillose epidermis described in *M. pohliana* (Stadelm.) A. Gentry (Appezzato-da-Glória, Estelita-Teixeira, 1992). According to Neinhuis and Bathlott (1997), convex epidermal cells favour water-repellency and therefore the removal of particulate contamination.

Concerning the epicuticular waxes and trichomes which are considered relevant in comparative systematic surveys (Metcalf, Chalk, 1988), the former with evident ornamentation was not seen in this investigation. Simple non-glandular trichomes were found in the petiole of *M. coccinea*, and similar appendages were reported in the leaf of *M. dardanoi*, *M. scabra*, *M. tenuifolia* (Martins, Alves, 2008), *P. rubra* (Araújo, Silva, Gil, 1984; Barreto *et al.*, 2001), *Allamanda nerifolia* Hook and *Vinca minor* L. (Fjell, 1983).

Apocynaceae members have anomocytic or paracytic stomata (Metcalf, Chalk, 1950; 1988), and the latter type was encountered in *M. coccinea* on both surfaces. Accordingly, in *M. pohliana* (Appezzato-da-Glória, Estelita-Teixeira, 1992) and *M. scabra* (Martins, Alves, 2008) the leaf is amphistomatic and the stomata are paracytic.

Dorsiventral mesophyll, single or multiple layered hypoderm and bicollateral vascular bundle in the midrib, are common in Apocynaceae (Metcalf, Chalk, 1950). *Mandevilla coccinea* shows a dorsiventral-like mesophyll as does *M. pohliana* (Appezzato-da-Glória, Estelita-Teixeira, 1992), *M. dardanoi*, *M. scabra* and *M. tenuifolia* (Martins, Alves, 2008). The sub-epidermal parenchymatic layer described herein was also reported for the three species of *Mandevilla* studied by the previous authors and corresponds to the hypoderm published in *M. pohliana* by Appezzato-da-Glória and Estelita-Teixeira (1992). The presence of a bicollateral vascular bundle in the midrib and minor collateral vascular bundles located in the mesophyll are assumed to be common, since internal phloem may not extend into the smaller veins in leaves (Esau, 1977). These findings were also registered in *M. pohliana* (Appezzato-da-Glória, Estelita-Teixeira, 1992), *M. dardanoi*, *M. scabra*, *M. tenuifolia* (Martins, Alves, 2008), *H. sucuuba* (Larrosa, Duarte, 2005) and *P. rubra* (Araújo, Silva, Gil, 1984). In addition, the distinctive arrangement of the external and internal phloem as well as the trache-

ary elements aligned in rows, were previously mentioned for the family (Metcalf, Chalk, 1950; Cronquist, 1981).

Together with the internal phloem, laticifers are anatomical characters universally occurring in Apocynaceae (Metcalf, Chalk, 1950). They may be branched or unbranched non-articulated laticifers (Esau, 1977; Mahlberg, 1993). Following the pattern, *M. dardanoi*, *M. scabra* and *M. tenuifolia* have shown branched non-articulated laticifers (Martins, Alves, 2008). Nevertheless, Lopes *et al.* (2009) have described articulated laticifers in *M. atrovioleacea* (Stadelm.) Woodson. The laticifer cell walls are primary and composed of cellulose, pectic substances and hemicellulose (Murugan, Inamdar, 1987; Fahn, 1990), and may be irregularly thick due to their plasticity (Mahlberg, 1993). The laticifers observed in *Plumeria alba* L., *M. illustris* and *M. velutina* are larger than the neighbouring cells and have polygonal or circular transverse section, conspicuous nucleus, dense cytoplasm and no starch (Murugan, Inamdar, 1987; Appezato-da-Glória, Estelita, 1997), features also found in this study. In *M. atrovioleacea*, the laticifer secretion contained an emulsion of lipophilic substances rich in neutral lipids, resin oils and possibly rubber (Lopes *et al.*, 2009).

Remaining caulinar epidermis was recorded for herbaceous species (Esau, 1977) and for *M. pohliana* (Appezato-da-Glória, Estelita-Teixeira, 1992), which also shows hypoderm containing phenolic substances, similarly to the sub-epidermal layer of *M. coccinea*. The caulinar organization of this species corresponds to the description for *M. illustris*, *M. velutina* (Appezato-da-Glória, 1993), *M. dardanoi*, *M. scabra* and *M. tenuifolia* (Martins, Alves, 2008). Regarding the reported idioblasts storing phenolic substances, Beckman (2000) attributed various roles in general defense responses to these, such as programmed cell death and periderm formation.

As expected, the described morpho-anatomical characters of *M. coccinea* follow the Apocynaceae pattern and are conducive to the identification of the species, while contributing valuable knowledge on the Brazilian flora. However, further research on the genus is required to evaluate whether the anatomical findings have taxonomical relevance and are useful to distinguish allied species.

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