Leaf morphoanatomy of the medicinal *Maytenus imbricata* (Celastraceae): an ecological approach

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Abstract

Background: The leaves of many species of *Maytenus* are used to prepare herbal drugs to treat stomach ulcers.
Questions: In order to define key anatomical characters for the unambiguous species identification, the present study aimed to describe the leaf morphoanatomy of *M. imbricata*.
Species study: *Maytenus imbricata* Mart. ex Reissek (Celastraceae)
Study site and dates: Collected in an area of 'campo rupestre' - one of the Brazilian Savannah vegetation (20º 22' 11.02" S, 43º 30' 22.81" W) in August 2015.
Methods: The plant material was processed according to commonly used techniques in plant anatomy studies.
Results: The major anatomical characters for several species of *Maytenus* identification also present in *M. imbricata* are marginal teeth, conspicuous epicuticular wax, ciclocytic stomata, thick external periclinal wall with large pits on epidermal cells and vascular system with a closed loop shape in cross-section. Strips on the anticlinal walls on epidermal cells and conspicuous gelatinous fibers sheath are diagnostic for *M. imbricata*.
Conclusions: Strips on the anticlinal wall of the epidermal cell are reported for the first time for the genus. The xeromorphic traits help the species to survive in arid environments and may contribute to quality control of the raw material used in the production of herbal medicines.
Keywords: cell wall strips, colleter, gelatinous fiber, xeromorphic.

Resumen

Antecedentes: Las hojas de muchas especies de *Maytenus* se utilizan para la preparación de medicamentos para el tratamiento de las úlceras de estómago.
Preguntas: Con la finalidad de contribuir a la identificación de la especie, este estudio tuvo como objetivo describir la morfología y anatomia de la hoja de *M. imbricata*.
Especie de estudio: *Maytenus imbricata* Mart. ex Reissek (Celastraceae)
Sitio y años de estudio: Recolecta de material en una zona de 'campo rupestre' - vegetación del cerrado brasileño (20º 22' 11.02" S, 43º 30' 22.81" W) en agosto del 2015.
Métodos: El material vegetal se procesó mediante las técnicas que se utilizan en los estudios de anatomía vegetal.
Resultados: Los principales caracteres anatómicos para la identificación de varias especies de *Maytenus*, que están presentes en *M. imbricata*, son los dientes marginales, la cera epicuticular gruesa, los estomas ciclocíticos, las células epidérmicas con la pared periclinal externa gruesa, con grandes punteaduras y la sección transversal del sistema vascular en un círculo cerrado. Las estrías en las paredes anticlinales y las fibras gelatinosas son caracteres diagnósticos para *M. imbricata*.
Conclusiones: Las estrías que se forman en la pared de las células epidérmicas se reportan por primera vez en el género. Estos caracteres xeromórficos ayudan a la especie a sobrevivir en ambientes áridos y pueden contribuir al control de la calidad de la materia prima utilizada en la producción de medicamentos.
Palabras clave: caracteres xeromórficos, coléteres, estrías en la pared celular, fibras gelatinosas.

Maytenus Molina is one of the largest genera of Celastraceae, found in tropical and subtropical regions, and distributed throughout the Brazilian territory (Lombardi *et al*. 2014). Many of the chemical components isolated from its species have pharmacological properties (Din *et al*. 2013, Duarte *et al*. 2013, Moo-Puc *et al*. 2014). A good example is *M. ilicifolia* Mart. ex Reissek, that is mentioned in the Brazilian Pharmacopoeia (ANVISA 2011) to treat gastric ulcer (Santos-Oliveira *et al*. 2009). Similarly, *M. imbricata* Mart. ex Reissek, (Silva *et al*. 2009, Rodrigues *et al*. 2012), *M. aquifolium* Mart., (Gonzalez *et al*. 2001), *M. salicifolia* Reissek, (Magalhães *et al*. 2011) and *M. truncata* Reissek, (Silva *et al*. 2005a) are used in folk medicine to treat the same stomach illness, and have the same popular name "espinheira-santa" (Niero *et al*. 2011). For these reasons it is necessary a careful quality control of raw material in the manufacture of drugs using *Maytenus* species and is required define anatomical key characters for the unambiguous species identification (Jacomassi & Machado 2004, Duarte & Debur 2005, Joffily & Vieira 2005, Nakamura *et al*. 2013). For five species of *Maytenus*, Den Hartog & Bass (1978) elected stomatal type and the presence of trichomes and crystals as safe parameters among others epidermal characteristics. Duarte & Debur (2005) proposed epidermal cells containing styloids, cuticular flanges and amphicrival vascular bundles as relevant characters for the diagnosis of *M. ilicifolia*.

M. imbricata is known for the presence of bioactive compounds (Silva *et al*. 2005b, 2007, Silva *et al*. 2009, Rodrigues *et al*. 2012) nevertheless there is no study to quality control. The goal of this work was to describe the morphological and anatomical features of the leaf and relate them to the environment where the species occurs.

Materials and methods

Fully expanded leaves of *M. imbricata* were obtained from three individuals in an area of 'campo rupestre' (one of the Brazilian Savannah vegetation, 20º 22' 11.02"S, 43º 30' 22.81"W) and they represent the morphological variation of the studied population. A voucher specimen (MCTB Messias, 2565) was deposited at the OUPR Herbarium (Universidade Federal de Ouro Preto, Minas Gerais, Brazil). Samples of the median region of the leaf blade and the petiole were fixed in Karnovsky solution (Karnovsky 1965) for 12 hours, dehydrated in a graded ethanol series (Jensen 1962), and cold-embedded (Paiva *et al*. 2011) in hydroxyethyl-methacrylate resin (Leica Microsystem Inc., Heidenbeg, Germany). Cross sections of the median rib, transverse and paradermal sections of the internerval region, and cross-sections of the margin and the petiole were obtained using a rotary microtome (Atago, Tokyo, Japan), stained with Toluidine blue pH 4.7, rutenium red (O'Brien *et al*. 1964, modified), basic Fuchsin and Astra blue and mounted in acrylic resin (Itacril, Itaquaquecetuba, Brazil). Phenolic compounds were identified by the red color obtained with basic Fuchsin and bright blue color obtained with Tolulidine blue, and acids polysaccharides with ruthenium red. Images were captured using an AxioCam MRc camera coupled to a AxioVision LE light microscope (Zeiss, Oberkochen, Germany).

Results

Morphology. The leaves are light green, congested, erect, imbricate, coriaceous and glabrous, and are coated on both sides by a conspicuous wax layer. Leaf shape varies from orbicular to oval, the base is slightly cordate and the apex is obtuse-emarginate. The margins are revolute, crenate, and have teeth. The petiole is 0.1 to 0.2 cm long, and the leaf blade is 1.5 to 3 cm long and 1 to 3 cm wide with a protruding central nerve on the adaxial side. Secondary nerves are evident at the leaf surface but are not protruding (Figure 1A). The venation is craspedodromous, and the marginal teeth contain multicellular secretory structures named colleters (Figure 1B-C).

Anatomy. At the midrib region the epidermal cells have elongated anticlinal wall and the vascular system has the shape of a closed loop in cross-section, and is surrounded by a conspicuous sheath of gelatinous sheath (Figure 2A). At the internerve region the cells of the adaxial face of the epidermis have thick external periclinal wall, and straight anticlinal wall with strips on

Author Contributions.
Silvia Ribeiro de Souza, Izabel Cristina Trindade and Ariadna Conceição dos Santos performed the botanical histological study.
Maria Olívia Mercadante-Simões, Lucienir Pains Duarte and Sidney Augusto Vieira-Filho conceived the work and guided its execution by the students.
Grácia Divina de Fátima Silva and Maria Cristina Teixeira Braga Messias carried out the ethnobotanical study, the field collection and the botanical identification.

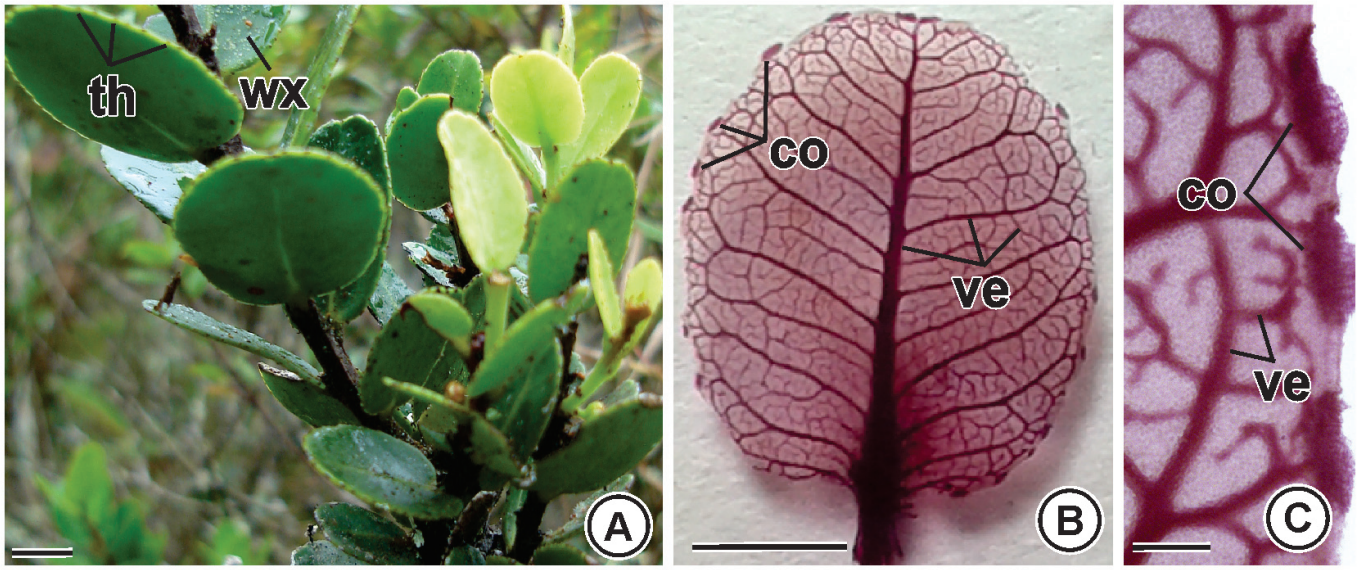

Figure 1. Leaf morphology of *Maytenus imbricata* Mart. ex Reiss. **A)** General view of the plant showing the rounded marginal teeth and epicuticular wax. **B)** Venation craspedodromous. **C)** Colleters on marginal teeth. co: colleters; th: teeth; ve: vein; wx: waxy. Bars: A-C = 1cm.

its innermost region (Figure 2B-C). The mesophyll is dorsiventral with palisade parenchyma composed of two layers of transversely elongated cells, and spongy parenchyma composed of globoid cells compactly arranged facing the abaxial surface (Figure 2D-E). The presence of phenols is observed throughout the whole leaf tissues (Figure 2A-E).

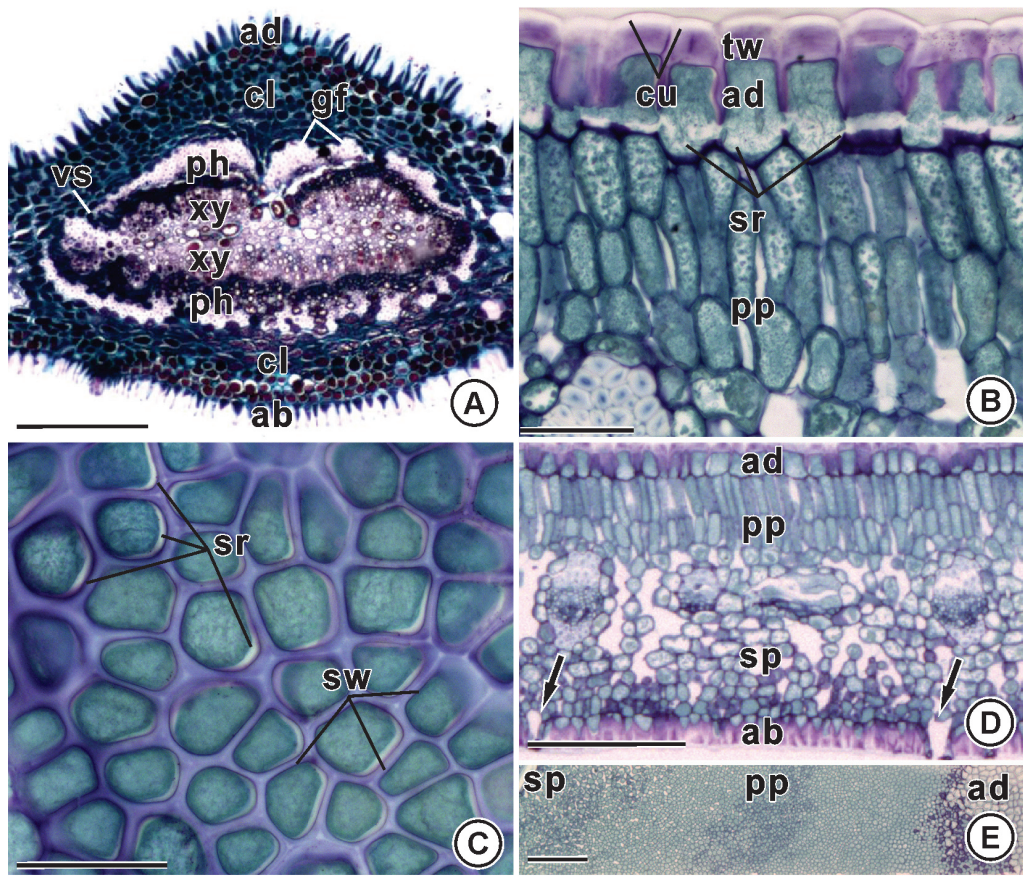

Figure 2. Leaf anatomy of *Maytenus imbricata* Mart. ex Reiss. **A)** Midrib region. Vascular system with a closed-loop shape (cross section). **B-C)** Cells of the adaxial epidermis with strips on the base of the straight anticlinal walls (cross section on B, and paradermic section – external view on C). **D-E)** Internerve region. Hypostomatic leaf (arrows). Dorsiventral mesophyll (cross section on D and paradermic-oblique section on E). ab: abaxial epidermis; ad: adaxial epidermis; cl: collenchyma; cu: cuticle; gf: gelatinous fibers; ph: phloem; pp: palisade parenchyma; sr: strips; sp: spongy parenchyma; sw: straigth anticlinal wall; tw: thick external periclinal wall; vs: vascular system; xy: xylem. Bars: A = 200 µm; B-E = 50 µm.

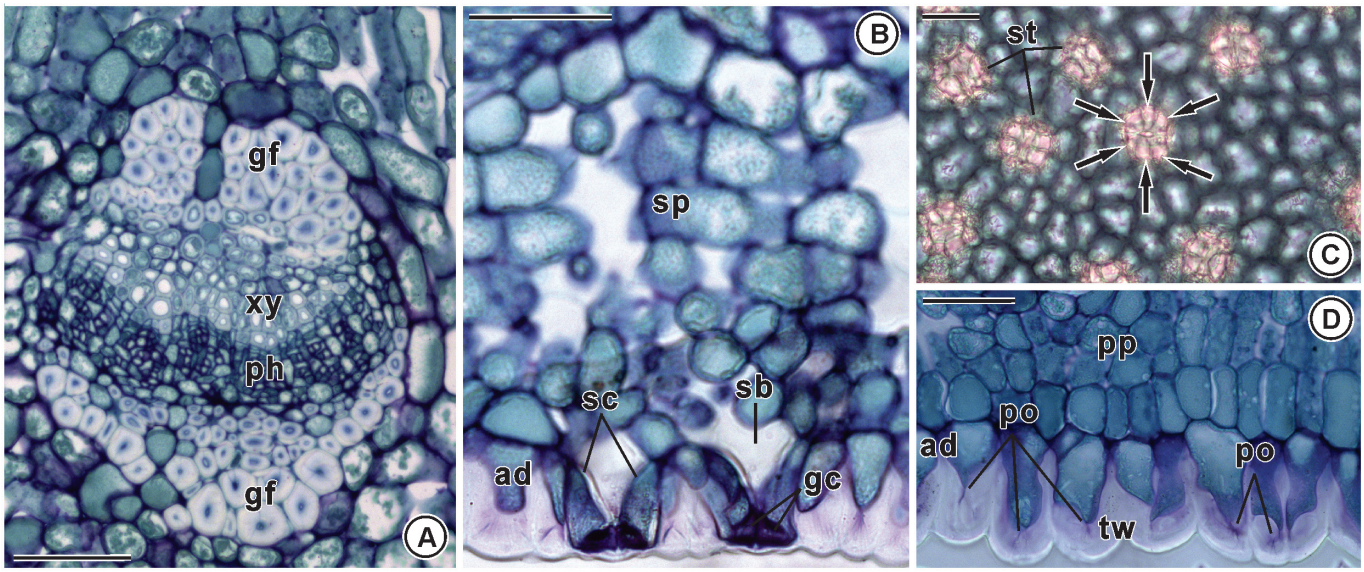

Figure 3. Leaf anatomy of *Maytenus imbricata* Mart. ex Reiss. **A)** Collateral vascular bundle with gelatinous fibers on both sides (cross section). **B)** Subsidiary pear-shaped cells with narrow diameter on the leaf surface, and voluminous internally (cross section). **C)** Ciclocytic stomata (paradermic section – internal view). **D)** Thick external wall of the ordinary cells of the abaxial epidermis with large pits as pores (cross section). gf: gelatinous fibers; ph: phloem; po: pore; sb: substomatal chamber; sc: subsidiary cell; sp: spongy parenchyma; st: stomata; tw: thick wall; xy: xylem. Bars = 50 μm.

The vascular bundles in secondary veins are collateral and gelatinous fibers are on both sides (Figure 3A). In cross section, subsidiary cells have pear-shaped and narrow diameter on the leaf surface, and are internally voluminous (Figure 3B). The leaf is hypostomatic displaying a higher number of ciclocytic stomata on the adaxial face (Figures 2D, 3B-C). The external walls of the ordinary cells of the abaxial epidermis are thick and show large pits such as pores (Figure 3B, D).

In leaf margin, the epidermal cells have thick cuticle and thick pectic walls. The mesophyll is homogeneous and involves a bicolateral vascular bundle with a prominent gelatinous fibers cap on one side (Figure 4A). The petiole outline is rounded with two lateral ribs on the adaxial side and is covered by epidermal cells with thick cuticle and thick pectic walls. The outer cortex is constituted by cells with periclinal divisions and the inner cortex has sclereids clusters. The vascular system is closed loop, and it is wrapped by a discontinuous sheath of gelatinous fibers and displays cambial activity. The secondary xylem is well developed and encircle a parenchymatous pith (Figure 4B).

Discussion

The structural features present in plant organs are useful tools for quality control in the manufacture of herbal medicines. The leaf margin teeth are an important feature in the identification of *Maytenus* species (Joffily & Vieira 2005, Lombardi & Groppo 2014). The thorns and scars present on the end of the teeth indicate the drop of the colleters observed at the primordial leaf margin. Some anatomical characters in leaves occur in response to environmental conditions (Voltolini & Santos 2011, Sack & Scoffoni 2013). Colleters secrete resin or mucilage to protect the stem apex against desiccation, which is favored by the low humidity of the air during the dry season in Brazilian Savannah (Mercadante-Simões & Paiva 2013).

Epidermal traits are particularly useful for quality control purpose, because they are well preserved even in pulverized material. In this work ciclocytic stomata were found in *M. imbricata* whereas anomoctic stomata were reported for *M. aquifolia* and *M. ilicifolia* (Jacomassi &

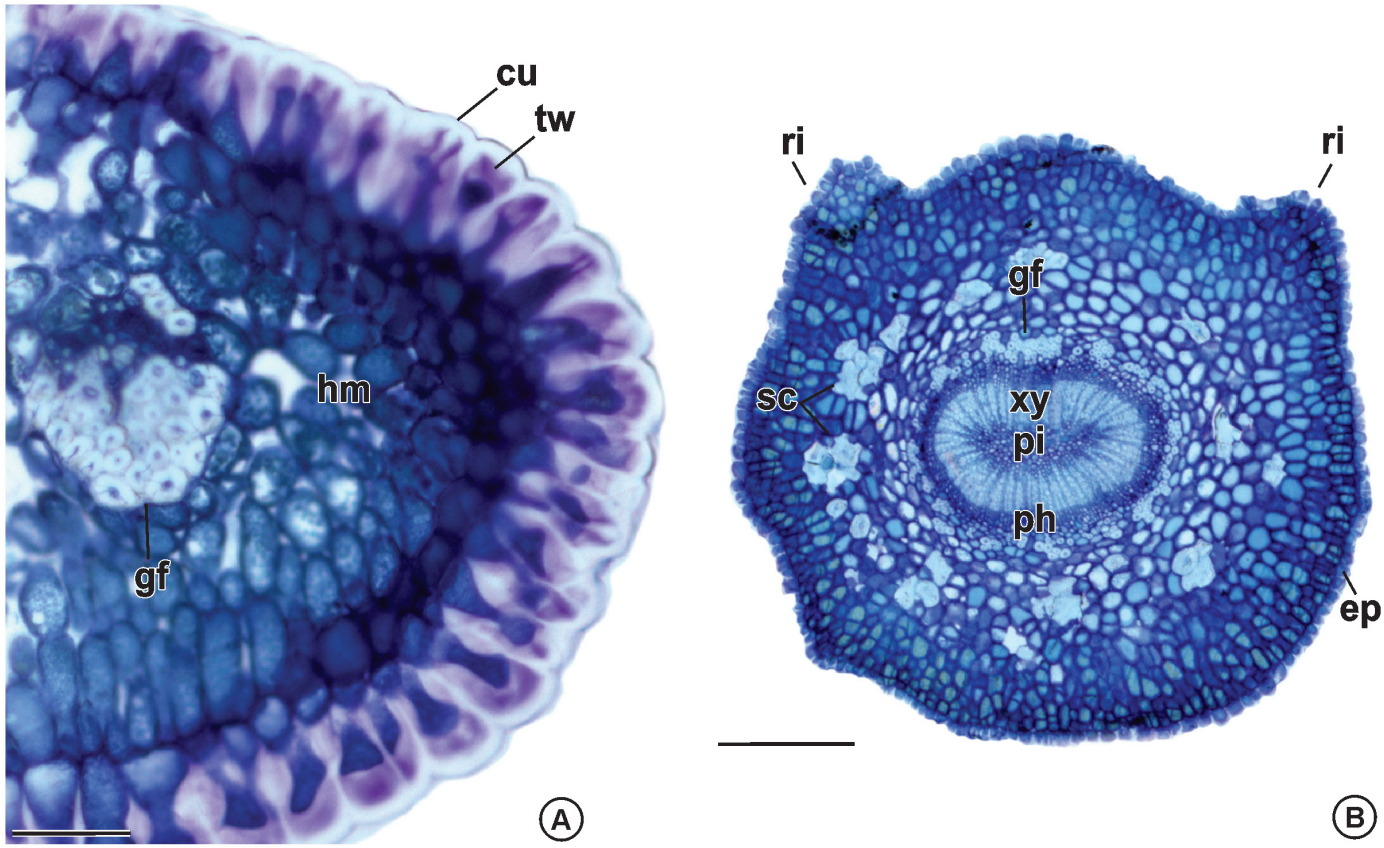

Figure 4. Leaf anatomy of *Maytenus imbricata* Mart. ex Reiss. **A)** Leaf margin: epidermal cells with thick cuticle and pectic walls, homogeneous mesophyll and a bicolateral bundle with gelatinous fibers on both sides. **B)** Petiole: ribs on the adaxial side, epidermal cells with thick cuticle and thick pectic walls, outer cortex cells with periclinal divisions, inner cortex with clusters of sclereids, vascular system closed loop, sheath of gelatinous fibers, cambial activity, secondary xylem and parenchymatous pith. cu: cuticle; ep: epidermis; gf: gelatinous fibers; hm: homogenous mesophyll; ph: phloem; pi: pith; ri: ribs; sc: sclereids; tw: thick wall; xy: xylem. Bars: A = 50 µm; B = 250 µm.

Machado 2004). Acicular crystals on both sides of the epidermis were recorded for *M. ardisiaefolia*, *M. brasiliensis*, *M. communis* and *M. obtusifolia* (Joffily & Vieira 2005) but not observed in *M. imbricata*. The presence of epicuticular wax, thick cuticle and thick periclinal wall on the epidermis of *M. imbricata* has also been recorded in other species of Maytenus (Duarte & Debur 2005, Joffily & Vieira 2005). The thickening of the epidermal cell walls of *M. imbricata* seems to be related to the restriction of water loss by transpiration. Also, the reduced area of exposure of the subsidiary cell on the leaf surface minimizes water loss under high solar radiation conditions (Voltolini & Santos 2011).

The presence of pores in the wall of epidermal cells and the occurrence of guard cells arranged in rosette and subsidiary cells positioned below the surface in *M. imbricata* have been reported for other species of Celastraceae (Metcalfe & Chalk 1957). However, strips on the epidermal cell wall are reported for the first time for *Maytenus* and their role in the control of water traffic in apoplast is well recognized (Moreira & Isaias 2008, Yang *et al*. 2011). The 'Phi thickening' on the cell walls was also related to the water traffic control (Fernandez-Garcia *et al*. 2009, Idris & Collings 2015). On the other hand, the occurrence of large pits such as pore on the wall in *M. imbricata* may favor the deposition of deterrent compounds throughout the whole leaf surface protecting against herbivory and microorganisms infection (Yeats & Rose 2013).

Cells containing phenolic compounds are common in *Maytenus* species (Gupta & Sharma 2012) and have also been reported for other genera of Celastraceae (Mercadante-Simões *et al*.

2014). The secondary metabolites are responsible for the relation of the plant with the biotic and abiotic environment. Bioactive compounds accumulation can be induced by the severe water constraint conditions in the Brazilian Savannah (Di-Ferdinando *et al*. 2014).

The major branching of the craspedodromous venation in *M. imbricata* is related to the need of an adequate water supply. The vein length for area has a strong correlation with environmental aridity and higher values were found to sun plants when compared to shade plants (Sack & Scoffoni 2013). In our work a sheath of gelatinous fibers was found in *M. imbricata*. These characters were not observed in other species of *Maytenus* but a vascular bundle with a sclerenchymatic sheath at the margin were reported for *M. aquifolia* and *M. ilicifolia* (Jacomassi & Machado 2004) and perivascular fibers near the vascular system were observed in *M. ardisiaefolia*, *M. brasiliensis*, *M. communis* and *M. obtusifolia* (Joffily & Vieira 2005). The conspicuous sheath of gelatinous fibers surrounding the vascular bundles of *M. imbricata* contributes to the better use of water absorbed by the xylem. The gelatinous fibers have thickened walls rich in acid polysaccharides allowing water retention (Melo *et al*. 2011, Sonsin *et al*. 2012).

Conclusions

The structural features on medicinal plants are useful for their unequivocal identification, particularly when they are in small pieces to be used in herbal medicine production. The key characters to *M. imbricata* are strips on the epidermal cell anticlinal wall, reported here for the first time for the genus, and conspicuous gelatinous fibers sheath. These structural characters are related to the dry Brazilian Savanna conditions.

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