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Stem and leaf morphoanatomy of *Maytenus ilicifolia*

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Abstract

Maytenus ilicifolia is a woody medicinal plant, employed mainly for its antiulcerogenic properties. The stem and leaf morphoanatomy has been studied, aiming to supply knowledge for the pharmacognostic and taxonomic species identification. The vegetative material was fixed, freehand sectioned and stained according to usual microtechniques. The stem organization, in secondary growth, shows periderm beneath the remaining epidermis, conspicuous sclerenchymatic ring in the cortex and cambium forming phloem outside and xylem inside. The leaf is simple, alternate and lanceolate and has sparsely spiny teeth along the margin. Epidermal cells containing calcium oxalate crystals, thick cuticle that forms cuticular flanges, dorsiventral mesophyll and amphicrival bundle in the midrib and petiole are observed.

Keywords: *Maytenus ilicifolia*; Morphoanatomy

1. Introduction

The Celastraceae family comprises approximately 50 genus and 800 species, distributed mostly in tropical and subtropical regions [1]. The genus *Maytenus* Molina consists of woody and shrubby species [2], often employed in the traditional medicine and

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investigated mainly for phytochemical and pharmacological purposes, such as *Maytenus aquifolium* Mart. [3–6], *M. chuchuhuasha* Raymond-Hamet et Colas [7,8], *M. evonymoides* Reiss. [9], *M. hookeri* Loes. [10], *M. macrocarpa* (Ruiz et Pav.) Briq. [11], *M. robusta* Reiss. [12] and *M. senegalensis* (Lam.) Exell. [13–16].

Various studies have been carried out with *Maytenus ilicifolia* Mart. ex Reiss. (Celastraceae), an indigenous tree to Brazil [17], to evaluate the antiulcerogenic [4,18], abortifacient [19], mutagenic [20] and toxicologic effects [21,22] and antifungal activities [23]. The presence of cytotoxic aromatic triterpenes [7,24], oxidant agents [25,26], flavonoids [27,28], triterpenes [12] and glucosides [29] was reported. Nevertheless, as morphological aspects have not been emphasized for aerial vegetative organs, this work has studied the stem and leaf morphoanatomy of this plant, aiming to supply knowledge for the medicinal plant identification and for the taxonomy of related species.

2. Experimental

2.1. Plant material

M. ilicifolia plant material was collected at the Horto de Plantas Medicinais, Departamento de Farmácia, Universidade Federal do Paraná (25°26′ 45″ S and 49°14′ 25″ W), in August 2002. The dried flowering material was identified, and a voucher herbarium specimen was registered under number MBM 253825 in the Museu Botânico Municipal de Curitiba.

2.2. Analysis

Fresh stem fragments, collected 10 cm from the apex, and fresh mature leaves were fixed in FAA 70 [30] and maintained in 70% EtOH solution [31]. The examined foliar region was the petiole and the lower third of the blade and midrib. Transverse and longitudinal freehand sections were stained either with toluidine blue [32] or with basic fuchsine and astra blue combination [33]. Histochemical reactions were applied with hydrochloric phloroglucin to reveal lignified elements [34], iodine–iodide for starch [31], Sudan IV for lipophilic substances [35] and ferric chloride for phenolic compounds [30].

The results were registered by botanical illustration, diagrams and photos taken by means of the Olympus BX40 optical microscope equipped with the control unit PM20.

3. Results

3.1. Stem morphoanatomy

M. ilicifolia Mart. ex Reiss. (Celastraceae; Fig. 1) reveals circular stem transection (Fig. 2a). In secondary growth, the epidermis may remain in the stem, although the

Fig. 1. Apical branch aspect of *M. ilicifolia*.

phellogen is installed in the first layers of the cortex, originating phellem outside. A few strata of cortical parenchyma containing amiloplasts and phenolic compounds are seen in the cortex, which is internally bounded by a sclerenchymatic ring (Fig. 2b), almost continuous and composed of fibers and stone cells (Fig. 2c). In the vascular cylinder, the cambium produces phloem toward the outside, and xylem, toward the inside, both showing one- to two-layered parenchymatic medullary rays (Fig. 2b). These rays may have amiloplasts and contain phenolic compounds. In the xylem, the tracheary elements, distributed in orderly rows, are often solitary and surrounded by fibers (Fig. 2d). At the centre of the stem, encircled by the xylem, lies the pith. It has an irregular contour and is formed by parenchymatic cells, which may be lignified and present evident simple pits. Calcium oxalate prisms are seen in the cortex and pith (Fig. 2c).

3.2. Leaf morphoanatomy

M. ilicifolia (Fig. 1) has simple and entire leaves, with alternate phyllotaxy, lanceolate shape, acute apex and round base, measuring about 5 cm long and 2 cm wide. The margin has sparsely spiny teeth, and the petiole is short. The foliar surface is coriaceous and glabrous, and the midrib is more prominent on the abaxial side.

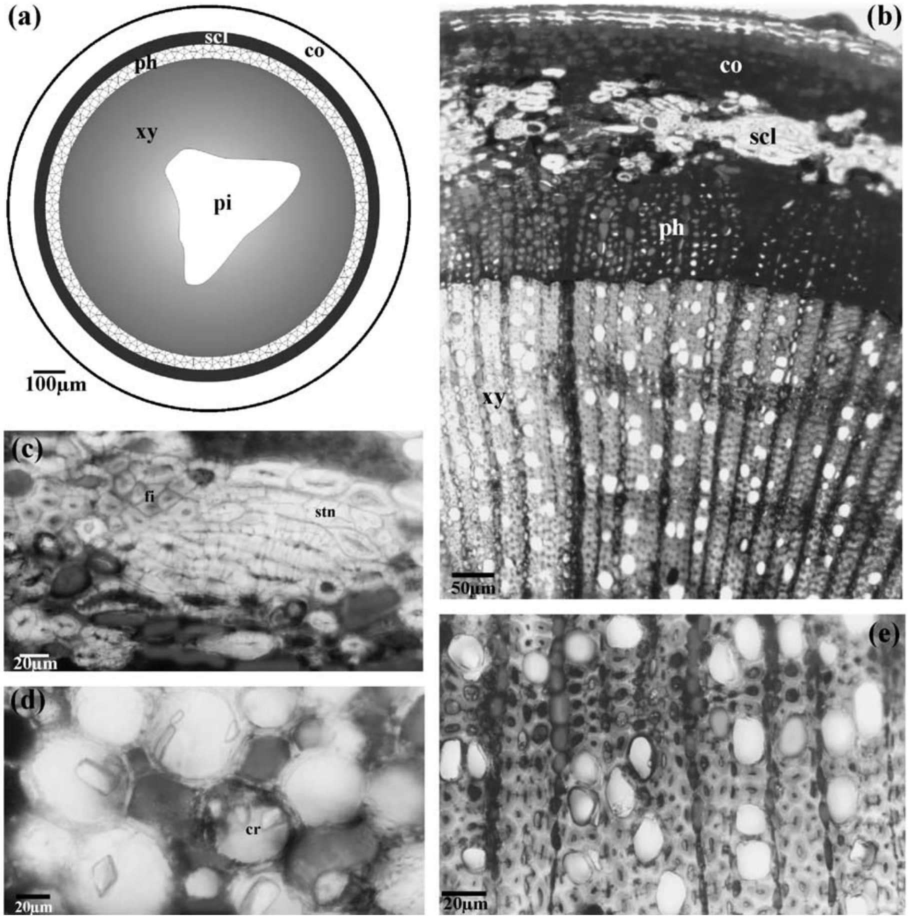

Fig. 2. Stem structure of *M. ilicifolia*. (a) Diagram of the stem organization in secondary growth; (b) transverse section; (c) detail of the sclerenchymatic ring, composed of fibers and stone cells; (d) oxalate calcium crystals in the pith; (e) detail of the xylem. Abbreviations—co: cortex; cr: crystals; ep: epidermis; fi: fibers; ph: phloem; pi: pith; scl: sclerenchymatic sheath, stn: stone cells; xy: xylem.

The blade, in face view, has polygonal epidermal cells, containing small calcium oxalate styloids and prisms (Fig. 3a). Anomocytic stomata are seen on the abaxial surface (Fig. 3b) and are even with the other epidermal cells (Fig. 3d). The guard cells present outer stomatal ledges, and the surrounding cells bear ridges of cuticle, which overlap the guard cells (Fig. 3b). The epidermis is uniseriate and coated by a smooth and thick cuticle, which extends into adjacent anticlinal cell walls, forming cuticular flanges (Fig. 3c).

The mesophyll reveals dorsiventral organization, comprising three to five strata of palisade parenchyma and various layers of spongy parenchyma, the latter occupying 60% of the mesophyll (Fig. 3e). Minor vascular bundles are enclosed by a sheath of parenchymatic cells and may present sclerenchymatic caps adjoining the phloem and,

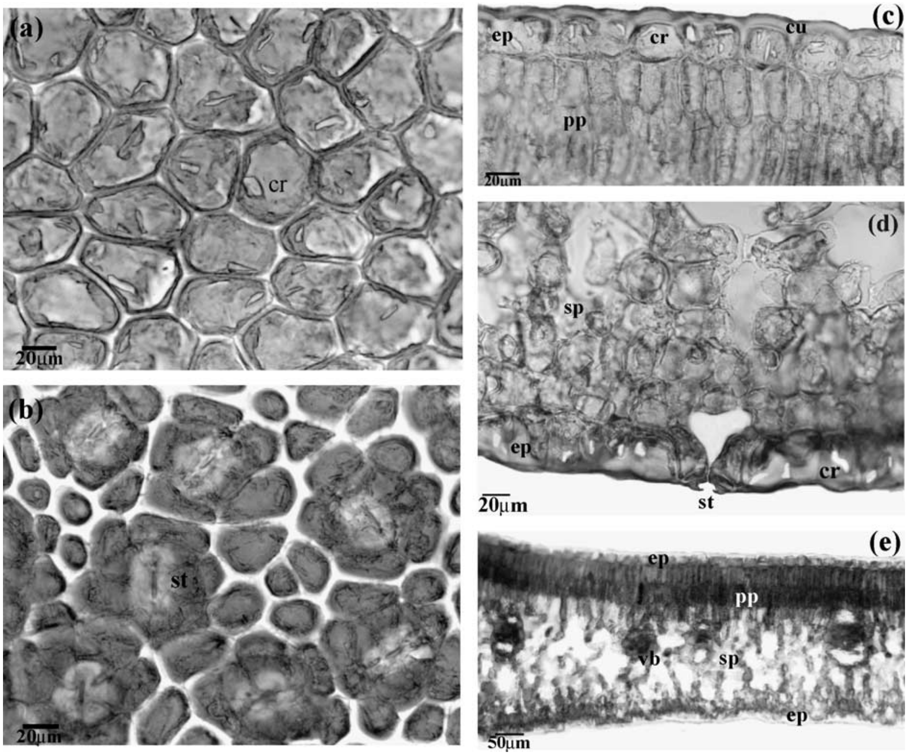

Fig. 3. Leaf structure of *M. ilicifolia*. (a) Adaxial side of the epidermis, in face view, showing the calcium oxalate styloids and prisms; (b) abaxial surface of the epidermis, in face view; (c) transverse section of the adaxial side, revealing cuticular flanges; (d) stomatum, even with the other epidermal cells; (e) dorsiventral mesophyll. Abbreviations—cr: crystals; cu: cuticle; ep: epidermis; pp: palisade parenchyma; sp: spongy parenchyma; st: stomata; vb: vascular bundle.

eventually, the xylem. Bundle sheath extensions are not observed. Idioblasts with prisms of calcium oxalate may be seen in the chlorenchyma, and cells containing phenolic compounds occur in the mesophyll, predominantly in the second palisade stratum.

The midrib, in transection, is biconvex (Fig. 4a). The epidermis has the same characteristics of the blade, and, beneath it, the angular collenchyma may occur. An amphicrival vascular bundle, nearly continuous and encircled by a conspicuous sclerenchymatic sheath, is embedded in the ground tissue. Idioblasts containing phenolic compounds are frequently present in the phloem and ground tissue.

The petiole, in transection, shows a circular contour, slightly plain on the adaxial surface (Fig. 4b). The epidermis is similar to the blade, and, embedded in the ground parenchyma, an amphicrival vascular bundle is present, showing a parenchymatic central region. An incomplete sclerenchymatic sheath encloses the vascular bundle (Fig. 4c and d), and, nearby, many amiloplasts are encountered (Fig. 4d). Distributed in the ground tissue, solitary or clustered stone cells are seen (Fig. 4c and e), as well as idioblasts containing phenolic compounds.

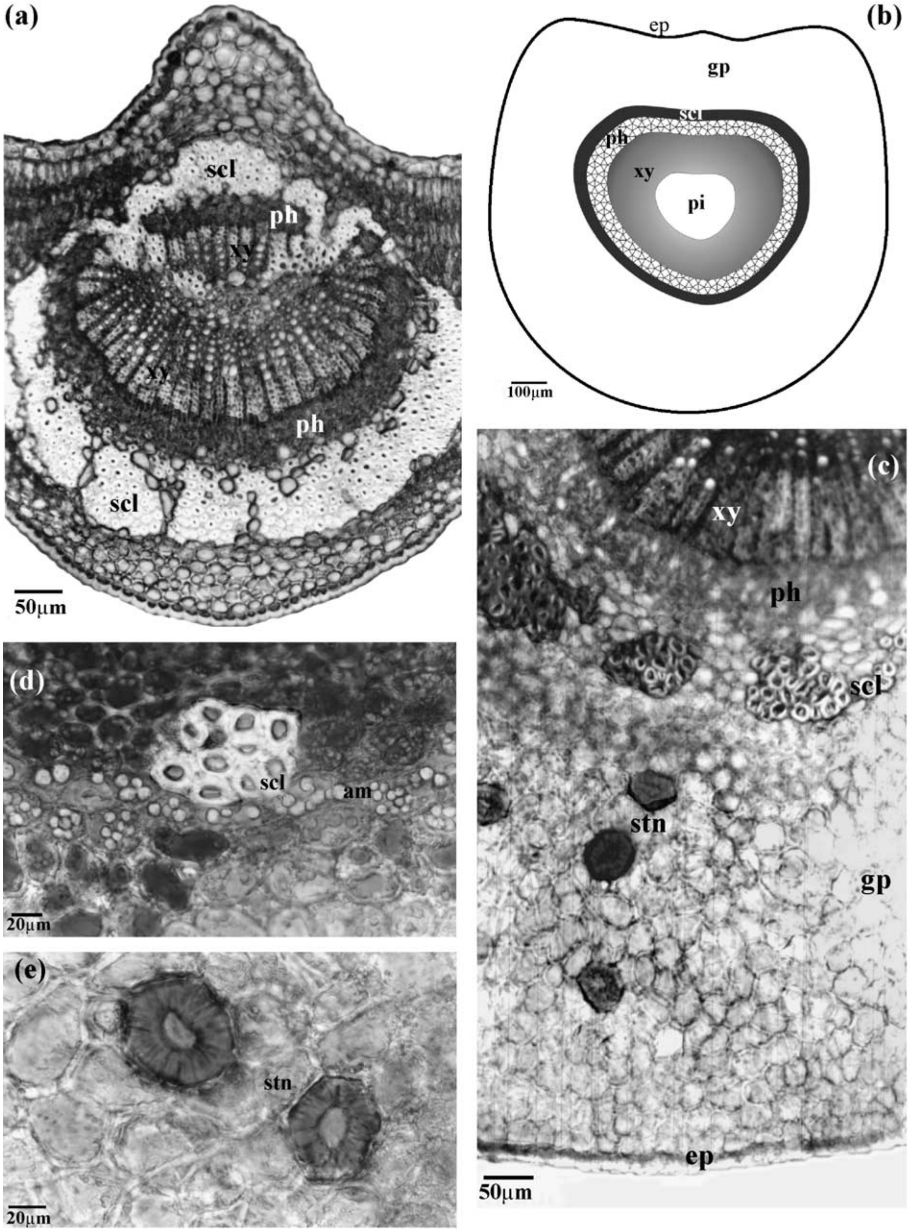

Fig. 4. Midrib and petiole structure of *M. ilicifolia*. (a) Transverse section of the midrib, presenting one amphicrival bundle surrounded by descontinuous sclerenchymatic sheath; (b) diagram of the petiole organization; (c) transverse section of the petiole; (d) detail of the incomplete sclerenchymatic sheath and the amiloplasts nearby; (e) stone cells in the ground parenchyma. Abbreviations—am: amiloplasts; ep: epidermis; gp: ground parenchyma; ph: phloem; pi: pith; scl: sclerenchymatic sheath; stn: stone cells; xy: xylem.

4. Discussion

The stem morphoanatomy of *M. ilicifolia* in secondary growth corresponds to the caulinar pattern reported for the family and the *Maytenus* genus by Metcalfe and Chalk [36]. The epidermis, usually representing the outermost cell layer of the young stem in woody plants, may briefly remain in the mature axis since it is sooner or later replaced by the periderm [37], or may never be replaced even in some perennial woody plants [38]. In the present study, despite the epidermis that has been observed in the stem at the level examined, the phellogen differentiation indicates that, at lower levels, the epidermis would become detached. As it was found here, the phellogen is often superficial, arising in the subepidermis or outer part of the cortex [37]. Although a sclerenchymatic ring in the cortex, composed of fibers and stone cells, has been described in this paper, Metcalfe and Chalk [36] have only mentioned stone cells in the cortical region for the genus without referring to this evident arrangement. The sclerenchymatic cell concentration is effective in withstanding environmental pressures, such as damage by wind and to fend off herbivores [39].

As observed herein, the establishment of the vascular cambium follows the common pattern of secondary growth, where phloem and xylem usually constitute closed cylinders traversed by narrow rays, which are often homogeneous [36]. With respect to these authors [37], pith usually consists of parenchyma, which may become partly or wholly lignified, and serve to store starch or secrete crystals and other ergastic substances. Based on their investigation [36], the pith of *Maytenus* stem sometimes contains stone cells, disagreeing with this analysis.

The leaf morphology and phyllotaxy of *M. ilicifolia* follows the description by Bernardi and Wasicky [40], Correa et al. [17], Alonso [41] and the Farmacopéia Brasileira [42]. The occurrence of spiny teeth along the leaf margin may be invoked as a mechanical character that prevents herbivory [39]. The leaf features observed are closely similar to *M. aquifolium* and mislead the correct species identification among the population. On the other hand, *M. evonymoides* Reiss. has elliptical and membranous leaf; in *M. robusta* Reiss., this appendage is elliptical and coriaceous, and the *M. salicifolia* leaf is oval-lanceolate and membranous [43].

The observation of a conspicuous thick cuticle coating the epidermis is a character reported for the Celastraceae family and for the *Maytenus* genus [36], and particularly emphasized in *M. ilicifolia* [40,42,44], *M. oleoides* (Lam.) Loes. [45] and *M. boaria* Molina [37]. According to the latter authors, the cuticle helps to prevent the water loss efficiently, as well as the leaf from collapsing when the cells dehydrate. Moreover, the cuticular ornamentation and the occurrence of cuticular flanges have been considered of taxonomic value and used for diagnostic purposes [37]. Differing from the current evidence, the leaf cuticle has been considered striated and papillose on *M. ilicifolia* monograph [42].

Crystals within epidermal cells are common in Celastraceae, being mentioned in *Elaeodendron*, *Catha* and *Maytenus* [36]. Plant crystal idioblasts have been recognized to assume different functions as storing the calcium ion, avoiding the oxalate toxic accumulation, contributing to the mechanical support and protecting against foraging animals [46].

The hypostomatic leaf with anomocytic stomata described in the present study and in the Farmacopéia Brasileira [42] contradicts partially Alquini and Takemori [44], who have mentioned paracytic stomata exclusively on the abaxial epidermal surface for the same species. Regarding Metcalfe and Chalk [36], trichomes are seldom in Celastraceae, while hypoderm is often seen in the *Maytenus* leaf. The present findings demonstrate that the *M. ilicifolia* leaf has no trichomes or hypoderm. Besides, the mesophyll is dorsiventral and the midrib has an amphicrival bundle, which correspond to the evidences pointed out by Bernardi and Wasicky [40], Alquini and Takemori [44] and the Farmacopéia Brasileira [42]. Nevertheless, Metcalfe and Chalk [36] have indicated isobilateral organization to *Maytenus*.

Concerning the phenolic-storing cells, Metcalfe and Chalk [36] have mentioned them in the mesophyll of species of *Cassine*, *Euonymus*, *Gymnosporia*, *Maytenus*, *Microtropis*, *Myginda*, *Pachystima*, *Siphonodon*, *Wimmeria* and *Zinowiewia*. From Beckman's [47] point of view, these specialized cells, distributed within most tissues, synthesize phenolics and store them in their vacuoles during the normal process of differentiation. By decompartmentation, they can seal off infections or injuries and produce a peridermal defence in depth. In addition, based on the review by Scalbert [48], phenolic compounds, especially the tannins, exhibit antimicrobial properties involving the inhibition of extracellular microbial enzymes or oxidative phosphorylation, or deprivation of the substrates required for microbial growth.

5. Conclusion

Despite the lack of outstanding morphoanatomical characters for *M. ilicifolia*, the stem showing a conspicuous sclerenchymatic ring composed of fibers and stone cells and the leaf diplaying spiny teeth along the margin, epidermal cells containing calcium oxalate styloids and prisms, cuticular flanges, dorsiventral mesophyll and amphicrival bundle in the midrib and petiole are relevant for the diagnosis of the species.

Acknowledgments

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References

[1] Cronquist A. An integrated system of classification of flowering plants. New York: Columbia University; 1981.
[2] Joly A. Botânica. 12th ed. São Paulo: Nacional; 1998.
[3] Pereia AM, Menezes A, Pereira PS, Cerdeira RM, Lancas FM. J Herbs Spices Med Plants 1995;3:43.
[4] Souza FML, Oliveira MG, Monteiro MG, Silveira NG, Carlini EA. J Ethnopharmacol 1991;34:21.

[5] Corsino J, Carvalho PRF, Kato MJ, Latorre LR, Oliveira OMMF, Araujo AR, et al. Phytochemistry 2000;55:741.
[6] Gonzalez FG, Portela TY, Stipp EJ, Di Stasi LC. J Ethnopharmacol 2001;77:41.
[7] Shirota O, Morita H, Takeya K, Itokawa H, Iitaka Y. J Nat Prod 1994;57:1675.
[8] Shirota O, Morita H, Takeya K, Itokawa H. Chem Pharm Bull 1998;46:102.
[9] Pullen CB, Schmitz P, Hoffmann D, Meurer K, Boettcher T, Bamberg D, et al. Phytochemistry 2003;62:377.
[10] Lu CH, Zhang JX, Gan FY, Shen YM. Acta Bot Sin 2002;44:603.
[11] Chavez H, Estevez BA, Ravelo AG, Gonzalez AG. J Nat Prod 1998;61:82.
[12] Niero R, Moser R, Busato AC, Yunes RA, Reis A, Filho VC. Z Naturforsch 2001;56:158.
[13] Gessler MC, Nkunya MHH, Mwasumbi LB, Heinrich M, Tanner M. Acta Trop 1994;56:65.
[14] El Tahir A, Satti GMH, Khalid SA. J Ethnopharmacol 1999;64:227.
[15] Hussein G, Miyashiro H, Nakamura N, Hattori M, Kawahata T, Otake T, et al. Phytother Res 1999;13:31.
[16] Hussein G, Nakamura N, Meselhy RM, Hattori M. Phytochemistry 1999;50:689.
[17] Correa Jr C, Ming LC, Scheffer MC. Cultivo de plantas medicinais, condimentares e aromáticas. Curitiba: Emater-PR; 1991.
[18] Queiroga CL, Silva GF, Dias PC, Possenti A, Carvalho JE. J Ethnopharmacol 2000;72:465.
[19] Montanari T, Bevilacqua E. Contraception 2002;65:171.
[20] Camparoto ML, Teixeira RO, Mantovani MS, Vicentini VEP. Gen Mol Biol 2002;25:85.
[21] Oliveira MG, Monteiro MG, Macaubas C, Barbosa VP, Carlini EA. J Ethnopharmacol 1991;34:29.
[22] Vargas VM, Guidobono RR, Henriques JA. Mem Inst Oswaldo Cruz 1991;86:67.
[23] Cunico MM, Cirio GM, Miguel OG, Miguel MD, Montrucchio DP, Auer CG, et al. Rev Bras Farmacogn 2002;12:69.
[24] Buffa Filho W, Corsino J, Bolzani SV, Furlan M, Pereira AM, Franca SC. Phytochem Anal 2002;13:75.
[25] Oliveira JF, Braga AC, Oliveira MBN, Avila AS, Araujo AC, Cardoso VN, et al. J Ethnopharmacol 2000;72:179.
[26] Melo F, Soares SF, Costa RF, Silva CR, Oliveira MBN, Bezerra RJAC, et al. Mutat Res, Genet Toxicol Environ Mutagen 2001;496:33.
[27] Vilegas JHY, Lancas FM, Wauters JN, Angenot L. Phytochem Anal 1998;9:263.
[28] Leite JP, Rastrelli L, Romussi G, Oliveira AB, Vilegas JH, Vilegas W, et al. J Agric Food Chem 2001;49:3796.
[29] Zhu N, Sharapin N, Zhang J. Phytochemistry 1998;47:265.
[30] Johansen DA. Plant microtechnique. New York: McGraw-Hill; 1940.
[31] Berlyn GP, Miksche JP. Botanical microtechnique and cytochemistry. Ames: Iowa State University; 1976.
[32] O'Brien TP, Feder N, McCully ME. Protoplasma 1965;59:368.
[33] Roeser KR. Mikrokosmos 1962;61:33.
[34] Sass JE. Botanical microtechnique. 2nd ed. Ames: Iowa State College; 1951.
[35] Foster AS. Practical plant anatomy. 2nd ed. Princeton: D. Van Nostrand; 1949.
[36] Metcalfe CR, Chalk L. Anatomy of dicotyledons: leaves, stem, and woods in relation to taxonomy with notes on economic uses. Oxford: Clarendon; 1950.
[37] Metcalfe CR, Chalk L. Anatomy of dicotyledons, vol. 1. Oxford: Clarendon; 1981.
[38] Mauseth JD. Plant anatomy. Menlo Park: Benjamin/Cummings; 1988.
[39] Lucas PW, Turner IM, Dominy NJ, Yamashita N. Ann Bot 2000;86:913.
[40] Bernardi HH, Wasicky M. Algumas pesquisas sobre espinheira-santa ou cancerosa *Maytenus ilicifolia* Martius, usada como remédio popular no RS. Santa Maria: Centro de Pesquisas; 1959.
[41] Alonso JR. Tratado de fitomedicina—bases clínicas y farmacológicas. Buenos Aires: Isis; 1998.
[42] Farmacopéia Brasileira. 4th ed. São Paulo: Atheneu; 2003.
[43] Okano RMC. Hoehnea 2000;27:27.
[44] Alquini Y, Takemori NK. Organização estrutural de espécies vegetais de interesse farmacológico. Curitiba: Herbarium; 2000.
[45] Hlwatika CNM, Johnson CT, Bhat RB. Biologia (Bratisl) 1998;53:73.
[46] Franceschi VR, Horner Jr HT. Bot Rev 1980;46:361.
[47] Beckman CH. Physiol Mol Plant Pathol 2000;57:101.
[48] Scalbert A. Phytochemistry 1991;30:3875.