Anais da Academia Brasileira de Ciências (2014) 86(3): 1167-1179
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201420130105
www.scielo.br/aabc

Structure, histochemistry and phytochemical profile of the bark of the sobol and aerial stem of *Tontelea micrantha* (Celastraceae - Hippocrateoideae)

MARIA OLÍVIA MERCADANTE-SIMÕES[1], HELLEN C. MAZZOTTINI-DOS-SANTOS[1], LAYS A. NERY[1], PERACIO R.B. FERREIRA[2], LEONARDO M. RIBEIRO[3], VANESSA A. ROYO[2] and DARIO A. DE OLIVEIRA[2]

[1]Laboratório de Anatomia Vegetal, Universidade Estadual de Montes Claros, Campus Darcy Ribeiro, Vila Mauricéia, 39401-089 Montes Claros, MG, Brasil
[2]Laboratório de Bioprospecção e Recursos Genéticos, Universidade Estadual de Montes Claros, Campus Darcy Ribeiro, Vila Mauricéia, 39401-089 Montes Claros, MG, Brasil
[3]Laboratório de Micropropagação Vegetal, Universidade Estadual de Montes Claros, Campus Darcy Ribeiro, Vila Mauricéia, 39401-089 Montes Claros, MG, Brasil

Manuscript received on March 18, 2013; accepted for publication on August 12, 2013

ABSTRACT

The bark of the underground stem of *Tontelea micrantha* (Mart. ex. Schult.) A. C. Sm., a native Brazilian Cerrado species, is used in folk medicine for treating kidney ailments. The structures of the underground- and the aerial stems were examined and their barks were analyzed for the presence of secondary metabolites. Bark fragments were processed according to conventional techniques in plant anatomy and their chemical compositions examined using histochemical and phytochemical tests, thin layer chromatography, and high-efficiency liquid chromatography. The underground stem is a sobol with unusual cambial activity. Laticifers that secrete terpenoids were present in the cortex and phloem of both organs and can contribute to the identification of the species in field. Druses were present in both barks, but mono-crystals were only observed in the sobol. Tannins, flavonoids, alkaloids, and terpenoids occurred in both types of bark, but carotenoids were only detected in the sobol. The similarities between these two organs indicate that the aerial stem bark has potential medicinal use and represents a plausible alternative to harvesting the sobol, which could contribute to the preservation of natural populations of this species.

Key words: natural products, pharmacognosy, secondary metabolites, sobol.

INTRODUCTION

Representatives of the family Celastraceae are well known in popular medicine for their pharmacological properties, especially species of the genus *Maytenus* used for treating gastric ulcers (Leite et al. 2010, Santos et al. 2007, Silva et al. 2011). *Cheiloclinium* and *Salacia* likewise comprise species that have been examined in the search for bioactive compounds with analgesic and anti-inflammatory activities or for controlling diabetes (Duarte et al. 2010, Tanabe et al. 2008).

Tontelea micrantha (Mart. ex. Schult.) A. C. Sm. is a native medicinal species of the Brazilian Cerrado (savanna), and alcoholic extracts of the bark of its underground stem are used for treating kidney problems. The oil extracted from its seeds

Correspondence to: Maria Olívia Mercadante-Simões
E-mail: omercadante@hotmail.com

is a potent anti-inflammatory and represents a significant source of income for people living in areas of Cerrado vegetation (Dias and Laureano 2010). Individuals of this species occur as clumps (clones) of shrubs whose aerial shoots emerge from an underground stem with a rather complex anatomical structure (Wanderley et al. 2003). This subterranean structure is responsible for re-sprouting above-ground structures damaged by fires (a common occurrence in the Cerrado biome) (Silva et al. 2009). The intensive human exploitation of these plant organs for medicinal purposes threatens natural populations of these plants. Although not usually considered, harvesting only the aerial portions of these plants instead of their subterranean structures could minimize negative effects on plant growth (Zschocke et al. 2000).

Governmental agencies responsible for regulating and registering the production and use of natural medicinal products require the correct botanical identification of the species in order to prevent their incorrect use and risks to consumer health (WHO 1987, Brasil 2000). Scientific research characterizing the anatomical structures and chemical contents of species utilized in traditional medicine has contributed to controlling the quality of the dried and powdered drugs obtained from them (Coelho et al. 2012, Cruz et al. 2012, Ferreira et al. 2011).

The present study sought to: (i) identify anatomical and chemical characteristics of the bark of the underground and aerial stems of *T. micrantha* that could be used as diagnostic features for the species; (ii) identify similarities in the distributions of the secondary metabolite classes found in the two organs in order to evaluate the potential medicinal use of aerial stem as a substitute for the underground system.

MATERIALS AND METHODS

Plant Material

The "bark", *sensu* Fahn (1990), of the underground and aerial stems and the shoot apices of *T. micrantha* were examined. Study material was collected from 10 individuals in a natural population of this species growing in the Cerrado (savanna) vegetation in the municipality of Montes Claros, state of Minas Gerais, Brazil (16° 52' 15" S, 44° 00' 58" W). Voucher material was deposited in the BHCB herbarium of the Departamento de Botânica of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais (Mercadante-Simões 2; registry number 214463; identified by Dr. Julio Lombardi).

Structural Evaluation

The material was fixed in Karnovsky's solution (Karnovsky 1965) under vacuum (560 mm Hg) for 12 hours, dehydrated in an ethanol series (Jensen 1962), and cold-embedded (Paiva et al. 2011) in glycol-methacrylate resin (Leica Microsystem Inc., Heidenbeg, Germany). Transversal and longitudinal sections (5 μm) were made using a rotary microtome (Atako, Japan) and stained with toluidine blue, pH 4.7 (modified from O'Brien et al. 1964), fuchsin (Johansen 1940), floroglucinol (Johansen 1940), and Sudan IV (Pearse 1980). Paradermal sections were cleared in a 20% hypochlorite solution and stained with safranin to examine the epidermal cells of the aerial stem in frontal view (Johansen 1940). The presence of calcium oxalate was verified using HCl (Chamberlain 1932). Permanent slides were mounted using Itacril acrylic resin (Itacril, Itaquaquecetuba, Brazil). Photo-documentation was conducted using a Canon A 620 digital camera (Canon, Tokyo, Japan) coupled on a Nikon Eclipse E-200 optical microscope (Nikon, Tokyo, Japan) and a digital camera (Zeiss AxioCam HRc, Göttinger, Germany), using Axion Vision image-capturing software, coupled on an Olympus Optical model AX70 TRF light microscope with a U-photo system.

Histochemical Analyses

Histochemical tests were performed on transverse sections of fresh material obtained from the bark of the underground and aerial stems of *T. micrantha* that had been sectioned on a table microtome (LPC

Rolemberg & Bhering, Belo Horizonte, Minas Gerais, Brazil) using the following reagents: Lugol's solution for starch (Jensen 1962), Sudan IV (Pearse 1980) for lipids, bromophenol blue (Mazia et al. 1953) and Xilidine Ponceau for proteins (Vidal 1977); vanillin-HCl for tannins (Mace and Howell 1974); DMACA (*p*-dimethylaminocinannamaldehyde) for flavonoids (Arnous 2002, Feucht et al. 1986); Dittmar and Wagner reagents for alkaloids (Furr and Mahlberg 1981); and NADI (naphtol and dimethyl-paraphenylene-diamine) for terpenoids (David and Carde 1964). Image documentation was performed using a Canon A 620 digital camera (Canon, Tokyo, Japan) coupled on a Nikon Eclipse E-200 optical microscope (Nikon, Tokyo, Japan) and a digital camera (Zeiss AxioCam HRc, Göttinger, Germany), using Axion Vision image-capturing software, coupled on an Olympus Optical model AX70 TRF light microscope with a U-photo system.

Phytochemical Prospection

Bark from the underground and aerial stems of *T. micrantha* was dried at room temperature and powdered in a Willey-TE 64 mill (TECNAL, Piracicaba, Brazil). The resulting powder was stored at -18°C. Aqueous and ethanol extracts of the bark were obtained by weighing the powder (using an analytical balance; Shimadzu BL320H, Tokyo, Japan) and macerating it in a water/ethanol solution (1:10,V/V) three times every 24 hours; the extracts were then filtered and concentrated under reduced pressure at 35°C. The protocols described by Barbosa et al. (2001) and Mouco et al. (2003) were employed for the extraction and identification of tannins (using ferric chlorate), flavonoids (using Shinoda and Bornträger's reagent), alkaloids (using Dragendorff and Mayer's reagent), and terpenoids (using Salkawski and Liberman-Burchard's reagent).

High Performance Liquid Chromatography (HPLC)

The chemical constituents of the barks of the underground and aerial stems of *T. micrantha* were identified using a Waters® chromatograph coupled to a photodiode detector array and a phenomenex ODS2 chromatography column (250 mm x 4.6 mm x 5 µm) (Waters, Minneapolis, USA) with a flux rate of 1 mL/minute and an injection volume of 20 µL. The identifications of the chemical compounds were performed by comparing their retention times with external standards (using Empower 2 software).

Tannins: 4.0 g of the bark powder was extracted with 10 mL of butanol. The liquid phase was then dried at 35°C, and 115 mg of the extract was subsequently diluted in 50 mL of methanol in an ultra-bath for 10 min. The chromatograph detector was adjusted to 270 nm, with butanol as the mobile phase and using gallic, tannic, and ellagic acids as standards; the run-time was 10 min at 25°C (Santos and Melo 2003).

Flavonoids: 20.0 g of the bark powder was extracted with 30 mL of 85% ethanol at 45°C. Extract was then filtered, dried at 35°C and resuspended in methanol/acetonitrile/water (40:15:45 v/v/v) + 1% acetic acid as the mobile phase. The chromatograph detector was adjusted to 257 nm, with rutin and quercetin as standards; the runtime was 15 min at 25°C (Lu et al. 2006).

Alkaloids: 3.0 g of the bark powder was extracted with 6.0 g of magnesium oxide in 100 mL of distilled water at 100°C for 15 min. The mixture was subsequently cooled and weighed, and any water lost through evaporation was replaced to 100 g above the original weight; chloroform was used as the mobile phase. The mixture was then centrifuged at 2000 rpm for 5 min and the supernatant filtered through a 0.45 µm membrane. The chromatograph detector was adjusted to 273 nm with a running time of 35 minutes at 25°C; caffeine and theophylline were used as internal standards (Alves and Bragagnolo 2002).

Spectrophotometry

Carotenoids present in the bark of the underground stems were identified using a Human Reader HS - catalog 16670 spectrophotometer (Human, Wiesbaden, Germany). The pigments were extracted

from 1.0 g of the bark powder using 5.0 mL of acetone. The extract was then filtered and dried at 35°C, resulting in a residue of 0.015 g that was resuspended in 3 mL of an ethanol/water solution (1:1) and diluted 1:30. The chromatograph detector was adjusted for absorbance measurements at 450 nm and carotenoid concentrations were determined in triplicate using standard beta-carotene (Kimura and Rodriguez-Amaya 2002).

Thin Layer Chromatography (TLC)

To identify terpenoids and other volatile compounds, 200 g of the bark powder was extracted in 500 mL of distilled water in four extraction sessions (50 g of powder each) using a Clevenger apparatus; the water phase was partitioned with hexane, which was subsequently stored under freezing conditions in the dark. The partition obtained was examined by TLC using hexane: ethanol (9:1) as the mobile phase; visualizations of the terpenoids and volatile compounds were performed using sublimated iodine.

RESULTS

Structure and Histochemistry

Underground stem

The underground stem of *T. micrantha* has a woody consistency with an intense natural orange external coloration that facilitates its identification in the field (Figs. 1A-C). The stem-like nature of this organ was confirmed by the presence of pith (Fig. 1B). Unusual cambial activity that produces concentric and alternating layers of secondary xylem and phloem can be observed in more advanced stages of

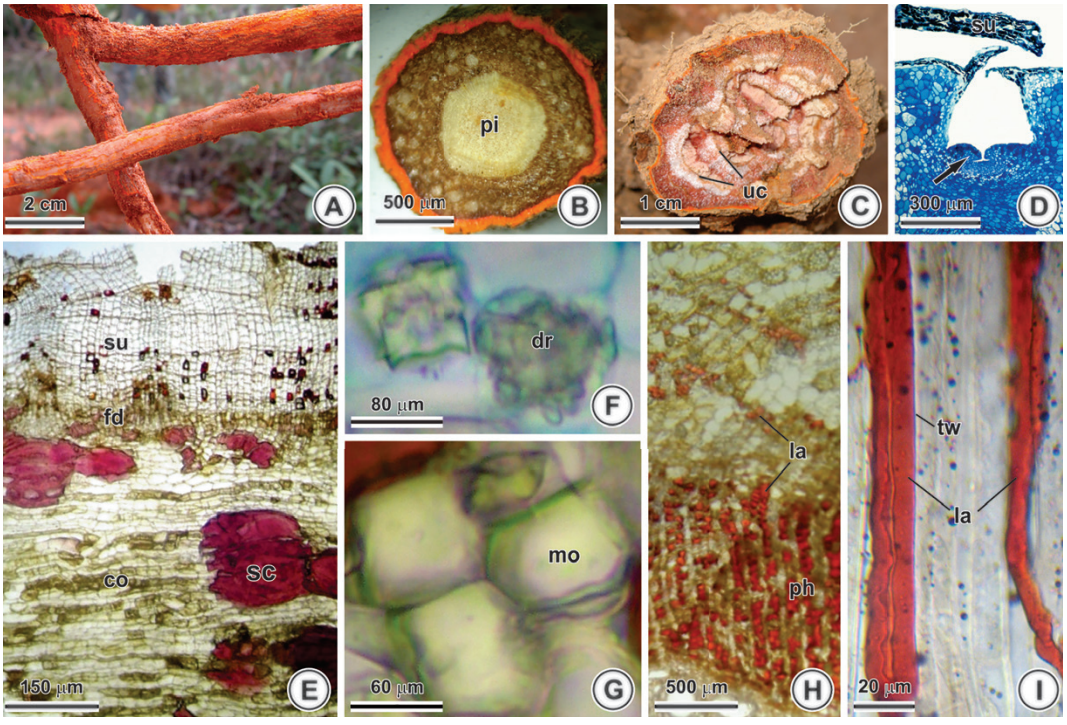

Figure 1 - Underground stem of *Tontelea micrantha*. **(A-C)** General aspect, showing its natural orange coloration (presence of carotenoids). **(D-G)** Transverse sections. **(I)** Longitudinal section. **(B)** Stem-like structure (presence of pith). **(C)** Unusual cambial activity. **(D)** Adventitious bud (arrow). Periderm and cortex. **(F)** Druses. **(G)** Mono-crystals. **(H-I)** Laticifers in the secondary phloem. co: cortex; dr: druse; fd: phelloderm; la: laticifer; pi: pith; mo: mono-crystal; sc: sclereid; su: suber; tw; thick wall; uc: unusual cambium.

development (Fig. 1C). Vegetative buds give rise to aerial branches that form interconnected clumps of plants that can cover large areas of land (Fig. 1D).

The periderm of the sobol has suber composed of thin-walled cells that are predominantly suberized, although some are lignified, with the orange color typical of that structure; lenticels can be observed; phellogen activity gives rise to a compact phelloderm composed of layers of radially disposed cells (Fig. E). The primary cortex is well-developed on the secondary structure (Figs. B, E). The parenchymatous layers of the phelloderm and cortex show isolated or grouped large irregularly shaped sclereids (with many pits); druses and mono-crystals of calcium oxalate were also observed (Figs. F-G). The secondary phloem shows uniseriate rays composed of radially elongated cells; non-articulated and non-anastomosing laticifers with thick walls and viscous lipophilic contents could be observed in the axially elongated parenchyma; laticifers also occur in the cortex and phelloderm (Figs. 1G-H).

Table I and Figure 2 show the results of histochemical tests performed on the underground stem bark. The natural color of the material can be seen in the sections not treated with reagents (Fig. 2A); the suber shows carotenoids (Fig. 2B); the cortex contain starch grains (Fig. 2C), protein reserves (Fig. 2D-E), and secondary metabolic compounds such as tannins (Fig. 2F), flavonoids (Fig. 2G), alkaloids (Fig. 2H) and terpenoids (Fig. 2I-J).

TABLE I
Results of the histochemical tests in the underground and aerial stems of *T. micrantha*. (+) presence; (-) absence.

Compounds	Reagent	Underground stem			Aerial stem		
		Suber	Phelloderm and cortex	Phloem parenchyma	Suber	Phelloderm and cortex	Phloem parenchyma
Starch	Lugol's solution	-	+++	+	-	+	+
Lipids	Sudan IV	-	++	+++	*	*	*
Protein	Bromophenol blue	-	+	+	-	-	-
	Xilidine Ponceau	-	+	+	-	-	-
Tannins	Vanillin-hydrochloric	-	++	++	-	++	++
Flavonoids	DMACA	-	++	++	-	+	+
Alcaloids	Dittmar reagent	-	+	+	*	*	*
	Wagner reagent	*	*	*	-	+	+
Terpenoids	NADI reagent	-	++	+++	-	++	+++

(+) presence; (-) absence; (*) test not performed.

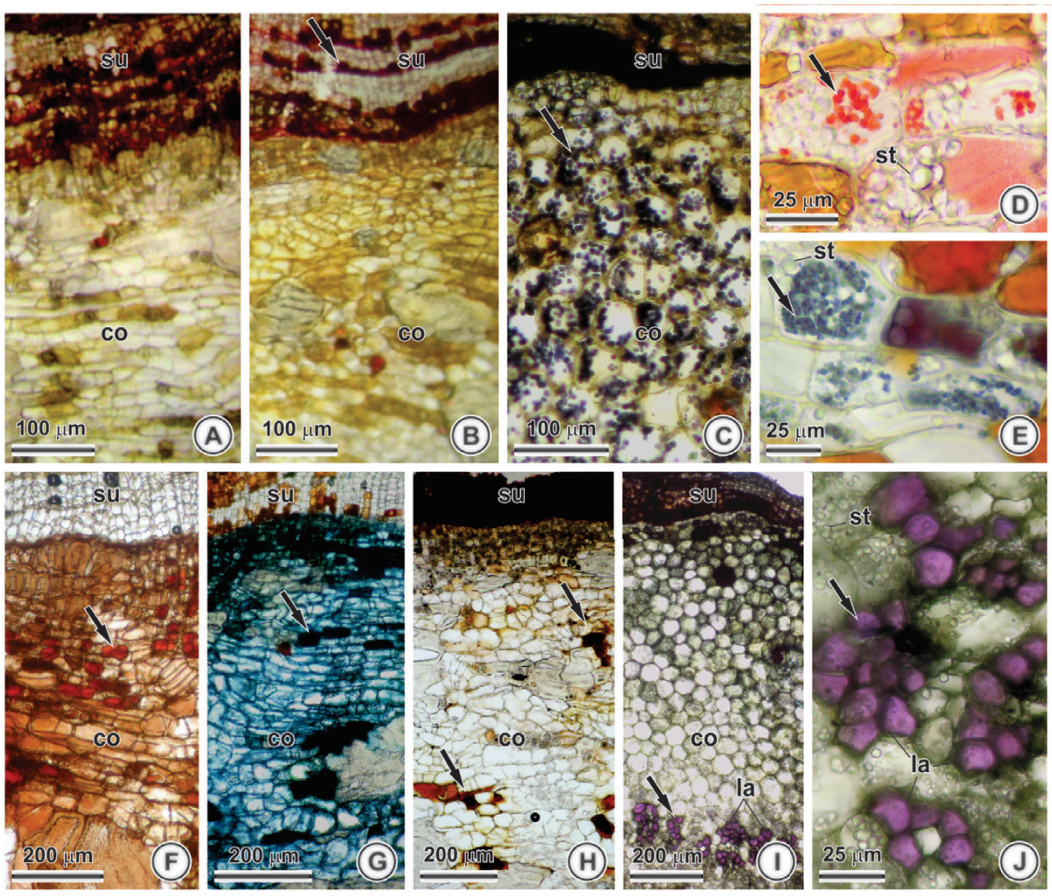

Figure 2 - Transverse sections of the bark of the underground stem of *Tontelea micrantha* subjected to different histochemical tests (arrows show positive reactions). **(A)** Material not subjected to any reagents (white). **(B)** Lipids stained red with Sudan IV (presence of carotenoids). **(C)** Starch grains stained purple with Lugol solution. **(D, E)** Proteins stained red with Ponceau xilidine and blue with bromophenol blue, respectively. **(F)** Tannin stained reddish-brown with vanillin hydrochloride. **(G)** Flavonoids stained blue with DMACA reagent. **(H)** Alkaloids stained reddish brown with Dittmar reagent. **(I.J)** Oleoresin stained pink with NADI reagent (arrow). co: cortex; la: laticifer; st: starch grain; su: suber.

Aerial stem

Adult individuals of *T. micrantha* produce numerous long and thin axially oriented branches that arise from the underground stem, forming clumps of clonal shrubs (Fig. 3A). The uniseriate epidermis on the young branches is composed of cells covered by a very thick cuticle that extends along the anticlinal and inner periclinal walls (Fig. 3B). A frontal view of the epidermis shows the polyhedral outlines of the cells and the presence of cyclocytic stomata (Fig. 3C). The subsidiary cells have periclinal external walls that are thinner than the internal walls, giving them pyramid shapes in transverse section (Fig. 3D).

The cortex has small intercellular spaces, cells with phenolic contents, druses and laticifers; the stem structure is eustelic; the primary phloem has a conspicuous cap of fibers with pectic-cellulosic walls and medullary rays that show accumulations of phenolic compounds (Fig. 3E). The secondary structure shows a multilayered suber that originated from phellogen activity in the sub-epidermal layers (Fig. 3F); the phelloderm is not well-developed and

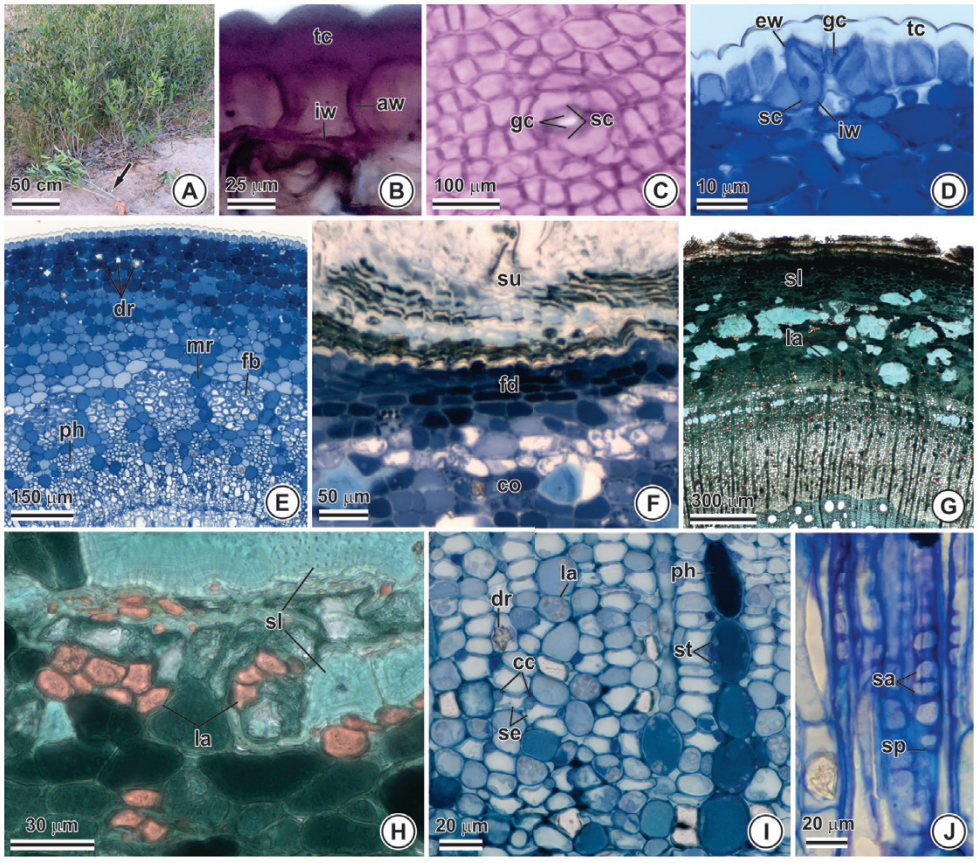

Figure 3 - Aerial stem of *Tontelea micrantha*. **(A)** General view. **(B, D-I)** Cross section. **(B)** Paradermal section. **(J)** Longitudinal section. **(A)** General aspect of the clone emerging from the underground stem (arrow). **(B)** Epidermal cells with the cuticle coating the anticlinal and periclinal walls. **(C-D)** Stomata. **(E)** Primary structure. **(F-J)** Secondary structure. **(F)** Periderm and cortex. **(G)** Periderm, cortex with sclereids and laticifers, and secondary phloem. **(H)** Detail of the cortex showing sclereids and laticifers. **(I-J)** Detail of the secondary phloem showing laticifers. aw: anticlinal wall; cc: companion cell, co: cortex; dr: druse; ew: external periclinal wall, fb: fiber; fd: phelloderm; gc: guard cell; iw: inner periclinal wall; la: laticifer; mr: medullary ray, ph: phenolic content; sa: sieve area, sc: subsidiary cell; se: sieve element; st: starch grain; sl: sclereid; sp: sieve plate; su: suber; tc: thick cuticle.

has tangentially elongated cells that are smaller than the cortical cells (Fig. 3G); the cortex is well-preserved in the secondary structure; groups of large sclereids of variable sizes with small lumens and walls with conspicuous pits can be observed, as well as laticifers with thickened walls and lipophilic contents (Fig. 3G-H). The secondary phloem is well-developed, with radial cells with phenolic contents, druses, and starch grains (Fig. 3I). The sieve elements have oblique sieve plates and numerous sieve areas (Fig. 3J). Laticifers can be observed with the naked eye when the stem is injured.

The results of the histochemical tests of the aerial stems are presented in Table I and Figure 4. The natural color of the material can be seen in the sections not treated with reagents. (Fig. 4A). The cortex shows the presence of starch grains (Fig. 4B), tannins (Fig. 4C), flavonoids (Fig. 4D) and alkaloids (Fig. 4E). A conspicuous presence of elongated laticifers was observed in the axial system (Figs. 4F-G); the laticifers have elastic walls (Fig. 4G).

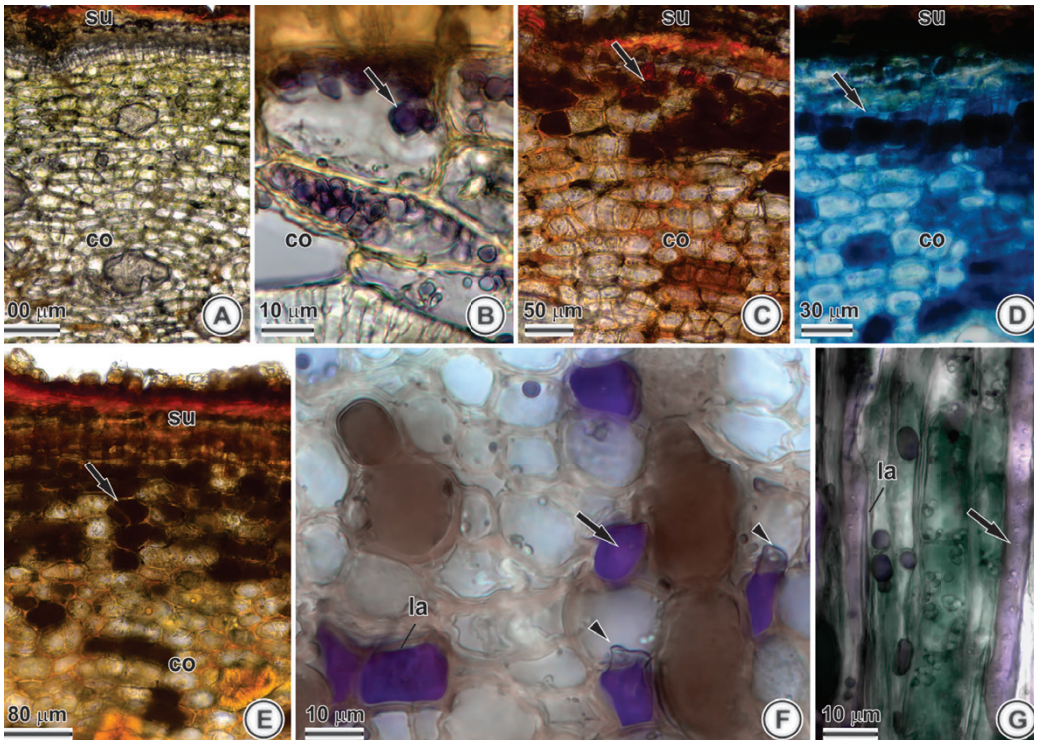

Figure 4 - Sections of the aerial stem of *Tontelea micrantha* subjected to different histochemical tests (arrows show positive reactions). **(A-F)** Transverse sections. **(G)** Longitudinal section. **(A)** Material not subjected to reagents (white). **(B)** Starch grains stained purple with Lugol's solution. **(C)** Tannins stained reddish-brown with vanillin hydrochloride. **(D)** Flavonoids stained blue with DMACA reagent. **(E)** Alkaloids stained reddish-brown with Wagner's reagent. **(F-G)** oil-resin stained lilac with NADI reagent (arrowheads show elastic walls of the laticifers). co: cortex; la: laticifer; su: suber.

Phytochemical Profile

The results of the phytochemical tests corroborated the results of the histochemical tests, indicating the presence of tannins (Shalcowski: purple; Lieberman Buchard: purple; and ferric chloride: green), flavonoids (Shinoda: red; and Bornträger: pink), and alkaloids (Dragendorff: purple; and Mayer: turbid) in both the underground and aerial stems.

High-performance liquid chromatography (HPLC), using the liquid phase standards listed in Table II, indicated the presence of tannins, flavonoids, and alkaloids. The retention times of the peaks, considering tannic acid as a standard, were different between the underground stem (3.683; 4.931; 6.998; and 7.466) and the aerial stems (2.487 and 2.631). The peaks for gallic acid and tannic acid were the same (3.683; 4.931; 6.998; and 7.466) in the underground stem; no retention peaks were observed in the aerial stem using gallic acid.

In terms of the presence of flavonoids, and considering the patterns generated by rutin, two peaks were shared by the underground stem and the aerial stems (2.843 and 3.276), with one additional peak exclusive to the aerial stem (3.897). No peaks were seen when using the quercetin standard. In terms of the analyzed alkaloids, and in relation to theophylline as a standard, three peaks were observed for the underground system (1.975; 2.845 and 3.426); and one different peak for the aerial stem (3.553). No peaks were seen using the caffeine standard.

Spectrophotometric values indicated the presence of carotenoids at concentrations of 0.0104 g/L (1% of the plant material), as compared to the standard. Carotenoids were only observed in the underground stem.

TABLE II
Results of the high-performance liquid chromatography (HPLC) in the underground and aerial stem of *T. micrantha*.

Metabolic group	Standard	Retention time (in minutes)	
		Underground stem	Aerial stem
Tannins	Tannic acid	3.683; 4.931[A]; 6.998 and 7.466	2.487 and 2.631[A]
	Gallic acid	3.683; 4.931[A]; 6.998 and 7.466	-
Flavonoids	Rutin	2.843 [A] and 3.276	2.843 [A]; 3.276 and 3.897
	Quercetin	-	-
Alcaloids	Teophylline	1.975; 2.845[A] and 3.426	3.553
	Caffeine	-	-

([A]) major peak.

Thin layer chromatography used to identify terpenoids and other volatile compounds indicated the presence of two bands from the extraction of the underground stem; the band with more apolar characteristics was not seen in the stem extract (Fig. 5).

Figure 5 - Thin layer chromatography (TLC) of terpenoids and other volatile compounds from extracts of bark powder of the underground stem (US) and aerial stem (AS) of *Tontelea micrantha.* ab: more apolar band; pb: more polar band.

DISCUSSION

The stem-like nature of the underground stem of *T. micrantha* is indicated by the inner position of the pith. The underground stem of this plant was described as a xylopodium by Wanderley et al. (2003), although xylopodium are tuberized structures derived from the hypocotyl and primary root. The morphology and anatomy of the underground stem of *T. micrantha,* as described here, suggests that its classification as being a sobol, to be more correct – a diffuse stem-like underground system growing horizontally below the soil surface (Appezzato-da-Glória 2003, Maroso et al. 2009).

Barks constitute the majority of all plant subproducts used for folk medicinal purposes (Sen et al. 2010). Extracts of the root bark of *M. illicifolia*, for example, have antifungal activity due to the presence of the triterpenoids maitenin and pristimerin (Gullo et al. 2012); the bark of the roots of *M. segalensis* has antimicrobial activity due to the presence of maitenonic acid (Lindsey et al. 2012).

Anatomical attributes of the periderm are often useful in identifying plant species (Pace et al. 2011).

Epidermis remnants of the bark situated externally to the suber have diagnostic properties in drugs prepared from those structures because of their high mechanical resistance and their preservation even in crushed and dehydrated material (Farias et al. 2009). Ciclocytic stomata, with pyramid-shaped subsidiary cells, have been observed on the epidermis of the leaves of various species of Celastraceae (Gomes et al. 2005).

Sclerenchyma organization is a taxonomic character in this group, and has special importance in identifying plant-derived drugs as they remain well-preserved even after fragmentation (Soffiatti and Angyalossy-Alfonso 1999). *T. micrantha* demonstrates a diffuse disposition of the sclereids and fibers present in the cortical strip, which is preserved in the secondary structures of both stems, different from the continuous arrangement seen in *M. ilicifolia* (Duarte and Debur 2005). The presence of druses is considered a universal characteristic within the family Celastraceae and therefore has no diagnostic value (Gomes et al. 2005), but the observed presence of mono-crystals only in the sobol could aid in distinguishing materials derived from either the underground system or aerial stem (Gomes et al. 2010).

The occurrence of laticifers in the Celastraceae family can be considered a diagnostic character for some species (Gomes et al. 2005, 2010). Their presence has been associated with the vascular system and with tissues that arose from the fundamental meristem (Gomes et al. 2005, Lopes et al. 2009), and they can accumulate secondary metabolic compounds of medicinal value (Monacelli et al. 2005). This is in agreement with their occurrence in the cortex and phloem of both, the underground and the aerial stem of *T. micrantha*. The distribution of laticifers within different plant organs constitutes an easily recognizable taxonomic character, and the chemical compositions of their protoplasts and the thicknesses of their cell walls allows them to be readily identified in any tissue in which they may occur (Jacomassi et al. 2007, Pickard 2008). The elastic aspect of the laticifer contents of *T. micrantha* can be used as a diagnostic character in the field (Dias and Laureano 2010) and is consistent with their terpenoid chemical nature. The well-developed phloem of *T. micrantha* holds significant numbers of laticifers.

Anatomical characters identified in the bark of both the sobol and the aerial stem of *T. micrantha* which can be used as diagnostic criteria for identifying that species include: the occurrence of lignified layers in the suber; the disposition and morphology of sclereids present in the cortex; and the occurrence of resiniferous laticifers in the phloem.

The occurrence of secondary metabolic compounds in the periderm, cortex and phloem seems to be related to the medicinal value of the bark of the sobol. The presence of these compounds in the aerial stem suggests the possibility of using those stems as an alternative to the underground structure, which would contribute to the preservation of the species.

Phenolic compounds have wide spectra of medicinal uses (Santos and Mello 2003) and tannins with anti-microbial activities have been identified in Celastraceae species (Silva et al. 2011) used to treat kidney inflammation (Pansera et al. 2003). These observations corroborate the popular use of *T. micrantha* in treating renal infections (Dias and Laureano 2010).

The presence of rutin in Celastraceae has been associated with the wound-healing properties and anti-oxidant activity of this plant group (Tiberti et al. 2007). Flavonoids act as co-factors of vitamin C, lending it anti-inflammatory and antibacterial properties (Zuanazzi and Montanha 2003).

The anesthetic and antiseptic activities of alkaloids are well-known (Henriques et al. 2003) and theophylline has been found to have important antitumor therapeutic properties (Barnes 2003). In spite of the presence of only relatively small quantities of alkaloids in plant tissues they can still be used as chemical markers due to their restricted

presence in only some plant groups. Their detection in *T. micrantha* and absence from the bark of *M. rigida* (Estevam et al. 2009) indicates their possible utility as a marker for these species.

Terpenoids have been described in various species of Celastraceae and are known to have numerous therapeutic properties (Costa et al. 2007, Lorenzi and Matos 2002), and a number of species of this family show potential for treating cancers (Wang et al. 2012). Carotenoids, a chemical group within the general class of tetraterpenoids, have known biological activities (Maoka 2009, Niizu and Rodriguez-Amaya 2005). The orange color of the suber of the sobol of *T. micrantha* is the result of the accumulation of carotenoids in that organ and can be used to identify it in the field (Wanderley et al. 2003, Dias and Laureano 2010). Considering the medicinal importance of this class of plant secondary metabolic products, the identification of terpenoids in *T. micrantha* indicates its potential value in anti-cancer screening projects.

CONCLUSIONS

The most distinctive difference between the sobol and the aerial stem is the presence of carotenoids in the former that can be easily identified by their strong orange color (visible to the naked eye) and the occurrence of mono-crystals. The presence of compounds with proven medicinal properties such as tannins, alkaloids, flavonoids, and terpenoids in *T. micrantha* indicates its potential value in bio-prospection to obtain new herbal medicines. The possibility of using the bark of the aerial stem of *T. micrantha* (in place of the sobol bark) would enable the sustainable use of this species – one of many Cerrado plants that are poorly known but severely threatened.

ACKNOWLEDGMENTS

The authors would like to thank the Fundação de Amparo a Pesquisa do Minas Gerais (FAPEMIG) for the Incentivo à Pesquisa e ao Desenvolvimento Tecnológico grants, awarded to M.O. Mercadante-Simões (CRA-BIPID-00152-12) and L. M. Ribeiro (CRA-BIPIT-00137-11); Suelaine Barbosa for her technical assistance; and Valdeci Leite Fonseca for indicating the collection locality.

RESUMO

Tontelea micrantha (Mart. Ex. Schult.) A. C. Sm. é uma espécie nativa do Cerrado brasileiro cuja casca do caule subterrâneo é utilizada como medicinal no tratamento de doenças renais. As estruturas dos caules subterrâneo e aéreo foram estudadas e suas cascas avaliadas para a presença de classes de metabólitos secundários. Fragmentos das cascas foram processados de acordo com metodologias usuais em anatomia vegetal e submetidos às análises fitoquímicas colorimétricas, cromatografia em camada delgada e identificação química por cromatografia líquida de alta eficiência. O caule subterrâneo é um sóbole e apresenta atividade cambial não usual. Laticíferos que secretam terpenóides estavam presentes no córtex e floema de ambos os órgãos e podem contribuir para a identificação da espécie no campo. Drusas estão presentes em ambas as cascas, mas mono-cristais são observados apenas no sóbole. Taninos, flavonóides, alcalóides e terpenóides ocorrem em ambas as cascas, mas carotenóides são detectados apenas no sóbole. As semelhanças entre estes dois órgãos indicam que a casca do caule aéreo tem potencial para uso medicinal, representando uma alternativa plausível para o uso do sóbole, o que pode contribuir para a preservação de populações naturais da espécie.

Palavras-chave: produtos naturais, farmacognosia, metabólitos secundários, sóbole.

REFERENCES

ALVES AB AND BRAGAGNOLO N. 2002. Determinação simultânea de teobromina, teofilina e cafeína em chás por cromatografia líquida de alta eficiência. Rev Bras Cienc Farm 38: 237-243.

APPEZZATO-DA-GLÓRIA B. 2003. Morfologia de sistemas subterrâneos: histórico e evolução do conhecimento no Brasil, Ribeirão Preto: A.S. Pinto, 80 p.

ARNOUS A. 2002. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. J Food Comp Anal 15: 655-665.

BARBOSA WLR. 2001. Manual para análise fitoquímica e cromatográfica de extratos vegetais, Belém: U Fed Pará.

BARNES PJ. 2003. Theophylline: new perspectives for an old drug. Am J Resp Crit Care 167(6): 813-818.

BRASIL MS. 2000. Farmacopéia Brasileira, Brasília: Ministério da Saúde.

CHAMBERLAIN CJ. 1932. Methods in plant histology, Chicago: University of Chicago Press, 416 p.

COELHO VPM, LEITE JPV, NUNES LG AND VENTRELLA MC. 2012. Anatomy, histochemistry and phytochemical profile of leaf and stem bark of *Bathysa cuspidata* (Rubiaceae). Aust J Bot 60: 49-60.

COSTA EA, SANTOS LR, PONTES IS, MATOS LG, SILVA GA AND LIÃO LM. 2007. Analgesic and anti-inflammatory effects of *Cheiloclinium cognatum* root barks. Braz J Pharmacog 17: 508-513.

CRUZ BP, CHEDIER LM, PEIXOTO PHP, FABRI RL AND PIMENTA DS. 2012. Effects of light intensity on the distribution of anthocyanins in *Kalanchoe brasiliensis* Camb. And *Kalanchoe pinnata* (Lamk.) Pers. An Acad Bras Cienc 84: 211-217.

DAVID R AND CARDE JP. 1964. Coloration différentielle dês inclusions lipidique et terpeniques dês pseudophylles du *Pin maritime* au moyen du reactif nadi. C R Acad Sci Paris D 258: 1338-1340.

DIAS JE AND LAUREANO LC. 2010. Farmacopéia popular do cerrado, Góias: Articulação Pacari, 352 p.

DUARTE LP, FIGUEIREDO RC, SOUSA GF, SOARES DBS, RODRIGUES SBV, SILVA FC AND SILVA GDF. 2010. Chemical constituents of *Salacia elliptica* (Celastraceae). Quim Nova 33: 900-903.

DUARTE MR AND DEBUR MC. 2005. Stem and leaf morpho-anatomy of *Maytenus ilicifolia*. Fitoterapia 76: 41-49.

ESTEVAM CS, CAVALCANTI AM, CAMBUI EVF, ARAÚJO-NETO V, LEOPOLDO PTG, FERNANDES RPM, ARAÚJO BS, PORFÍRIO Z AND SANT'ANA AEG. 2009. Perfil fitoquímico e ensaio microbiológico dos extratos da entrecasca de *Maytenus rigida* Mart. (Celastraceae). Rev Bras Farmacog 19: 299-303.

FAHN A. 1990. Plant anatomy, Oxford: Butteerworth-Heinemann Ltd.

FARIAS V, ROCHA LD, PREUSSLER KH AND MARANHO LT. 2009. Leaf structural organization of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum, Myrtaceae. Acta Bot Bras 23: 398-406.

FERREIRA PRF, MENDES CSO, REIS SB, RODRIGUES CG, OLIVEIRA DA AND MERCADANTE-SIMÕES MO. 2011. Morphoanatomy, histochemistry and phytochemistry of *Psidium guineense* Sw. (Myrtaceae) leaves. J Pharm Res 4: 942-944.

FEUCHT W, SCHMID PPS AND MARTINS RP. 1986. Distribution of flavonols in meristematic and mature tissues of *Prunnus avium* shoots. J Plant Physiol 125: 1-8.

FURR M AND MAHLBERG PG. 1981. Histochemical analyses of laticifers and glandular trichomes in *Cannabis sativa*. J Nat Prod 44: 153-159.

GOMES SMA AND LOMBARDI JA. 2010. Leaf anatomy as a contribution to the taxonomy of Salacioideae N. Hallé ex Thorne & Reveal (Celastraceae). Plant Syst Evol 289: 13-33.

GOMES SMA, SILVA EAM, LOMBARDI JA, AZEVEDO AA AND VALE FHA. 2005. Anatomia foliar como subsidio à taxonomia de Hippocrateoideae (Celastraceae) no Sudeste do Brasil. Acta Bot Bras 19: 945-961.

GULLO FP ET AL. 2012. Antifungal activity of maytenin and pristimerin. Evid Based Complement Alternat Med 2012: 1-6.

HENRIQUES AT, LIMBERGER RP, KERBER VA AND MORENO PRH. 2003. Alcalóides: generalidades e aspectos básicos. In: SIMÕES CMO ET AL. (Eds), Farmacognosia: da planta ao medicamento, Porto Alegre/Florianópolis: UFRGS/ UFSC, Rio Grande do Sul/Santa Catarina, BR, p. 765-792.

JACOMASSI E, MOSCHETA IS AND MACHADO SR. 2007. Morfoanatomia e histoquímica de *Brosimum gaudichaudii* Trécul (Moraceae). Acta Bot Bras 21: 575-597.

JENSEN WA. 1962. Botanical histochemistry: principles and practice, San Francisco: W.H. Freeman & Company.

JOHANSEN DA. 1940. Plant microtecnique. New York: Macgraw-Hill Book.

KARNOVSKY MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol: 27: 137-138.

KIMURA M AND RODRIGUEZ-AMAYA DB. 2002. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoids. Food Chem 78: 389-398.

LEITE JPV, BRAGA FC, ROMUSSI G, PERSOLI RM, TABACH R, CARLINI EA AND OLIVEIRA AB. 2010. Constituents from *Maytenus ilicifolia*. Leaves and bioguided fractionation for gastroprotective activity. J Brazil Chem Soc 21: 248-254.

LINDSEY KL, BUDESINSKY M, KOHOUT L AND VAN STADEN J. 2012. Antibacterial activity of maytenonic acid isolated from the root-bark of *Maytenus senegalensis*. S Afr J Bot 72: 473-477.

LOPES KLB, THADEO M, AZEVEDO AA AND MEIRA RMSA. 2009. Articulated laticifers in the vegetative organs of *Mandevilla atroviolaceae* (Apocynaceae, Apocynoideae). Can J Bot 87: 202-209.

LORENZI H AND MATOS FJA. 2002. Plantas medicinais no Brasil: nativas e exóticas cultivadas. Instituto Plantarum de Estudos da Flora Ltda, São Paulo: Pantarum.

LU J, PAPP LV, FANG J, RODRIGUEZ-NIETO S, ZHIVOTOVSKY B AND HOLMGREN A. 2006. Inhibition of Mammalian Thioredoxin Reductase by Some Flavonoids: Implications for Myricetin and Quercetin Anticancer Activity. Cancer Res 66: 4410-4418.

MACE ME AND HOWELL CR. 1974. Histochemistry and identification of condensed tannin precursor in roots of cotton seedlings. Can J Bot 52: 2423-2426.

MAOKA T. 2009. Sterically hindered carotenoids with 3 Z, 5 Z configuration from the seeds of oriental bitter sweet. *Celastrus orbiculatus*. Phytochemistry 70: 920-923.

MAROSO RP, CARNEIRO CM, SCHEFFER-BASSO SM AND FAVERO D. 2009. Morphological and anatomical aspects of birdsfoot trefoil and big trefoil. Rev Bras Zootectn 38: 1663-1667.

MAZIA D, BREWER PA AND ALFERT M. 1953. The cyto-chemistry staining and measurement of protein with mercuric bromophenol blue. Biol Bull 104: 57-67.

MONACELLI B, VALLETTA A, RASCIO N, MORO I AND PASQUA G. 2005. Laticifers in *Camptotheca acuminata* Decne: distribution and structure. Protoplasma 226: 155-161.

MOUCO GB, BERNARDINO MJ AND CORNÉLIO ML. 2003. Controle de qualidade de ervas medicinais. Biotecnol Ciênc Desenvolv 31: 68-73.

NIIZU PY AND RODRIGUEZ-AMAYA DB. 2005. New data on the carotenoid composition of raw salad vegetable. J Food Comp Anal 18: 739-749.

O'BRIEN TP, FEDER N AND MCCULLY ME. 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59: 368-373.

PACE MR, LOHMANN LG AND ANGYALOSSY V. 2011. Evolution of disparity between the regular and variant phloem in Bignonieae (Bignoniaceae). Am J Bot 98: 602-618.

PAIVA EAS, PINHO SZ AND OLIVEIRA DMT. 2011. Large plant samples: how to process for GMA embedding? In: CHIARINI-GARCIA H ET AL. (Eds), Light microscopy: methods and protocols, New York: Springer/Humana Press, New York, USA, p. 37-49.

PANSERA MR, SANTOS ACA, PAESE K, WASUM R, ROSSATO M, ROTA LD, PAULETTI GF AND SERAFINI LA. 2003. Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul. Rev Bras Farmacog 13: 17-22.

PEARSE AGE. 1980. Histhochemistry theoretical and applied. Longman Group Limited.

PICKARD WF. 2008. Laticifers and secretory ducts: two other tube systems in plants. New Phytol 177: 877-888.

SANTOS SC AND MELLO JCP. 2003. Taninos. In: SIMÕES CMO ET AL. (Eds), Farmacognosia: da planta ao medicamento, Porto Alegre: UFSC, p. 615-656.

SANTOS VL, COSTA VBM, AGRA MF, SILVA BA AND BATISTA LM. 2007. Pharmacological studies of ethanolic extracts of *Maytenus rigida* Mart (Celastraceae) in animal models. Rev Bras Farmacog 17: 336-342.

SEN A, MIRANDA I, SANTOS S, GRAÇA J AND PEREIRA H. 2010. The chemical composition of cork and phloem in the rhytidome of *Quercus cerris* bark. Ind Crop Prod 31: 417-422.

SILVA FC, DUARTE LP, SILVA GDF, FILHO SAV, LULA IS, TAKAHASHIC JA AND SALLUM WST. 2011. Chemical constituents from branches of *Maytenus gonoclada* (Celastraceae) and evaluation of antimicrobial activity. J Brazil Chem Soc 22: 943-949.

SILVA IA, VALENTI MW AND SILVA-MATOS DM. 2009. Fire effects on the population structure of *Zanthoxylum rhoifolium* Lam (Rutaceae) in a brazilian savanna. Braz J Biol 69: 813-818.

SOFFIATTI P AND ANGYALOSSY-ALFONSO V. 1999. Estudo anatômico comparativo do lenho e da casca de duas espécies de *Eugenia* L. (Myrtaceae). Rev Bras Bot 22: 175-184.

TANABE G, SAKANO M, MINEMATSU T, MATUSDA H, YOSHIKAWA M AND MURAOKA O. 2008. Synthesis and elucidation of absolute stereochemistry of salaprinol, another thiosugar sulfonium sulfate from the ayurvedic traditional medicine *Salacia prinoides*. Tetrahedron 64: 10080-10086.

TIBERTI LA, YARIWAKE HJ, NDJOKO K AND HOSTETTMANN K. 2007. Identification of flavonols in leaves of *Maytenus ilicifolia* and *M. aquifolium* (Celastraceae) by LC/UV/MS analysis. J Chrom B 846: 378-384.

VIDAL BC. 1977. Acid glycosaminoglycans and endochondral ossification: microespectrophotometric evaluation and macromolecular orientation. Cell Mol Biol 22: 45-64.

WANDERLEY MG, SHEPHERD GJ, GIULLIETTI AM AND MELLHEM TS. 2003. Flora Fanerogâmica do Estado de São Paulo, 3, São Paulo: Rima, p. 367.

WANG Y ET AL. 2012. Pristimerin causes G1 arrest, induces apoptosis, and enhances the chemosensitivity to gemcitabine in pancreatic cancer cells. Plos One 7: e43826.

WHO. 1987. Quality control methods for medicinal plant materials. Genebra: World Health Organization.

ZSCHOCKE S, RABE T, TAYLOR J, JAGER A AND VAN STADEN J. 2000. Plant part substitution - a way to conserve endangered medicinal plants? J Ethnopharmacol 71: 281-292.

ZUANAZZI JAS AND MONTANHA JA. 2003. Flavonóides. In: SIMÕES CMO ET AL. (Eds), Farmacognosia: da planta ao medicamento, Porto Alegre/Florianópolis: UFRGS/UFSC, Rio Grande do Sul/Santa Catarina, BR, p. 577-614.