



Original Article

Anatomy and histochemistry of leaves and stems of *Sapium glandulosum*



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ABSTRACT

Sapium belongs to Euphorbiaceae family and comprises 23 species. *Sapium glandulosum* (L.) Morong is popularly known in Brazil as “pau-leiteiro” and “leitosinha” and it is used in traditional medicine to cicatrization. Its leaf extracts have shown analgesic, anti-inflammatory and antibacterial activities. The preliminary set of pharmacognostic tools used for quality assessment of medicinal plant parts is macro- and micro-anatomy and *S. glandulosum* has not anatomical and histochemical description. Thus the aim of this study was to investigate the anatomical and histochemical characteristics of the leaf and stem of *S. glandulosum* as a means of providing information for quality assessment of herbal industry. The leaves and stems were investigated by employing field emission scanning electron microscopy, light microscopy, and histochemistry techniques. The analysis showed that *S. glandulosum* had the following anatomical features: dorsiventral and amphistomatic leaves; paracytic stomata; tabular crystal druses; non-articulated and branched laticifers; midrib's biconvex shape with vascular systems in open arc with invaginated ends; petiole with a round shape and slight concavity on the adaxial side; six collateral vascular bundles in U-shaped organisation; a circular stem shape and a sclerenchymatous ring. In the histochemical tests lipophilic components were found in cuticle and in the latex; phenolic compounds were met in the mesophyll and in the latex; starch grains were found in the parenchymatous sheath; lignified elements were met in the sclerenchymatous ring in the cortex and in the perivascular sclerenchymatous caps, beyond in the vessel elements. These features are helpful when conducting a quality control process.

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Introduction

Sapium Jacq. is one of the most important genus of Euphorbiaceae. It consists of 23 accepted species (The Plant List, 2015) and deserves consideration because of the complexity involved in delimiting its species (Secco et al., 2012). It is formed mainly by neotropical species and is distributed in fields, savannas, seasonal forests, rainforests and woodlands (Sátiro and Roque, 2008; Pscheidt and Cordeiro, 2012).

This genus presents several species that are used in popular medicine, such as *S. chihsonianum* S.K. Lee, *S. discolor* (Champ. ex Benth) Muell. Arg., *S. rotundifolium* Hemsl., and *S. sebiferum* (L.) Roxb, which are used mainly to cicatrization (Al Muqarrabun

et al., 2014). Some species of *Sapium* have been chemically and pharmacologically studied. Extracts and single components from this genus were reported to have promising biological activities such as antioxidant, antimicrobial, and cytotoxic effects (Hajdu and Hohmann, 2012; Al Muqarrabun et al., 2014).

Sapium glandulosum (L.) Morong, which is popularly known in Brazil as “pau-leiteiro” and “leiteiro”, is a tree that can reach 3–8 m in height and is among the most polymorphic species of *Sapium*. It is used in traditional medicine to treat hernias (Hajdu and Hohmann, 2012; Al Muqarrabun et al., 2014) and its use has been potentially recommended for the recovery of degraded areas (Ferreira et al., 2009).

The leaves of *S. glandulosum* contain anthracene derivatives, monoterpenes, tannins and flavonoids (da Silva et al., 2011, 2012). This species is latex-bearing and the latex has proteins with considerable proteolytic activity. This activity is notably inhibited by a serine protease inhibitor (Sobottka et al., 2014). The leaf extracts

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have shown analgesic, anti-inflammatory (Valle and Kaplan, 2000) and antibacterial activities (da Silva et al., 2012).

Classifying medicinal plants is a serious problem because of their common names. A single medicinal species frequently has a number of popular names and a popular name can occasionally be used for a range of plants (Upton et al., 2011). Some species of *Sapium*, such as *S. glandulosum*, *S. arbutum* (Müll.Arg.) Huber and *S. sellowianum* (Müll.Arg.) Klotzsch ex Baill (Agra et al., 2008), are popularly known in Brazil as “pau-leiteiro”, “leiteiro” or “burra-leiteira”. In this context the most important consequence in regard to the use of inappropriate folk names is the substitution of therapeutic and safe herbs by toxic vegetable species (Upton et al., 2011).

The preliminary set of pharmacognostic tools used for quality assessment of medicinal plant parts is macro- and micro-anatomy (Upton et al., 2011). Consequently, the aim of this study was to investigate the anatomical and histochemical characteristics of the leaf and stem of *S. glandulosum* as a means of providing information for quality control in the herbal industry. Furthermore, there are no previous papers in the literature about the pharmacobotanical characteristics of this taxon.

Materials and methods

Plant material

The leaves and stems of *Sapium glandulosum* (L.) Morong, Euphorbiaceae, were collected from grown specimens in open and sunny areas in the Campos Gerais region of Paraná (24° 18' S and 49° 37' W), Brazil in October 2013. Mature leaves and stems (at least ten samples) obtained from the sixth node and below (median, intercostal and margin regions), as well as stem fragments from 5 to 15 cm from the shoot were prepared for the pharmacobotanical assays. The plant material containing inflorescences was used to prepare a voucher specimen, which was identified by Osmar dos Santos Ribas and stored at the Museu Botânico de Curitiba under the number 390589 MBM.

Pharmacobotanical assays

The leaves and stems of *S. glandulosum* were placed in a solution of FAA 70 (Johansen, 1940), and stored in 70% ethanol (Berlyn and Miksche, 1976). For the examination of leaf and stem material free-hand longitudinal and cross-sections were prepared. In the leaves it was included the midrib, interneural regions, and lateral veins. These materials were stained using Astra blue and basic fuchsin (Roeser, 1972) and toluidine blue (O'Brien et al., 1964) to obtain semi-permanent slides. The diaphanisation of the leaves was performed by following the technique of Fuchs (1963). For the crystals descriptions He et al. (2012) were used.

Histochemical tests

The following standard solutions were employed in the histochemical tests: methylene blue to test for mucilage (Oliveira et al., 2005); hydrochloric phloroglucin to reveal traces of lignin (Sass, 1951); Sudan III for testing lipophilic compounds (Foster, 1949); Hoepfner–Vorsatz test, modified by Reeve (1951) (aqueous 10% sodium nitrate, aqueous 10% acetic acid, aqueous 10% urea and, 2 N NaOH) and ferric chloride to test for phenolic substances (Johansen, 1940); Bouchardat reactive for nitrogen compounds (Borio, 1959); methylene blue to test mucilage (Oliveira et al., 2005) and iodine-iodide to reveal starch (Berlyn and Miksche, 1976).

Photomicrographs were captured using a Olympus CX 31 light microscope that was equipped with a C7070 digital camera. The semi-permanent and histochemical test slides were then analysed

in the Laboratory of Pharmacognosy at the State University of Ponta Grossa (UEPG) for a detailed description of the leaf and stem tissues.

Field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDS)

For the field emission scanning electron microscopy (Mira 3 Tescan) fresh leaves and stems were used. The samples were submitted in high vacuum with high accelerating voltage (15 kV). This method required the samples to be previously dehydrated using increasing amounts of ethanol then dried in a critical point dryer. Afterwards, they were submitted to metallisation with gold (Quorum, modelo SC7620). Qualitative X-ray microanalyses were performed on certain crystals and in cells without crystals (control) using an EDS machine (Mira 3 Tescan) on the same variable-pressure microscope. This procedure was carried out at the multi-user laboratory (LABMU) of UEPG.

Results and discussion

The leaves of *S. glandulosum* (Fig. 1A, B), in frontal view, showed epidermal cells with straight to slight wavy anticlinal walls (Fig. 1C, F), which were relatively thin on both sides. The leaves were amphistomatic and the paracytic stomata were observed predominantly on the abaxial side (Fig. 1C–E). On the adaxial side, they appeared only near the midrib as observed in Fig. 1F, G. They measured 35 µm in length on average and the striate cuticle was tangentially positioned in the subsidiary cells (Fig. 1C–E). Metcalfe and Chalk (1950) reported paracytic stomata in the Euphorbiaceae tribe. Valle and Kaplan (2000) reported that *S. glandulosum* had amphistomatic leaves, while *S. sellowianum* (Müll.Arg.) Klotzsch ex Baill. had hypostomatic leaves. These authors affirmed that the distribution of stomata was a taxonomic feature that helps to separate these two species.

In cross-section, the epidermis was uniseriate and the cells were larger on the adaxial side. The cuticle was smooth and thin and reacted with Sudan III in the histochemical test (Fig. 1H). Druses were found in the epidermal cells (Fig. 1H). The mesophyll was dorsiventral and was formed by one layer of palisade parenchyma and about eight layers of spongy parenchyma. Small collateral vascular bundles were immersed in the mesophyll and they were surrounded by a parenchymatous sheath. Druses were also observed in the mesophyll (Fig. 1H).

Phenolic compounds are secondary metabolites responsible for adaptation and resistance to hostile environment factors. They are implicated not only in the defense mechanisms of plants against fungal pathogens but also against insect herbivores (Lattanzio et al., 2006). In the present study, phenolic compounds reacted positively with ferric chloride and Hoepfner–Vorsatz test and they are found in the mesophyll.

The midrib, in transection, was biconvex; however, the convexity was more conspicuous on the abaxial surface (Fig. 2A). The epidermis is uniseriate and it is covered by a striate and thick cuticle. The cuticle reacted with Sudan III (Fig. 2C). The cuticle is the most important barrier against uncontrolled water loss from leaves, stems, fruits and other parts of higher plants (Riederer and Schreiber, 2001). Cutin is the main component of the cuticle and is a lipophilic polymer that is deposited in and the top of the outer wall epidermal cells (Upton et al., 2011). Cuticle ornamentation is one of the most useful taxonomic characteristics of epidermis in leaves appearing as striations, ridges, or papillae (Barthlott et al., 1998; Upton et al., 2011).

Beneath the epidermis, on both sides, the chlorenchyma was interrupted and about six strata of angular collenchyma were apparent (Fig. 2A, C). The vascular system was represented by an

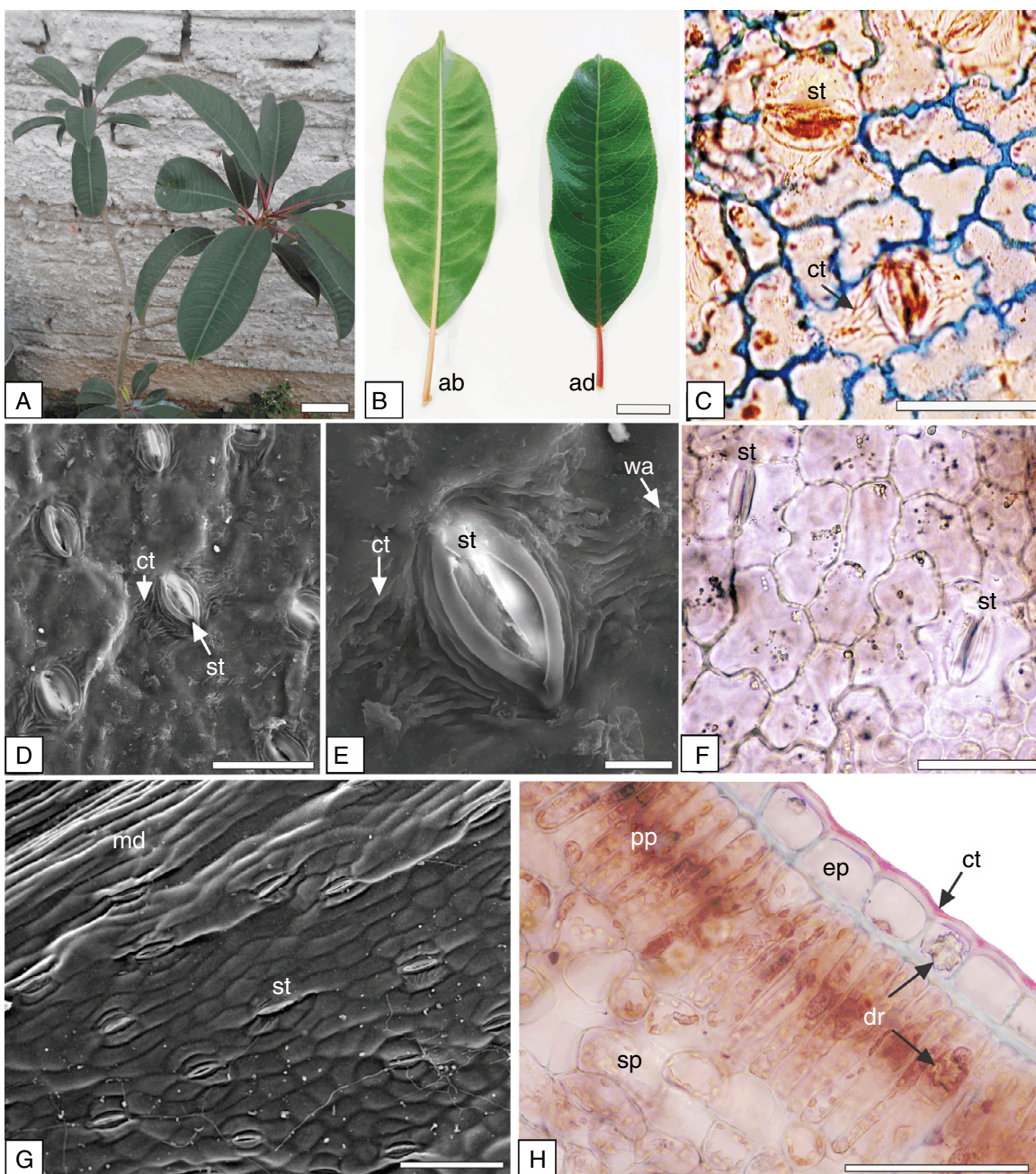


Fig. 1. *Sapium glandulosum* (L.) Morong, Euphorbiaceae. (A) Aspect of aerial vegetative organs, in habit. (B) Leaves, showing abaxial (ab) and adaxial (ad) sides. (C) Abaxial side of epidermis in frontal view, showing stomata (st) and striate cuticle (ct). (D) Abaxial side of epidermis in frontal view, showing stomata (st) and striate cuticle (ct) (FESEM – field emission scanning electron microscope). (E) Detail of the stomata (st), epicuticular waxes (wa) and striate cuticle (st), showing measure of the stomata in the abaxial epidermis – FESEM. (F) Adaxial side of epidermis in surface view, indicating stomata (st). (G) Adaxial side of epidermis in surface view, indicating stomata (st) near the midrib (md) – FESEM. (H) Leaf in cross-section indicating cuticle (ct), druses (dr), epidermis (ep), palisade parenchyma (pp), spongy parenchyma (sp). Scale bar = 1 cm (A, B), 10 μm (E), 50 μm (C, D, F, H), and 100 μm (G).

open arc with invaginated ends, which was surrounded by a sclerenchymatic sheath (Fig. 2A, B). The organisation of vascular system is relevant feature of species characterisation and differentiation (Almeida et al., 2017; Bobek et al., 2016). In the midrib of the leaf of Euphorbiaceae, the organisation of the vascular tissues was variable (Gaucher, 1902). The vascular system organisation can help *S. glandulosum* identification.

Several druses were evident in the ground parenchyma, mainly near the sclerenchymatic sheath (Fig. 2B, E, G). Druses are considered cluster crystals and are formed by aggregates having several sides and sharp points (Upton et al., 2011) as blockys, tabulars, styloids, and tetrahedral crystals (He et al., 2012). In the present

work these druses were considered to be tabular crystal druses, as described by aforementioned authors for the Mimosoideae family.

Numerous laticifers were observed near the sclerenchymatic sheath and the vascular system (Fig. 2D–F); they were non-articulated and branched, as can be seen in the longitudinal section shown in Fig. 2F. Latex-secreting cells are named laticifers and occur in several families, including some Apocynaceae (Demarco et al., 2006), Campanulaceae (Folquitto et al., 2014), Papaveraceae (Upton et al., 2011) and Euphorbiaceae species (Demarco et al., 2013; Luz et al., 2015). The cell walls of the laticifers vary in shape, size, thickness, distribution and occurrence in the tissues and organs and in their occurrence in particular structures (Gales and Toma, 2007).

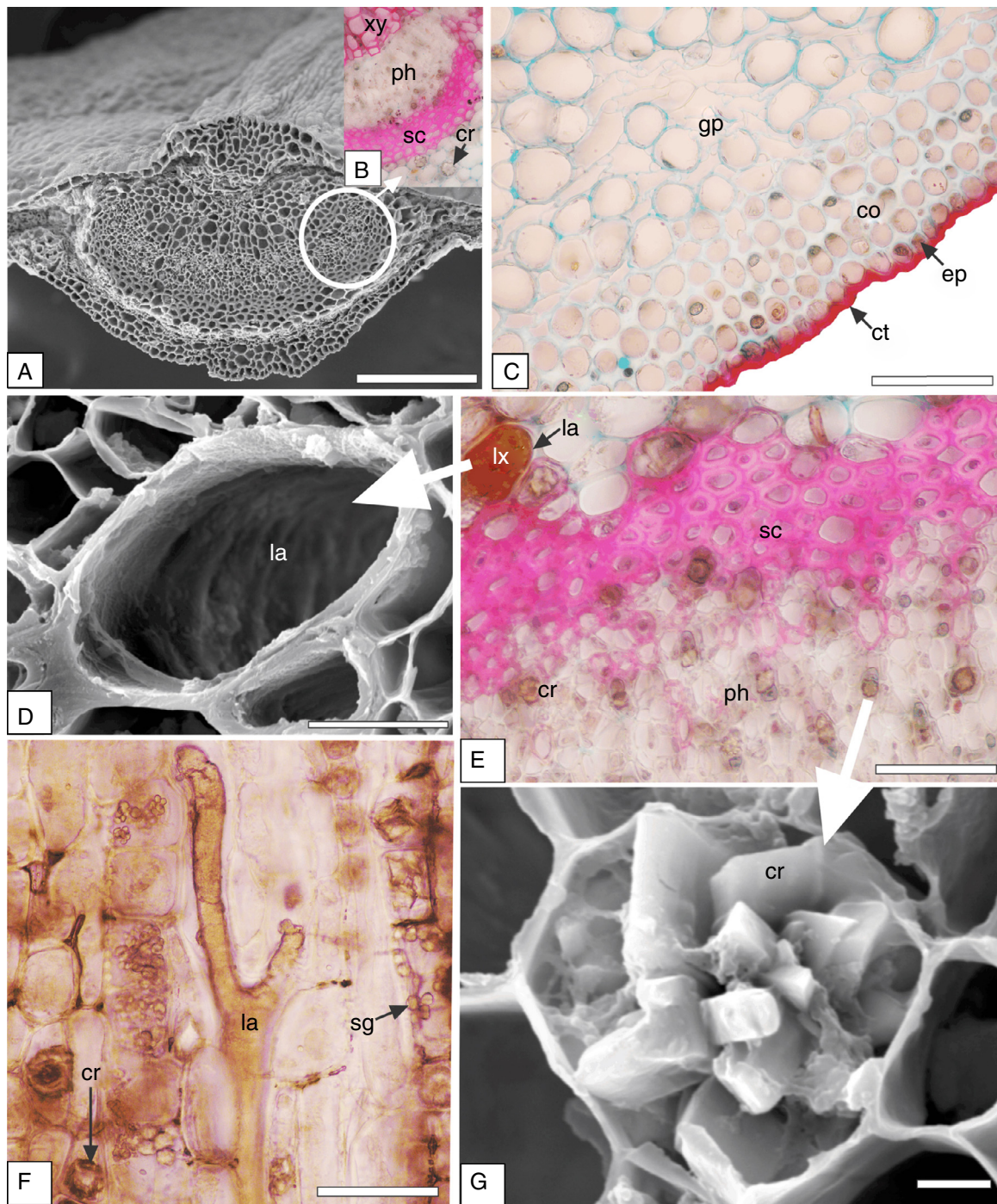


Fig. 2. *Sapium glandulosum* (L.) Morong, Euphorbiaceae – Midrib. (A) General aspect in cross-section (FESEM). (B) Detail of the vascular system in reaction with hydrochloric phloroglucin, indicating crystal (cr), sclerenchyma (sc), phloem (ph) and xylem (xy). (C) Detail of the abaxial side, showing collenchyma (co), cuticle (cu) in reaction with Sudan III, epidermis (ep), ground parenchyma (gp). (D) Laticifer in FESEM. (E) Phloem (ph) region, evidencing crystals (cr), sclerenchyma (sc), laticifers (la) with latex (lx). (F) Laticifer in longitudinal section in reaction with Hoepfner–Vorsatz modified reagent, crystals (cr) and starch grains (sg). (G) Tabular crystal druse (cr) – FESEM. Bar = 2 μm (G), 10 μm (D), 50 μm (C, E, F), 100 μm (B), and 200 μm (A).

Pompert (1989) reported that the laticifers were smaller and less frequent in *S. haematospermum* than in *S. longifolium*.

According to Demarco et al. (2013), Euphorbiaceae presents non-articulated branching and articulated anastomosing laticifers. These authors reported that *S. haematospermum* presented two articulated laticifers systems in the leaf and stem, one that was formed of narrow laticifers and the other shaped by wide laticifers. Non-articulated laticifers are initialised from single cells at an early period in seedling growth; articulated laticifers are formed

by chains of cells whose adjoining walls can occasionally break down, forming vessels (Rudall, 1987).

In the present study, the latex was a milky white fluid. In the histochemical tests it reacted positively in relation to lipophilic components with Sudan III (Fig. 3F); and to phenolic compounds in the Hoepfner–Vorsatz test (Fig. 2F) and with ferric chloride (Fig. 3E). The latex reacted negatively for mucilage and nitrogen components. Latex is a white fluid that is commonly exuded from the points of plant damage caused not only mechanically but also by

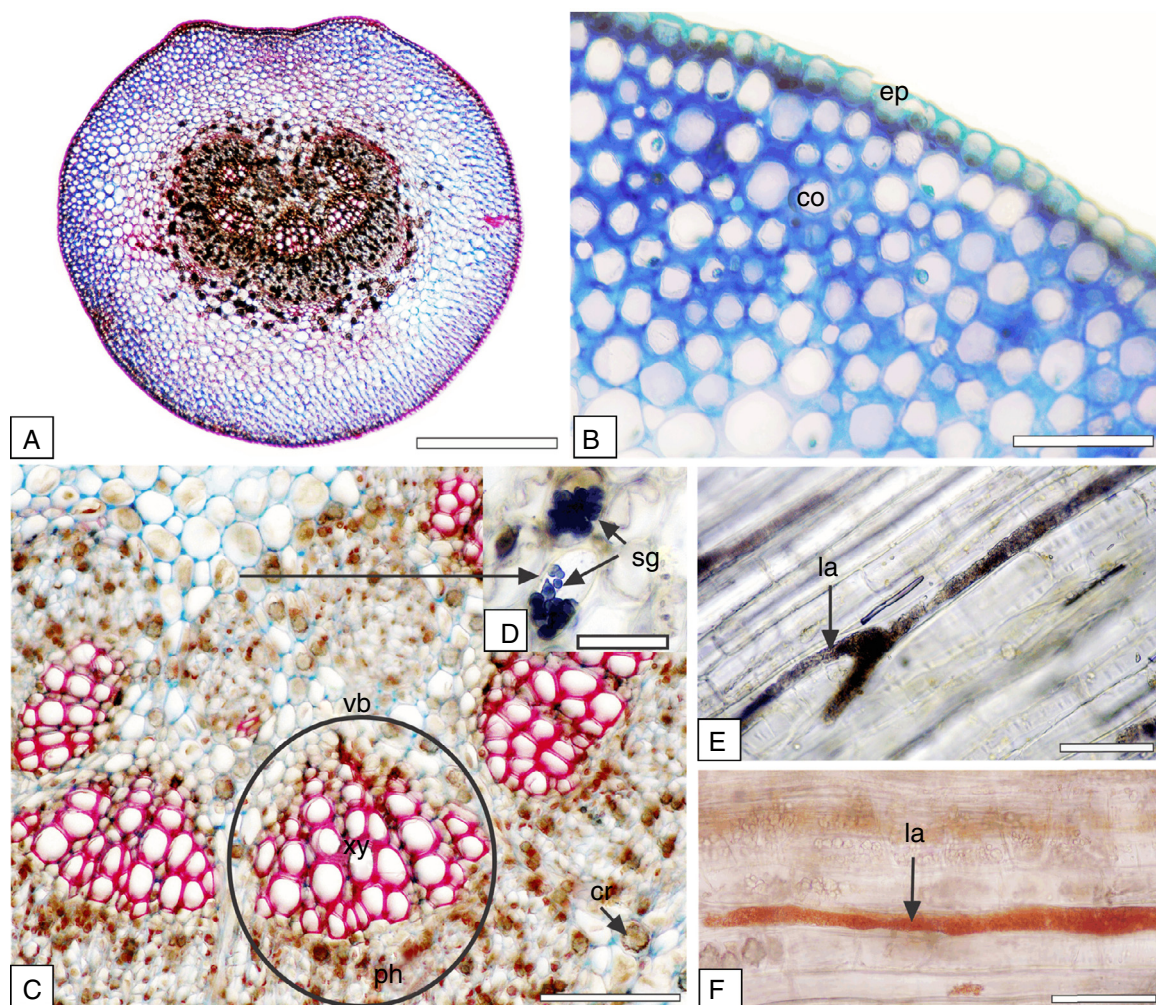


Fig. 3. *Sapium glandulosum* (L.) Morong, Euphorbiaceae – Petiole. (A) General aspect. (B) Collenchyma (co) and epidermis (ep). (C) Vascular system, showing vascular bundle (vb), phloem (ph), xylem (xy) and crystals (cr). (D) Detail of the starch grains in reaction with iodine-iodide. (E) Longitudinal section, showing laticifer (la) in reaction with ferric chloride. (F) Longitudinal section indicating laticifer (la) in reaction with Sudan III. Bar = 25 μm (D), 50 μm (B, C, E, F), and 200 μm (A).

insect herbivores (Konno, 2011). It is found in the vacuole of secretory cells known as laticifers, which include a complex combination of compounds such as phenolics, enzymes, terpenes, alkaloids, vitamins, mucilage, and lipids (Hagel et al., 2008; Folquitto et al., 2014; Luz et al., 2015). Latex has been attributed with cytotoxic (Luz et al., 2015), anti-tumour (Biscaro et al., 2013), anti-ulcer (Costa et al., 2012), and proteinase (Sobottka et al., 2014) activities, among others.

The petiole in the middle part and in cross-section had an almost round shape, however, with a slight concavity on the adaxial side (Fig. 3A). The epidermis had the same characteristics as the leaf blade and reacted with Sudan III in the microchemical test. Beneath the epidermis, there were about eight layers of angular collenchyma (Fig. 3B). Laticifers (Fig. 3E, F) as previously described for the midrib, could be observed near the vascular bundles. Druses were found in the ground parenchyma. The vascular system was formed by about six collateral vascular bundles (Fig. 3C) in a U-shaped organisation. Almeida et al. (2016) and Bobek et al. (2016) indicated the importance of the shape and vascular pattern of the petiole in cross-section and affirmed that these characteristics can be used as good markers in plants.

Starch is widely distributed throughout plant tissues, but is commonly found in highest concentrations in roots, rhizomes, and fruits (Upton et al., 2011). In the present study starch grains were met in the ground parenchyma of the midrib (Fig. 2F) and in the

vascular bundle sheath of petiole and reacted with iodine-iodide in the histochemical test (Fig. 3D). Not only the presence but also the structure of starch granules can be important for taxon identification (Upton et al., 2011). In *S. glandulosum* they were very small and rounded and/or ovate and appeared compound aggregates of two or more granules.

In transection, the stem presented a circular shape (Fig. 4A). The epidermis appeared in a single series with thickened cuticle. There were several layers of cells in the cortex (Fig. 4B, C). The sclerenchyma was formed by thickened cells containing lignin, leading to a sclerenchymatous ring in the cortex (Fig. 4C) and in the perivascular sclerenchymatous caps (Fig. 4B). They reacted with hydrochloric phloroglucin reagent (Fig. 4C) and with methylene blue (Fig. 4B). Lignin is polymer high phenolics which confers water resistance, strength, and elasticity. It is deposited among the cellulose microfibrils of primary and/or secondary cell walls (Upton et al., 2011). The presence of perivascular sclerenchymatous caps helped the *S. glandulosum* identification.

The endodermis was formed by a layer of cells. The vascular cylinder presented cambia, forming phloem outward and xylem inward. As expected, lignin was also found in vessel elements (Figs. 2B, 3C, 4B). Laticifers, as previously reported for the midrib and petiole, appeared close to the vascular system and to the sclerenchyma (Fig. 4D). The pith was composed of relatively small parenchymatous cells with thin walls (Fig. 4A).

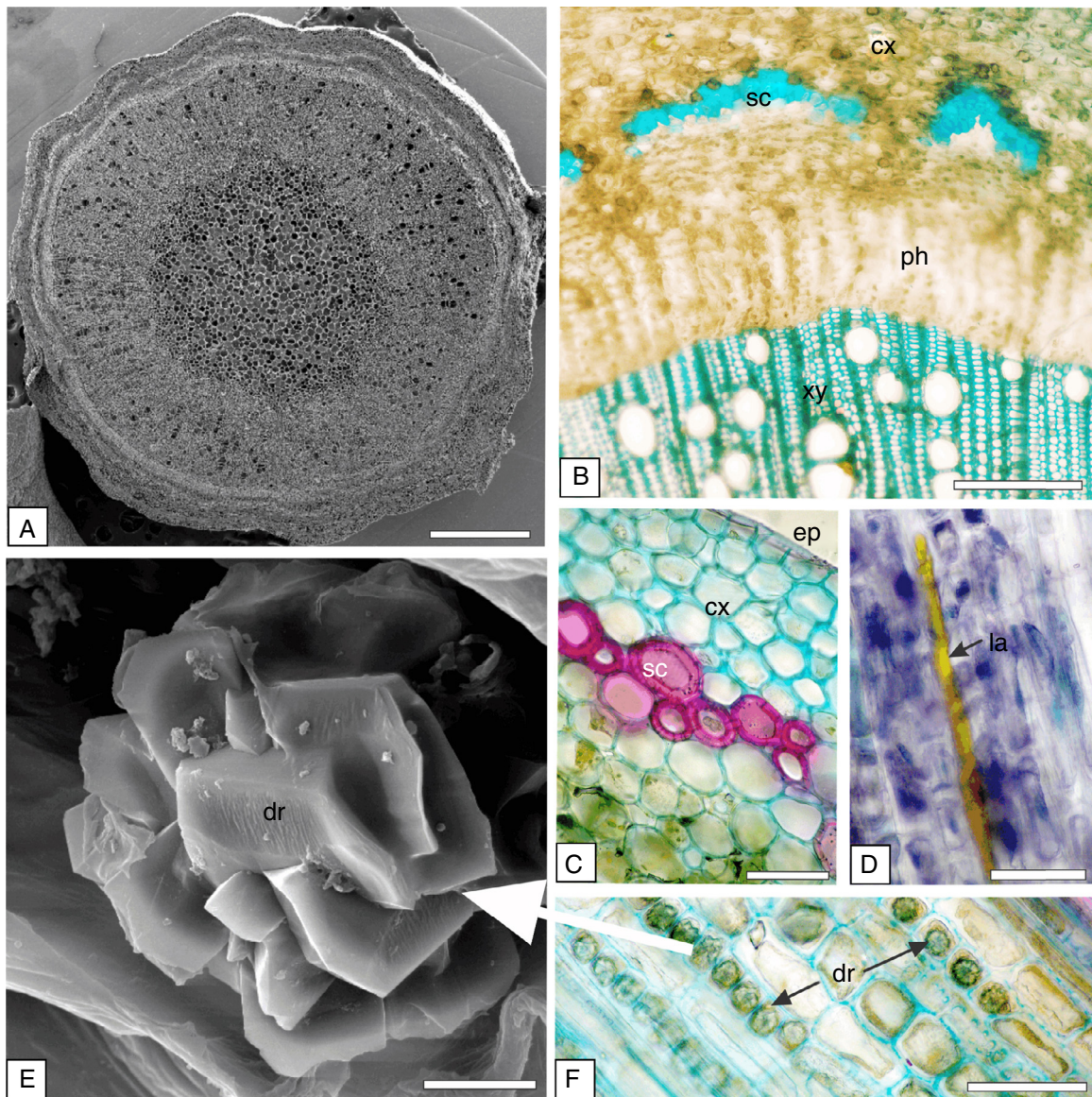


Fig. 4. *Sapium glandulosum* (L.) Morong, Euphorbiaceae – Stem. (A) General aspect in FESEM. (B) Cross-section, showing cortex (cx), phloem (ph), sclerenchyma (sc) and xylem (xy). (C) Detail of the cells of the sclerenchymatous ring, cortex (cx) and epidermis (ep). (D) Longitudinal section, showing laticifers (la). (E) Tabular crystal druses (cr) in FESEM. (F) Cortex showing several crystals (cr). Scale Bar = 2 μm (E), 50 μm (B–D, F), and 200 μm (A).

Crystal idioblasts were gathered in the stem (Fig. 4E, F), as observed in the mesophyll, midrib and petiole. In the present study, the crystals were analysed for their elemental composition and the spectra showed prominent peaks for calcium (27.74%), carbon (16.7%) and oxygen (55.56%), as can be seen in Fig. 5, indicating that these crystals were formed of calcium oxalate. Crystals have been

identified in some studies as calcium oxalate by using EDS (He et al., 2012; Almeida et al., 2016; Swiech et al., 2016).

The functions of crystals in plants are to act as an internal reservoir for calcium, to provide tissue rigidity, ionic balance, to remove calcium, magnesium, oxalic acid, aluminium and other heavy metals, and also to act as a protective device against for-

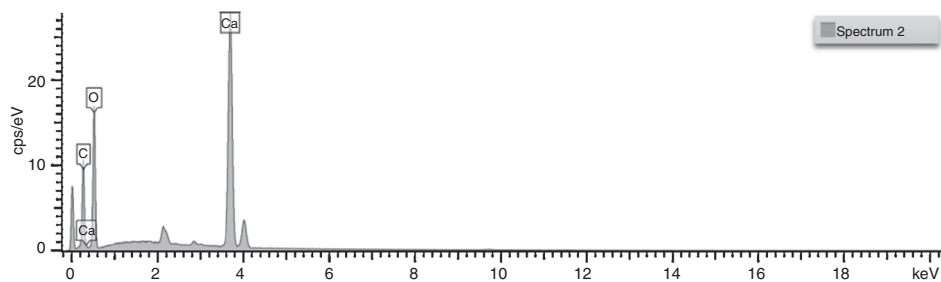


Fig. 5. EDS (energy-dispersive X-ray spectroscopy) spectra of crystals of *Sapium glandulosum*.

aging animals (Franceschi and Nakata, 2005; He et al., 2012; Silva et al., 2014). The presence or absence of crystals, their type and their chemical composition, can be characterised as taxonomic features (Meric, 2009). With reference to the chemical composition, excess calcium is habitually precipitated in calcium salts such as carbonate, citrate, malate, oxalate, phosphate, silicate and sulphate (Weiner and Dove, 2003).

Crystals of calcium oxalate are most commonly reported in higher families and they occur in most organs and tissues in the vegetable species. However, their size and number are responsive to changes in the concentration of calcium in the environment (Nakata, 2003; Franceschi and Nakata, 2005). Crystals of calcium oxalate are formed from endogenously synthesised oxalic acid and Ca taken from the environment, and they are formed and accumulated in species-specific morphologies (Meric, 2009).

Anatomical characterisation is an inherent part of practically all pharmacopoeias and is one of the primary identification tests necessary for pharmacopoeial compliance. The individual structural elements are comparatively frequent within the same plant organs, but the ways in which the tissues, elements and cells are set within a plant organ permits diagnosis to be performed and lend support to the quality assessment of herbal drugs (Upton et al., 2011).

The main anatomical characters were highlighted in this pharmacobotanical study, which was performed to provide more information about the standardisation of the *S. glandulosum* species in order to support the quality control of this vegetable material. The following characteristics are helpful when conducting the quality control process: dorsiventral and amphistomatic leaves; paracytic stomata; calcium oxalate tabular crystal druses; non-articulated and branched laticifers; biconvex with vascular systems in open arc with invaginated ends; petiole with round shape and slight concavity on the adaxial side; six collateral vascular bundles in U-shaped organisation; circular stem shape, sclerenchymatous ring in the cortex and perivascular sclerenchymatous caps.

The histochemical test showed the presence of the lipophilic and phenolic compounds in the latex; phenolic compounds in the mesophyll; starch grains small and rounded and compound aggregates of two or more granules; lignified elements were met in the sclerenchymatous ring in the cortex and in the perivascular sclerenchymatous caps, beyond in the vessel elements.

Authors' contributions

EAA, DGF, LECL and KSP assisted in carrying out the laboratory work. EAA contributed in collecting the plant material and its identification. PVF performed the scanning electron microscopy (FESEM) analysis. JMB created the project, supervised the laboratory work, and wrote the paper. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Agra, M.F., Silva, K.N., Basílio, I.J.L.D., de Freitas, P.F., Barbosa-Filho, J.M., 2008. Survey of medicinal plants used in the region Northeast of Brazil. *Rev. Bras. Farmacogn.* 18, 472–508.

- Al Muqarrabun, L.M.R., Ahmat, N., Aris, S.R.S., 2014. A review of the medicinal uses, phytochemistry and pharmacology of the genus *Sapium*. *J. Ethnopharmacol.* 155, 9–20.
- Almeida, V.P., Hirt, A.A., Raeski, P.A., Mika, B.E., Justus, B., dos Santos, V.L.P., Franco, C.R.C., de Paula, J.P., Farago, P.V., Budel, J.M., 2017. Comparative morphoanatomical analysis of *Mikania* species. *Rev. Bras. Farmacogn.* 27, 9–19.
- Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I., Wilhelm, H., 1998. Classification and terminology of plant epicuticular waxes. *Bot. J. Linn. Soc.* 126, 227–236.
- Berlyn, G.P., Miksche, J.P., 1976. *Botanical Microtechnique and Cytochemistry*. Iowa State University, Ames.
- Biscaro, F., Parisotto, E.B., Zanette, V.C., Günther, T.M.F., Ferreira, E.A., Gris, E.F., Correia, J.F.G., Pich, C.T., Mattivi, F., Wilhelm Filho, D., Pedrosa, R.C., 2013. Anticancer activity of flavonoid and flavan-3-ol rich extracts from *Croton celtidifolius* latex. *Pharm. Biol.* 51, 737–743.
- Bobek, V.B., Heiden, G., Oliveira, C.F., Almeida, V.P., de Paula, J.P., Farago, P.V., Nakashima, T., Budel, J.M., 2016. Comparative analytical micrographs of vasculas (Baccharis, Asteraceae). *Rev. Bras. Farmacogn.* 26, 665–672.
- Borio, E.B.L., 1959. *Lobelia langeana* Dusén. Contribuição para o estudo farmacognóstico. da Faculdade de Farmácia da Universidade do Paraná, Brasil, 86 pp. Tese para concurso à docência livre da cadeira de farmacognosia, Faculdade de Farmácia da Universidade do Paraná.
- Costa, L.L.G., David, V.C., Pinto, R.M.C., Minozzo, B.R., Kozłowski Jr., V.A., Campos, L.A., Silva, R.Z., Beltrame, F.L., 2012. Anti-ulcer activity of *Synadenium grantii* latex. *Rev. Bras. Farmacogn.* 22, 1070–1078.
- da Silva, C.H.T.P., Sobrinho, T.J.S.P., Saraiva, A.M., Pisciotano, M.N.C., de Amorim, E.L.C., 2012. Phytochemical profile and antibacterial activity of bark and leaves of *Caesalpinia pyramidalis* Tul. and *Sapium glandulosum* (L.) Morong. *J. Med. Plants Res.* 6, 4766–4771.
- da Silva, C.H.T.P., Sobrinho, T.J.S.P., e Castro, V.T.N.A., Lima, D.C.A., de Amorim, E.L.C., 2011. Antioxidant capacity and phenolic content of *Caesalpinia pyramidalis* Tul. and *Sapium glandulosum* (L.) Morong from Northeastern Brazil. *Molecules* 16, 4728–4739.
- Demarco, D., Castro, M.M., Ascensão, L., 2013. Two laticifer systems in *Sapium haematospermum* – new records for Euphorbiaceae. *Botany* 91, 545–554.
- Demarco, D., Kinoshita, L.S., Castro, M.M., 2006. Laticíferos articulados anastomosados: novos registros para Apocynaceae. *Rev. Bras. Bot.* 29, 133–144.
- Ferreira, B.G.A., Zuffellato-Ribas, K.C., Carpanezzi, A.A., Tavares, F.R., Koehler, H.S., 2009. Metodologias de aplicação de AIB no enraizamento de estacas semilenhosas de *Sapium glandulosum* (Vell.) Pax. *Rev. Bras. Plantas Med.* 11, 196–201.
- Folquitto, D.G., Budel, J.M., Pereira, C.B., Brojan, L.E.F., Folquitto, G.G., Miguel, M.D., Silva, R.Z., Miguel, O.G., 2014. Analytical micrography and preliminary phytochemistry of the leaves and stems of *Lobelia exaltata* Pohl. (Campanulaceae). *Lat. Am. J. Pharm.* 33, 245–250.
- Foster, A.S., 1949. *Practical Plant Anatomy*, 2nd ed. D. Van Nostrand, Princeton.
- Franceschi, V.R., Nakata, P.A., 2005. Calcium oxalate in plants: formation and function. *Annu. Rev. Plant Biol.* 56, 41–71.
- Fuchs, C.H., 1963. Fuchsin staining with NaOH clearing for lignified elements of whole plants or plants organs. *Stain Technol.* 38, 141–144.
- Gales, R.C., Toma, C., 2007. Researches regarding the morphology, structure and distribution of vegetative organs of some *Euphorbia* species from Romania's flora. *Biol. Veg.* 1, 40–45.
- Gaucher, L., 1902. Recherches anatomiques sur les Euphorbiacées. *Ann. Sci. Nat. Bot.* 15, 161–309.
- Hagel, J.M., Yeung, E.C., Facchini, P.J., 2008. Got milk? The secret life of laticifers. *Trends Plant Sci.* 13, 631–639.
- Hajdu, Z., Hohmann, J., 2012. An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paraguá Indian Reservation, Bolivia. *J. Ethnopharmacol.* 139, 838–857.
- He, H., Bleby, T.M., Veneklaas, E.J., Lambers, H., Kuo, J., 2012. Morphologies and elemental compositions of calcium crystals in phylloides and branchlets of *Acacia roborum* (Leguminosae: Mimosoideae). *Ann. Bot.* 109, 887–896.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw Hill Book, New York.
- Konno, K., 2011. Plant latex and other exudates as plant defense systems: roles of various defense chemicals and proteins contained therein. *Phytochemistry* 72, 1510–1530.
- Lattanzio, V., Lattanzio, V.M.T., Cardinali, A., 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato, F. (org). *Phytochemistry: Advances in Research*. Research Signposts, Trivandrum, pp. 23–67.
- Luz, L.E.C., Paludo, K.S., Santos, V.L.P., Franco, C.R.C., Klein, T., Silva, R.Z., Beltrame, F.L., Budel, J.M., 2015. Cytotoxicity of latex and pharmacobotanical study of leaves and stem of *Euphorbia umbellata* (Janaúba). *Rev. Bras. Farmacogn.* 25, 344–352.
- Meric, C., 2009. Calcium oxalate crystals in some species of the tribe Inuleae (Asteraceae). *Acta Biol. Crac. Ser. Bot.* 51, 105–110.
- Metcalfe, C.R., Chalk, L., 1950. *Anatomy of the Dicotyledons*. Clarendon Press, Oxford.
- Nakata, P.A., 2003. Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Sci.* 164, 901–909.
- O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59, 368–373.
- Oliveira, F., Akisue, G., Akisue, M.K., 2005. *Farmacognosia*. Atheneu, São Paulo.
- Pompert, M.G., 1989. Estudio morfo-anatomico de dos especies de *Sapium* (Euphorbiaceae). *Bonplandia* 6, 197–210.

- Pscheidt, A.C., Cordeiro, I., 2012. Sinopse da tribo Hippomaneae (Euphorbiaceae) no Estado de São Paulo, Brasil. *Hoehnea* 39, 347–368.
- Reeve, R.M., 1951. Histochemical tests for polyphenols in plant tissues. *Stain Technol.* 26, 91–96.
- Riederer, M., Schreiber, L., 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. *J. Exp. Bot.* 52, 2023–2032.
- Roeser, K.R., 1972. Die Nadel der Schwarzkiefer-Massenprodukt und Kunstwerk der Natur. *Mikrokosmos* 61, 33–36.
- Rudall, P.J., 1987. Laticifers in Euphorbiaceae conspectus. *Bot. J. Linn. Soc.* 94, 143–163.
- Sass, J.E., 1951. *Botanical Microtechnique*, 2nd ed. Iowa State College, Ames.
- Sátiro, L.N., Roque, N., 2008. A família Euphorbiaceae nas caatingas arenosas do médio rio São Francisco, BA, Brasil. *Acta Bot. Bras.* 22, 99–118.
- Secco, R.S., Cordeiro, I., Senna-Vale, L., Sales, M.F., Lima, L.R., Medeiros, D., Haiad, B.S., Oliveira, A.S., Caruzo, M.B.R., Carneiro-Torres, D., Bigio, N.C., 2012. An overview of recent taxonomic studies on Euphorbiaceae s.l. in Brazil. *Rodriguésia* 63, 227–242.
- Silva, R.J.F., de Aguiar-Dias, A.C.A., de Mendonça, M.S., 2014. Rosetas e concreções cristalinas silicificadas em *Piper* (Piperaceae): registros inéditos de macropadrões. *Acta Amazon.* 44, 435–446.
- Sobottka, A.M., Tonial, F., Sytwala, S., Melzig, M., 2014. Proteinase activity in latex of three plants of the family Euphorbiaceae. *Braz. J. Pharm. Sci.* 50, 559–565.
- Swiech, J.N.D., Bobek, V.B., Folquitto, D.G., Silva, R.Z., Budel, J.M., Farago, P.V., Miguel, M.D., Miguel, O.G., 2016. Morpho-anatomy of the vegetative organs of *Philodendron meridionale* (Araceae). *Lat. Am. J. Pharm.* 35, 2142–2148.
- The Plant List, 2015. *Sapium*, <http://www.theplantlist.org/browse/A/Euphorbiaceae/Sapium/> (accessed October 2015).
- Upton, R., Graff, A., Jolliffe, G., Länger, R., Williamson, E., 2011. *American Herbal Pharmacopoeia: Botanical Pharmacognosy – Microscopic Characterization of Botanical Medicines*. CRC Press, Boca Raton.
- Valle, L.S., Kaplan, M.A.C., 2000. *Sapium glandulatum* complex (Euphorbiaceae). *An. Acad. Bras. Cienc.* 72, 293–294.
- Weiner, S., Dove, P.M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Rev. Mineral. Geochem.* 54, 1–29.