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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 cicatrisation. 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.

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. chihsinianum* S.K. Lee, *S. discolor* (Champ. ex Benth) Muell. Arg., *S. rotundifolium* Hemsl., and *S. sebiferum* (L.) Roxb, which are used mainly to cicatrisation (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 fuchsine (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 Euphorbieae 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