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# Microscopy and Histochemistry of *Styrax leprosus* Hook. & Arn.: a Native Brazilian Tree Species

**Marco Franceschi**[1]
https://orcid.org/0000-0002-3184-9803

**Lorene Armstrong** [2*]
https://orcid.org/0000-0003-4078-8990

**Leandro Taborda da Rocha**[1]
https://orcid.org/0000-0002-5210-339X

**Jane Manfron**[2]
https://orcid.org/0000-0003-1873-2253

**Cintia Aparecida dos Anjos**[1]
https://orcid.org/0000-0001-5716-8646

**Karine Amorim Fladzinski**[1]
https://orcid.org/0000-0003-4346-8079

**Juliana de Fátima Garcia**[1]
https://orcid.org/0009-0003-3417-3997

**Deise Prehs Montrucchio**[1]
https://orcid.org/0000-0003-1440-7007

**Josiane de Fátima Gaspari Dias**[1]
https://orcid.org/0000-0002-8548-8505

**Marilis Dallarmi Miguel** [1]
https://orcid.org/0000-0002-1126-9211

**Obdulio Gomes Miguel**[1]
https://orcid.org/0000-0002-2231-9130

[1]Universidade Federal do Paraná, Departamento de Farmácia, Curitiba, Paraná, Brasil; [2]Universidade Estadual de Ponta Grossa, Programa de Pós-graduação em Ciências Farmacêuticas, Ponta Grossa, Paraná, Brasil.

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*Correspondence: larmstrong@uepg.br Tel.: +55-42-3220-3247 (L.A.).

**HIGHLIGHTS**

- Presence of lipids, lignified and phenolic compounds, starch grains, and alkaloids in *Styrax leprosus*;
- Presence of styloids, prismatic crystals, and druses of calcium oxalate;
- Peltate trichome was observed in the leaf and stem.

**Abstract:** *Styrax leprosus* Hook. & Arn. known as "*cuia-do-brejo, estoraque-do-campo, pau-de-remo*, *caujuja*", is a species of Styracaceae and can be found in South America. Reports on the medicinal use of the species have demonstrated its application in the treatment of diabetes and hypercholesterolemia. The aim of this work was to investigate the anatomy of leaves and stem, using the standard light and scanning electron microscopy techniques, and histochemical tests. The leaf is hypostomatic, dorsiventral, and present anomocytic stomata. Peltate trichomes are on abaxial side of leaf, petiole and stem. Midrib is flat-convex shaped with collateral vascular system arranged in open arch with a plate V-shaped. Petiole is concave-convex shaped with collateral vascular bundle in an open arc with invaginated ends. The stem has a circular shape, showing brachysclereids, styloids, prismatic crystals, and druses of calcium oxalate. Lipids, lignified and phenolic compounds, starch grains, and alkaloids were detected in the histochemical tests. Therefore, these data are relevant for the identification and differentiation of species within the genus, providing essential taxonomic information that acts as auxiliary tools in species identification.

**Keywords:** Anatomy; Crystal; Micromorphology; Peltate trichome; Styracaceae.

## INTRODUCTION

The family Styracaceae DC. & Spreng. encompasses 12 genera and 163 species and is distributed in North America, South America, Mediterranean region of Europe, Southeast Asia, India, and Japan. This family, on the American continent, is dispersed from North America to Argentina [1;2;3,4].

The genus *Styrax* L., one of the major representatives of Styracaceae, includes 129 species worldwide [4], with 25 species occurring in Brazil [5]. The name "*Styrax*" it is a word of Greek origin which means "fragrant resin", that is largely produced in the species of the genus [6]. The genus has economic value and pharmacological properties, such as to treat cardiovascular diseases, cough, peptic ulcers, pain and skin disturbs, and as antiseptic [7]. *Styrax officinalis* L. showed biopesticide potential, nutricional value [1] and, *Styrax tonkinensis* (Pierre) Craib ex Hartwich is employed as an expectorant, antiseptic, and analgesic [8].

The barks of some species produce an exudative resin obtained by incision, composed by esters and triterpenes that induced differentiation and antiproliferative effects in HL-60 cells [2;7]. Furthermore, the resin is utilized in Traditional Chinese Medicine, as well as in perfumery and incense production. Some species used for these purposes include *Styrax parallelloneurus* Perkins, *Styrax benzoin* Dryand., *Styrax tonkinensis*, and *S. officinalis* [1;2].

Lignans, neolignans, terpenoids, sterols, saponins, flavonoids, phenylpropanoids and others metabolites were detected in *Styrax* species [2;3]. Important biological activities have also been reported, for instance, that the benzofurans found in *Styrax agrestis* (Lour.) G.Don have demonstrated potential anticholinesterase inhibitory activity. In addition to, antimicrobial, anti-inflammatory, antioxidant, antiaging, anticancer activities were reported [2;7]. Additionally, a recent review described the diversity of secondary metabolites (benzoates, flavonoids, terpenes) in several *Styrax* species, including antioxidant and antimicrobial potential [3].

*Styrax leprosus* Hook. & Arn., popularly known in South America as "*cuia-do-brejo*, *estoraque-do-campo*, *pau-de-remo*, and *caujuja*" [8], is found in the Northeast of Argentina, Southeast and South of Brazil with its distribution spanning the states of Minas Gerais, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. According to Carvalho, the native habitat of *S. leprosus* corresponds to the Atlantic Forest biome, characterized by tropical deciduous forest, dense ombrophilous forest, and mixed ombrophilous forest. This species is commonly found in moist soils rich in organic matter [9]. It is a shrub (4-5 m tall) or tree (up to 8 m tall) with young branches covered in yellowish-silvery scales. When mature, the branches become glabrous, dark to almost black in color. The leaves are simple, alternate, petiolate, lanceolate or elliptical-oblong, generally with a rounded apex, a cuneate base and entire/flat margin, reaching up to 10 cm in length and 4 cm in width. They have a papery texture, prominent veins and are covered in small silvery scales, occasionally yellowish-silvery [5; 9]. The bark is used by the Paraguayan population in folk medicine to treat diabetes and hypercholesterolemia [10; 11].

The study of the morphoanatomy and histochemistry of *S. leprosus* is justified by several reasons that converge in botanical, pharmacological and biotechnological application. The microscopical features of leaves (lamina and petiole) and stems have not yet been fully described for this native species of Brazil and endemic to South American tropical forests. Furthermore, histochemical analysis allows the localization of group of secondary metabolites, such as phenolics, lipids and lignified compounds, whose tissue distributions may indicate potential pharmacognostic and ethnobotanical uses that have not yet been explored and, few studies in the species reported histochemical tests, which was complemented by this work. However, detailed histochemical reports for the genus are limited. A foundational study on *S. camporum* [12] described secretory tissues in the bark, but comprehensive histochemical profiling for species like *S. leprosus* remains unreported, highlighting the contribution of the present study. This framing acknowledges the existing work while clearly positioning our research as a novel contribution. These compounds, often accumulated in specific region of plant parts, have demonstrated anti-inflammatory, antimicrobial and antioxidant activities in related *Styrax* species, indicating opportunities for the development of herbals, cosmetics and bioproduction of aromatic resins [3;13].

By mapping these botanical structures in detail, it is also possible to provide support for targeted extraction protocols, *in situ* conservation, such as, leaf and stem architectures and the structure of secretory canals, serves as a reliable morphological fingerprint for the correct field identification of the species, which is essential for monitoring its populations and defining its true geographic distribution. Furthermore, the identification of defensive compounds, such as lipophilic compounds, resins and phenolics, within its tissues helps us understand the species interactions with herbivores and pathogens, informing its resilience and survival strategy in the ecosystem. Finally, the demonstration of these bioactive compounds with

bioprospecting potential adds tangible economic and pharmacological value to the species, strengthening the argument for preserving its natural habitat and promoting sustainable management practices to ensure this genetic resource is not lost. Therefore, the integration of morphological, anatomical and histochemical evidence will contribute to consolidating *S. leprosus* as a source of new bioactive compounds, guiding future tissue culture studies and reinforcing the importance of this species in the natural products innovation chain.

## MATERIAL AND METHODS

### *Plant material*

The aerial vegetative parts were collected in a Mixed Ombrophilous Forest belonging to the Atlantic Forest biome of the Federal University of Paraná, Curitiba – PR, in June 2022, at geographical coordinate: -25.44'93.5" S and 49.23'99.8" W. from a 12-meter-tall tree in an mature stage. The plant is located at an altitude of 915 meters above sea level. The material was identified by comparison with the voucher n° EFC 14317 registered at the Escola de Florestas Herbarium of UFPR (Federal University of Paraná) Campus III, Center for Forestry and Wood. The access to genetic heritage was registered and authorized by the Brazilian system SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) and registered under the code A11D443.

### *Light microscopy preparation*

Fresh vegetal material was fixed in FAA 70% (formalin, acetic acid, 70% ethanol (v/v/v), 5:5:90) for 36 h, then washed with distilled water and stored in 70% (w/v) ethanol [14]. The cross-sections of leaves and stem were made freehand using performed using a razor blade, and the thickness of the manually obtained cuts was approximately 30-50 µm. The sections were stained with Astra blue and basic fuchsin [15].

To analyse the epidermis features, leaves were immersed in a sodium hypochlorite (2.5% v/v) diluted in water at 70% (v/v) for three days. After clarification, the sections were washed six times with distilled water and 5% acetic acid (v/v). They were then stained with 1% safranin. The semi-permanent slides were made with 50% glycerin (v/v) and, sealed with clear nail polish.

### *Histochemical characterization*

The previously fixed material was sectioned and tested with Sudan III for the detection of lipophilic compounds [16]; phloroglucinol/HCl for lignified elements [17]; ferric chloride (2%) and potassium dichromate (10%) for phenolic compounds [18]; iodine solution for starch grains [16]; and, methylene blue for polysaccharides [19]. Untreated cross-sections were used as negative control.

Microphotographs were obtained using an Olympus CX31 photomicroscope equipped with an Olympus C-7070 digital camera at the pharmacognosy laboratory of the State University of Ponta Grossa.

### *Field emission scanning electron microscopy (FESEM) analysis*

Rectangular fragments (0.6 cm wide and 0.4 cm long) of the leaf blade and transverse sections of the stem and petiole were sonicated for five minutes in an ultrasonic bath sonicator (SolidSteel, SSBu 3,8L, Brazil). Then, they were dehydrated using solution with ethanol at 80%, 90% and 100% (*v/v*). They were mounted on aluminum stubs with double-sided adhesive tapes, dried at room temperature in the desiccator and then metallized in gold using a sputter coater (Balzers Sputtering SSCD 030). A Tescan Mira 3 FESEM was used for micro-morphological analysis, and micrographs were prepared in high vacuum mode and, obtained on a high-performance field emission scanning electron microscope (FESEM) for fast image acquisition processing with coupled with an Energy Dispersive X-ray Spectroscopy Detector (Oxford Instruments, Oxford, UK)) for crystal analysis, an accelerating voltage at 15 kV [20]. Electromicrographs were obtained in c-LABMU of State University of Ponta Grossa.

## RESULTS AND DISCUSSION

### *Anatomical and micromorphological analyses*

### *Leaf*

In frontal view, both of the epidermal surfaces of *S. leprosus* leaves are straight and thick anticlinal cell walls (Figure 1a, c) and are covered by a smooth cuticle (Figure 1d). The leaves are hypostomatic with anomocytic stomata (Figure 1c, f). Straight and thick anticlinal cell walls, hypostomatic and anomocytic

stomata were reported by Arambarri and coauthors [21] in *S. leprosus*, corroborating the findings of this study. Thick anticlinal cell walls were also found in *Styrax argenteus* C.Presl, *Styrax camporum* Pohl, *Styrax macrophyllus* Schott ex Pohl (*Styrax heteroclitus* J.F.Macbr., synonym) and, *Styrax trichocalyx* Perkins (*Styrax macrotrichus* Perkins, synonym) [22; 23]. As observed in the present study, anomocytic stomata were present exclusively on abaxial side in *Styrax* species. However, on adaxial epidermis, anticlinal cell walls were wavy to sinuous in *Styrax glabrescens* Benth. and *Styrax serrulatus* Roxb. [22].

The abaxial side is covered by a large number of scale-like peltate trichomes (Figure 1b, e, g, h). They have a circular or disc-shaped structure, composed of a flattened multicellular head and a short central pedicel. Peltate trichomes are not exclusive to all *Styrax* species, but have been reported in *S. leprosus* [21], *Styrax glaber* Sw., *Styrax ochraceus* Urb. and *S. portoricensis* Krug & Urb. [22]. Stellate non-glandular trichomes have also been described in other species of the genus, such as *S. camporum* [23]. The presence, micromorphology and location of trichomes on plants are valuable anatomical characteristics for the identification and differentiation of species within the same genus or between related species. These epidermal structures, which can vary widely in shape, density and distribution, provide important taxonomic information [24; 25; 26]. In addition, trichomes play a significant role in protecting the leaf surface, particularly the epidermis, by preventing the attack and germination of pathogenic species, reduction of mechanical damage and signal transmission [27]

In cross-section, the leaves exhibited a uniseriate epidermis, with adaxial epidermal cells larger than those on abaxial surface (Figure 1g), covered by a thin cuticle (Figure 1g). A uniseriate epidermis was also observed in *Styrax pohlii* A.DC. (*Styrax ferrugineus* Pohl, synonym), whereas other species of the genus, such as *S. glaber* (*Styrax micrasterus* Perkins, synonym), *Styrax portoricensis* Krug & Urb., and *Styrax pavonii* A.DC. (*Styrax pseudargyrophyllus* Sleumer,synonym), exhibited a subepidermal layer [22].

The mesophyll is dorsiventral and formed by one layer of palisade parenchyma and, around four layers of spongy parenchyma (Figure 1g, h). The minor vascular bundles were surrounded by a sclerenchymatic sheath which extended to the epidermis, on both sides (Figure 1g, h). Similar features were found in *Styrax* species [21; 22; 23]. However, *S. ochraceus* presented 2-3 distinct palisade layers. In contrast, *Styrax macrotrichus* Perkins, *Styrax tomentosus* Bonpl., and *Styrax portoricensis* Krug & Urb. had only a differentiated mesophyll, consisting of irregular to more or less rectangular parenchyma cells [22]. Species of *Styrax* showed bundle sheath extensions, occurring only on adaxial, or both adaxial and abaxial sides [22].

The midrib, in cross-section, has flat-convex shape. The epidermis is unilayered and covered by a thin and smooth cuticle. Beneath the epidermis, 3-4 layers of angular collenchyma are present in both sides. The vascular system is collateral, arranged in open arch with a plate V-shaped, and encircled by some layers of fibers (Figure 1h, i). Prismatic crystals and druses are present in the collenchyma and ground parenchyma (Figure 1i). The vascular system is variable across the family, presenting in three primary configurations: a vascular arc which is typically with invaginated ends; a medullated cylinder, or a medullated cylinder containing internal medullary vascular tissue [22]. Although perivascular sclerenchymatous fibers is common in *Styrax* species, it was not found in *S. americanus* [22].

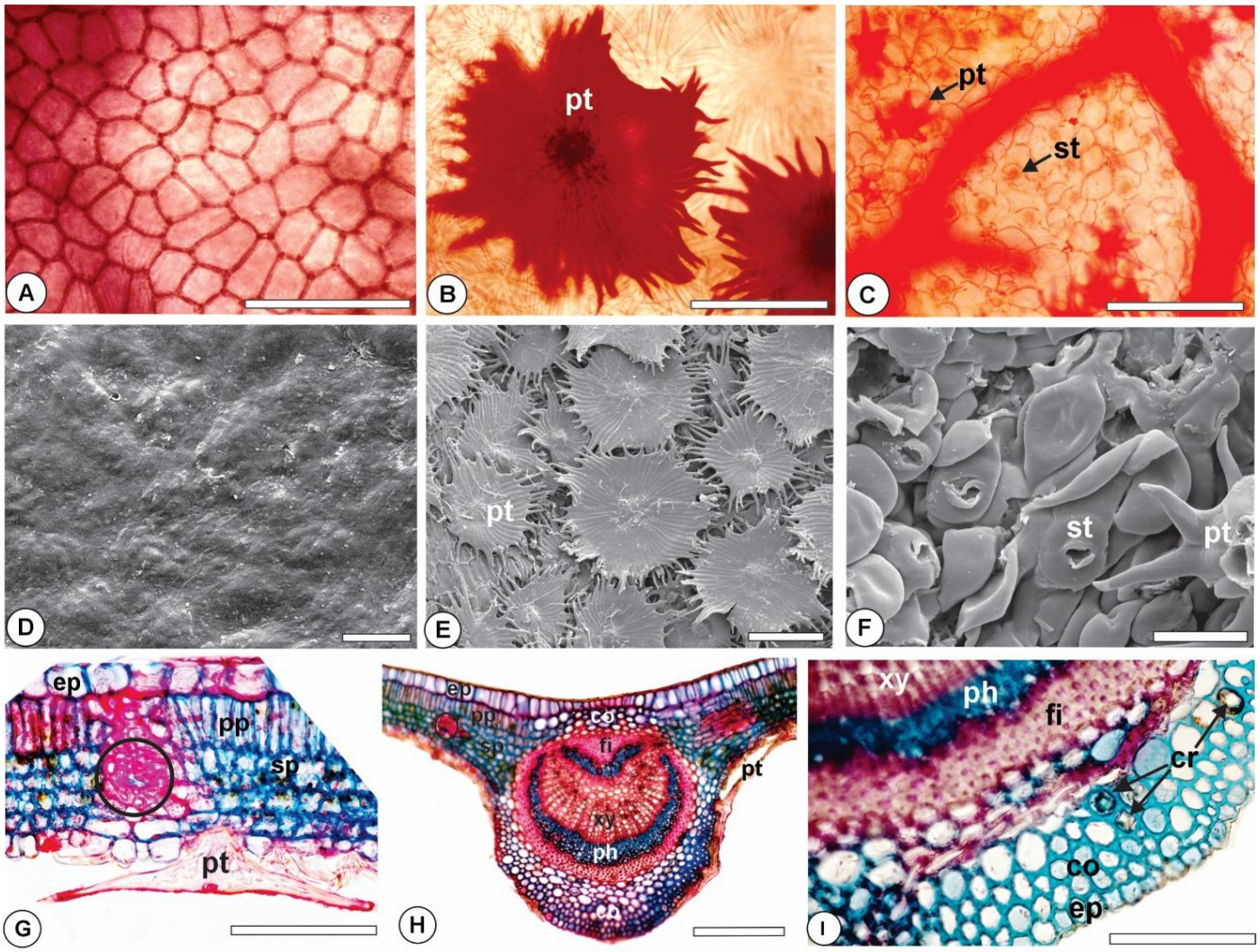



**Figure 1.** Anatomy of *Styrax leprosus* leaves [a-c: frontal view (stained with safranin), g-j: transverse sections (stained with Astra blue and fuchsin). a-c, g-i: light microscopy; d-f: FESEM]. a, d. epidermal cells on adaxial side. b, e. peltate trichome on abaxial side. c, f: stomata on abaxial surface. g. lamina. h. general view of the leaf. i. abaxial side of the midrib. [co: collenchyma; cr: crystal; ct: cuticle; ep: epidermis; fi: fibers; pp: palisade parenchyma; ph: phloem; pt= peltate trichome; sp: spongy parenchyma; st: stomata. xy: xylem]. Scale bars: a-c, e, g = 100 µm; d, f, h = 20 µm; i= 50 µm.

The petiole, in cross-section, has a concave-convex shape. The epidermis is unilayered with rectangular to irregular cells and possess peltate trichomes (Figure 2a, d, i), as previously described for the lamina. The vascular system is a collateral vascular bundle in an open arc with invaginated ends (Figure 2a, b). Prismatic crystals and druses (Figs 2c, d, k) and, styloid crystal (Figure 2j) was observed in the ground parenchyma. The same vascular pattern was found in *S. americanus, S. hypargyreus* Perkins, and *S. paralleloneurus* Perkins. [22] However, other patterns were found, such as arc with invaginated ends and two dorsal cortical bundles in *S. argenteus*, *Styrax benzoin* Dryand., *Styrax camporum* Pohl and *Styrax formosanus* Matsum*;* [22] medullated cylinder in *S. agrestis* and *Styrax faberi* Perkins*;*[22] medullated cylinder with medullary vascular tissue and two dorsal cortical bundles in *Styrax pohlii* A.DC*., Styrax lasiocalyx* Perkins and *Styrax conterminous* Donn.Sm. [22] All vascular bundles are collateral throughout the genus, with the exception of *S. argenteus*, *S. davillifolius*, and *S. pohlii*, in which amphicribral bundles are found [22]. The vascular system features can be used as anatomical markers to help the species differentiation [28; 29].

*Stem*

The stem, in transverse section, has a circular outline (Figure 2e). The epidermis is uniseriate and covered by several peltate trichomes (Figure 2e, i). It is then followed by about seven layers of cortex and, formed by about 9 to 12 layers of phloem, xylem rays can be observed, arranged radially with larger cells that differ from the others and pith (Figure 2e, f). The pith contains starch grains and crystals similar to those

observed in the midrib and petiole, druses and prismatic crystals of calcium oxalate (Figure 2 g, h, I) The presence of brachysclereids were seen in the cortex (Figure 4c). Absence of fibers and, the presence of brachysclereid cells was seen similar of our findings. Prismatic crystals were also observed in the xylem rays of *S. camporum* stem [12].

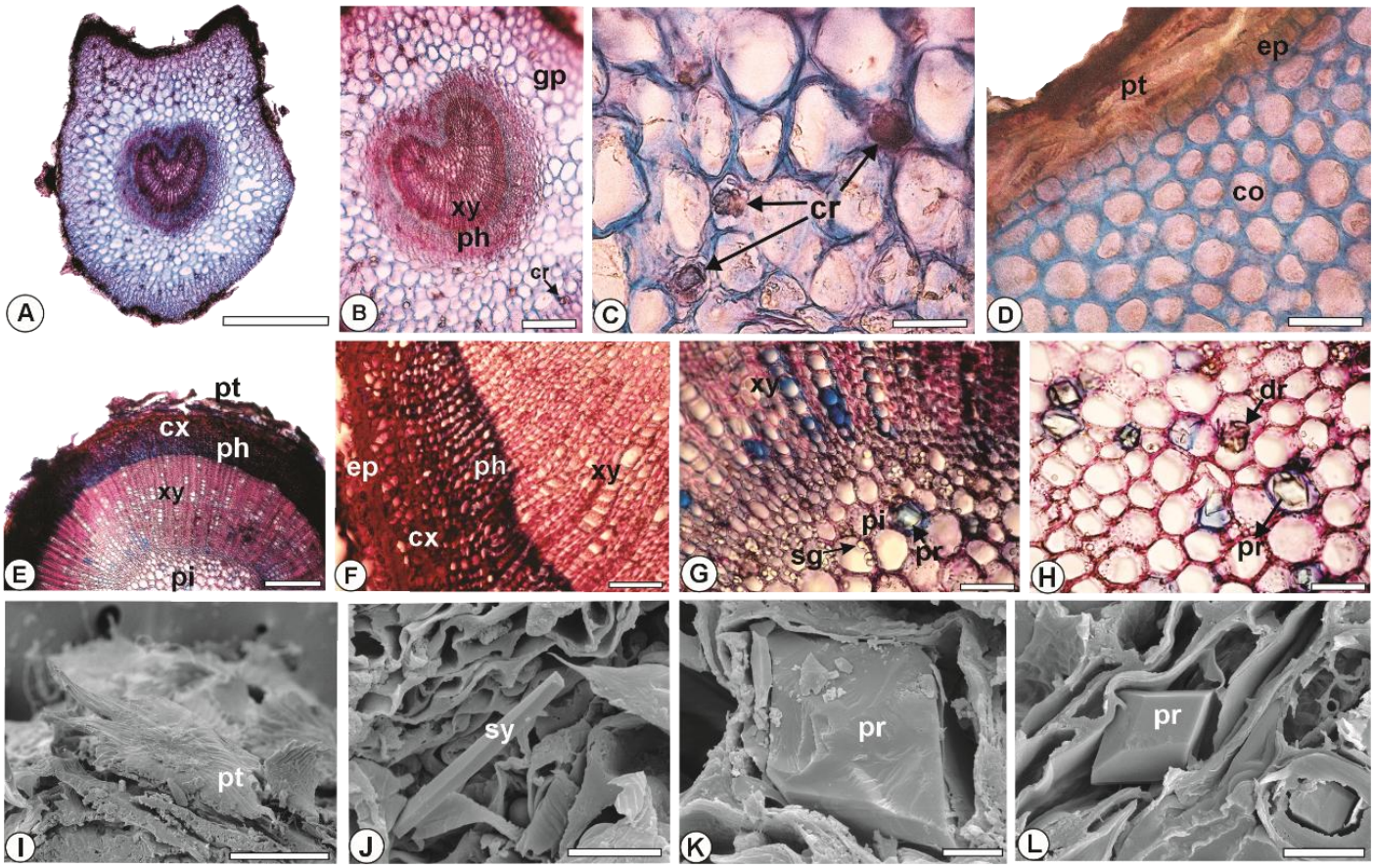



**Figure 2.** Anatomy of *Styrax leprosus* leaves and stem [a-h (stained with Astra blue and fuchsin): light microscopy; i-l: FESEM]. a-d, i-l: petiole; e-h: stem. A. general view of petiole. b. detail of vascular bundle. c. crystals. d. trichomes, epidermis and collenchyma. e. general view of the stem. f. xylem and pith details. g. xylem and pith. h. crystals in the pith. i. peltate trichomes. j. styloid crystal; k, l. prismatic crystals). [co: collenchyma; cr: crystal; cx: cortex; dr: druse; ep: epidermis; fi: fibers; gp: ground parenchyma; pt= peltate trichome; ph: phloem; pi: pith; sg: starch grain; sty: styloid crystal. xy: xylem]. Scale bars: a = 150 µm; b-d, f,g, h= 50 µm; e= 200 µm; i= 100 µm; j, l = 10 µm; k= 5 µm.

The elemental composition of the crystals was determined using Energy-dispersive X-ray spectroscopy (EDS) (Figure 3A-C). The prismatic crystals contain carbon (C), oxygen (O), and calcium (Ca) in varying proportions: 15.8% C, 58.98% O, and 25.22% Ca (Figure 3a), and 5.92% C, 42.11% O, and 51.97% Ca (Figure 3c), respectively. The styloid crystal exhibits a distinctly profile, with higher carbon content (62.09%), followed by oxygen (30.34%) and a lower proportion of calcium (7.75%). The presence of Ca, C, and O in these proportions strongly proposes that the crystals are formed by calcium oxalate, which is a common biomineral in plant tissues [30]. Different morphotypes of Ca oxalate crystals occur in plant species, such as prismatic, druse, pyramidal, styloid or sand, and play roles in Ca regulation, tissue support, and herbivore prevention [31]. The variation in elemental ratios, mainly the higher calcium content in the prismatic forms, may reflect differences in crystal structure or developmental stage.

The presence of the crystals may indicate plant defense strategies against herbivores and pathogens, since the accumulation of oxalates in crystals hinders digestion or intoxicates natural enemies. From a taxonomic and systematic point of view, the distribution pattern and morphotypes of crystals serve as diagnostic markers for distinguishing species and understanding phylogenetic relationships within the Styracaceae family. In the ecological context, the quantification of crystals in different organs and at different stages of development helps to understand the adaptation of *S. leprosus* to soil and climate variations, since the precipitation of salts in crystalline form is associated with ionic regulation processes and tolerance to the acidic soils [32]. Although much remains to be clarified regarding the function of calcium oxalate crystals,

they are believed to play a primary role in plant defense, being also involved in the detoxification of heavy metals and in the photoprotection of the photosynthetic apparatus under stress conditions [33].

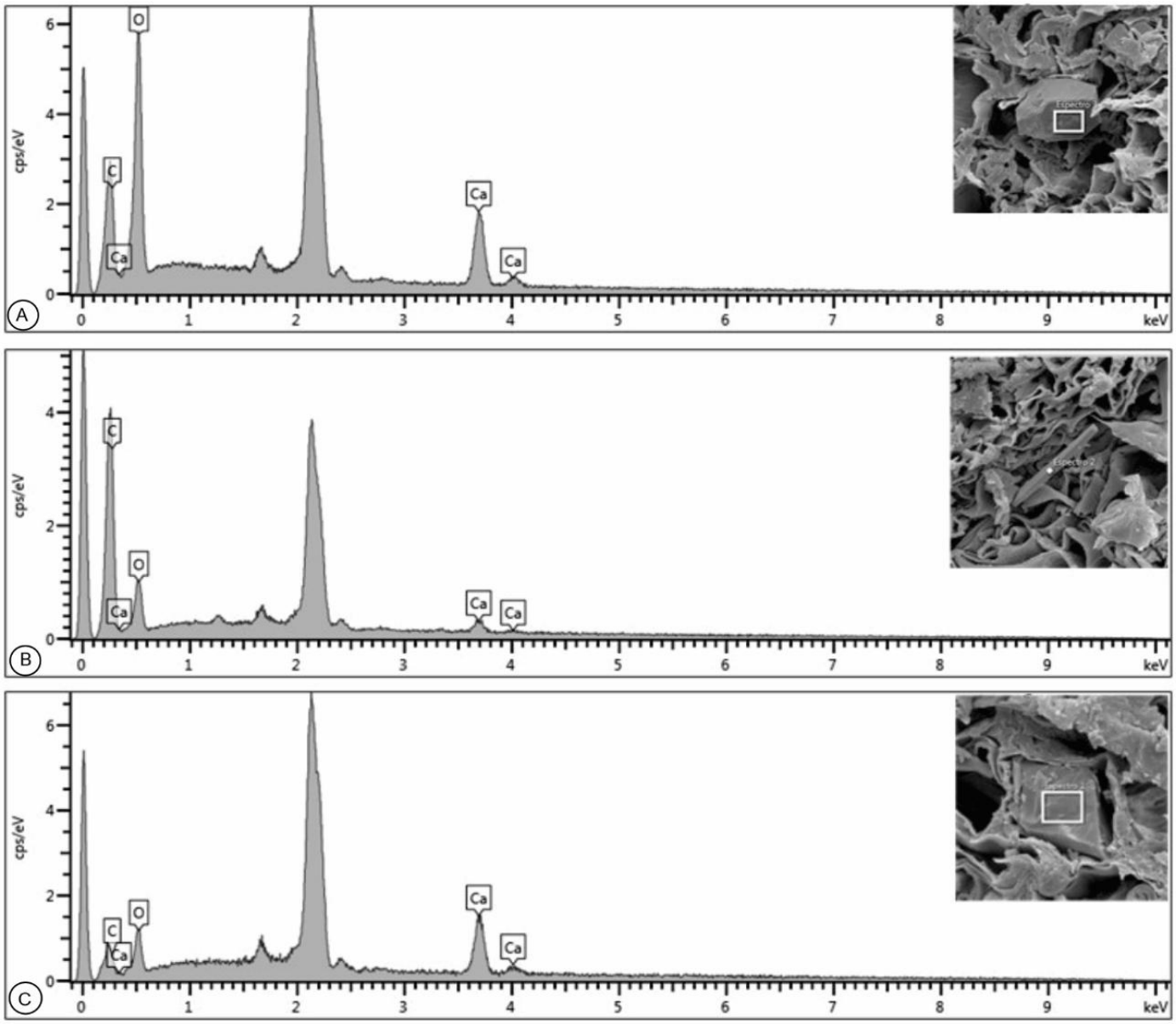



**Figure 3.** Energy-dispersive X-ray spectroscopy (EDS) spectrum of the petiole crystals of *Styrax leprosus*: a, c. prismatic crystals; b. styloid crystal. (0 keV is the noise peak, and at approximately 2.2 keV, correspond to the gold element used in the sputter coating sample).

*Histochemical analysis*

The histochemical data suggest important insights into the physiological roles of specialized etabolites and reserve compounds in different plant organs [34]. Lipophilic compounds react positively to Sudan III and are observed in the leaf cuticle, petiole and stem, suggesting their involvement in limiting water loss and forming protective barriers against pathogens and herbivores. Oil bodies are detected in the midrib and suberin is evident in the endodermis of stem (Figures 4a-c) point to the role of lipophilic components in mechanical protection and the regulation of apoplastic transport. In *S. camporum*, they were also detected in the xylem rays [12]. The essential oil of *S. officinalis* presented liphophilic classes of compounds, as like as, lipids, terpenes, terpenoids, triterpenoids saponins, hydrocarbons, and aromatic substances [3]. The detection of compounds in essential oils from other species has also been reported in *S. japonicus*, *S. grandiflora* and *S. calvescens* [3].

The reaction with phloroglucinol/HCl is more prominent in the leaf, especially in the midrib, which contained fibers that surrounded the phloem (Figure 4d). Polyphenols like lignin is vital for mechanical support

and water conduction, and its presence in the vascular tissues of the leaf blade, petiole, and stem indicates their role in preserving plant inflexibility and hydraulic conductivity [35].

In addition, the presence of lignified fibers in the suber and brachysclereids in the stem cortex (Figure 4e) indicates local support, possible contributing to the structural integrity of the stem. Pauletti and coauthors [36] reported the presence of lignan compounds, such as benzofuran, tetrahydrofuran, and furfuran lignans in *S. officinalis* stem.

Phenolic compounds are observed mainly in the spongy parenchyma in the leaf mesophyll and in the stem cortex region (Figures 4f,g). Phenolics are known for their diverse physiological roles, including UV protection (antioxidant activity) and defence against pathogens [37]. Phenolics and flavonoids, such cinnamyl cinnamate, cinnamic acid, benzyl cinnamate, cinnamaldehyde, eugenol, methoxy eugenol, vanillin, vanillic acid, cinnamyl benzoate and, ferulic acid were reported in extracts to the close species *S. officinalis* [38]. Machado and coauthors [39] reported the occurrence of phenolic compounds in the axial parenchyma of the stem wood and also in the rays of the root wood of *S. leprosus.*

The presence of phenolic compounds in *S. leprosus* is particularly relevant for the herbal medicine industry, as these secondary metabolites exhibit recognized antioxidant, anti-inflammatory and antimicrobial activities [40]. Furthermore, due to the antimicrobial action of these compounds, there is potential for application in natural preservatives of plant extracts, contributing to the stability and safety of products. In other areas, such as cosmetics, phenolics from *S. leprosus* can be incorporated into creams and lotions to combat skin aging and protect the skin against damage caused by free radicals [41]. In the food industry, phenolic extracts have applications as natural antioxidants, extending the shelf life of foods and conferring functional benefits to the diet [42]. Thus, the abundance of phenolic compounds in *S. leprosus* opens up numerous opportunities for the development of innovative, safe and sustainably sourced products. In *S. officinalis,* phenolic compounds such as, anthraquinones, flavonoids, lignans and tannins have already been described for this species [3].

The iodine solution reveals starch grains in small quantities in the petiole around the vascular system and, mainly in the stem, in the region of the xylem tissue and the pith (Figure 4h). Starch grains represents energy storage that can be mobilized under high metabolic demand or in response to environmental stress [43].

Wagner's reagent reacts with alkaloids present in the leaf mesophyll (Figure 3i). Alkaloids present strong defensive functions against herbivores and microbial pathogens, serving to avoid foliar herbivory [44]. Also, in both the leaf and stem, the reaction was negative with methylene blue. Table 1 shows the main structures in which the metabolites were detected with the reagents studied.

In *S. camporum*, lipophilic compounds were detected in the xylem rays. Phenolic compounds and starch grains were also identified in the parenchyma [29]. In *S. leprosus*, lipophilic compounds are noted in the trichomes, cortex and xylem rays; phenolic compounds were also identified in the parenchyma and starch grains, and were more evident in the xylem rays and pith.

Inclusive, the combination of structural and chemical characteristics reflects a complex and well-organized physiological system that integrates protection (against herbivores, microorganisms or competing plants), support, regulation of transport, and supply storage to certify plant subsistence and adaptation.

It is important to highlight that *Styrax* species possess resin ducts, which are present mainly in phloem tissues (cortex and bark) and, in some species, also in the xylem. These ducts are responsible for the production, preservation, storage and protection of the aromatic resin, known as benzoin, which has economic and medicinal importance [45]. Resin formation is induced in xylem and bark tissues after injury to the cambium. Early wounds do not produce much resin, but later wounds generate larger quantities of the aromatic compounds. The resin forms in globular or pyriform masses, yellowish-white in color, with the whitest 'tears' being the most valued [46]. *Styrax hypoglauca* Perk, produces balsamic (benzoin) and the ducts are initiated via a schizolysigenous mechanism, in which parenchyma cells of the secondary xylem both separate (schizogeny) and undergo localized cell death (lysigeny) to create an intercellular lumen lined by secretory epithelial cells [45]. In this study, ducts were not observed, it is suggested that there were no tissue injuries.

Microscopical analysis of the traumatic and constitutive resin ducts, through serial sections to characterize the mechanism of formation of these channels; the establishment of controlled resin tapping protocols to evaluate how frequency, and depth of wounds influence the number, diameter and yield of resin, can not only enrich the taxonomic knowledge of *S. leprosus*, but will also pave the way for sustainable pharmaceutical, cosmeceutical and biotechnological applications, enhancing the biodiversity of the Atlantic Forest.

**Table 1.** Histochemistry of *Styrax leprosus*

| Reagent | Leaf's lamina | Petiole | Stem |
|---|---|---|---|
| Sudan III | cuticle, spongy and palisade parenchyma, oil bodies in the midrib | cuticle, oil bodies in the ground parenchyma | cuticle, endodermis, oil bodies in the cortex |
| Phloroglucinol/HCl | fibers, xylem | xylem | xylem |
| Ferric Chloride | spongy parenchyma | weak in the scales | scales, epidermis,cortex and vascular system |
| Iodine solution | - | surrounding the vascular system | xylem rays and pith |
| Wagner's reagent | mesophyll | - | - |

Source: The Author, 2026

To the best of our knowledge, the description of microscopical features of the stem, along with the histochemical tests for leaves and stems have not been previously reported in the literature for *S. leprosus*, contributing to its accurate taxonomic identification. The identification of phenolic compounds and alkaloids contributes to the identification of chemical compounds in the species.

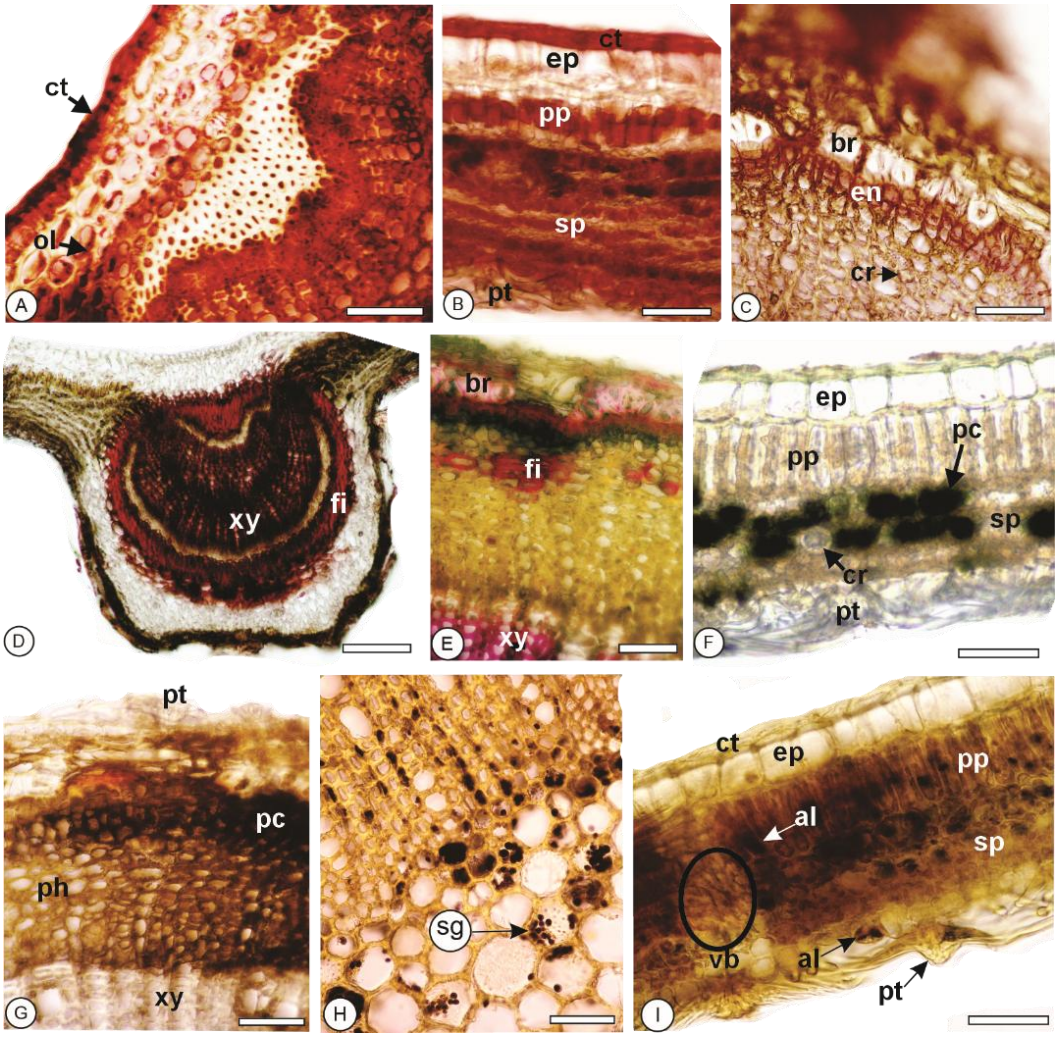



**Figure 4.** Histochemistry of *Styrax leprosus* (light microscopy). [a-c= Sudan III]. a. midrib b. mesophyll. c. stem. [phloroglucinol/HCl=d, e] d. general view of the leaf; e. sclereids in the stem [ferric chloride=f, g]. e. phenolic compounds in the mesophyll and, h. stem details. [Iodine solution=g]. i. starch grains in the stem. [Wagner's reagent= h]. [al: alkaloid; br: brachysclereid; ct: cuticle; cr: crystal; cx: cortex; ep: epidermis; en: endodermis; fi: fibers; pp: palisade parenchyma; pc: phenolic compound; ol: oil; ph: phloem; sp: spongy parenchyma; vb: vascular bundle; xy: xylem]. Scale bars: a-c, e-i= 50 µm; d: 200 µm.

## CONCLUSION

Based on the characteristics found in *S. leprosus*, we can consider together the anatomical characters described for the species, as well as anomocytic stomata, peltate trichome, presence of prismatic crystals, styloids, druses of calcium oxalate, shape of the midrib, petiole and stem, and arrangement of the vascular system of midrib and petiole. Also, the presence and localization of alkaloids, lipophilic compounds, lignified elements, starch grains and phenolics characterize *S. leprosus*.

The description of the morphoanatomy and histochemistry of *S. leprosus* adds scientific value as aid in taxonomic refinement, generate chemotaxonomic data, support the development of new drugs and herbals, contribute to the sustainable conservation of the species and provide support for biotechnological use. Thus, this study not only expands basic knowledge about the species, but also paves the way for innovations in management, pharmaceutical application and conscious exploitation of natural resources.

The lack of specific studies on *S. leprosus* indicates a gap in scientific research that could be explored, especially considering the traditional uses of other species of the same genus. Future investigations could focus on the identification of bioactive compounds and evaluation of their pharmacological activities. Furthermore, new studies in different environments could also verify the stability of anatomical and histochemical characteristics, as well as studies in which the plant suffers some injury or incision to verify resin production.

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### Use of Generative Artificial Intelligence

The authors declare that large language models and other generative artificial intelligence (AI) or AI-assisted technologies cannot be credited as authors and have not been listed as authors of this paper.

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## REFERENCES

1. Jaradat N. Phytochemistry, traditional uses and biological effects of the desert plant *Styrax officinalis* L. J. Arid Environ. 2020 Nov; 182, 104253. https://doi.org/10.1016/j.jaridenv.2020.104253;
2. Son NT, Linh NTT, Tra NT, Ha NTT, Anh LTT, Cham BT, Ahn DTT, Van Tuyen N. Genus *Styrax*: a resource of bioactive compounds. Studies. Nat Prod Chem 2021; 69: 299-347. https://doi.org/10.1016/B978-0-12-819487-4.00008-2;
3. Paparella A, Serio A, Shaltiel-Harpaz L, Revuru B, Kongala P R, Ibdah M. *Styrax* spp.: Habitat, Phenology, Phytochemicals, Biological Activity and Applications. Plants 2025; 14(5): 746. https://doi.org/10.3390/plants14050746;

4. POWO (2024). Kew: Facilitated by the [internet] [Place: Plants of the World Online: Publisher: Royal Botanic Gardens] [Updated: 2025 Jul 22; cited: 2025 Jul 22]. http://www.plantsoftheworldonline.org/.
5. Romão GO, Cabral A, Fritsch PW, Santos FBD. Styracaceae *in* Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro [internet]. 2025. [cited 2025 Mai 07]; Available from https://floradobrasil.jbrj.gov.br/FB14891.
6. Fritsch PW. Phylogeny and biogeography of the flowering plant genus *Styrax* (Styracaceae) based on chloroplast DNA restriction sites and DNA sequences of the internal transcribed spacer region. Mol Phylogenet Evol. 2001; 19(3): 387-408. https://doi.org/10.1006/mpev.2001.0933
7. Xia, DD, Han, XY, Zhang, Y, Zhang, N. Chemical constituents and their biological activities from genus *Styrax*. Pharmaceuticals. 2023; 16(7): 1043. https://doi.org/10.3390/ ph16071043;
8. Corrêa MP. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Vol.2. Rio de Janeiro: Ministério da Agricultura, IBDF; 1984. 707 p.
9. Carvalho PER. Espécies arbóreas brasileiras Vol.3. Embrapa, Brasília 2008. 155-161 p;
10. Kujawska M, Pardo-de-Santayana M. Management of medicinally useful plants by European migrants in South America. J Ethnopharmacol, 2015; 172: 347-355. https://doi.org/10.1016/j.jep.2015.06.037;
11. Kujawska M, Schmeda-Hirschmann G. The use of medicinal plants by Paraguayan migrants in the Atlantic Forest of Misiones, Argentina, is based on Guaraní tradition, colonial and current plant knowledge J Ethnopharmacol, 2022; 283, 114702. https://doi.org/10.1016/j.jep.2021.114702;
12. Machado S R, Marcati C R, De Morretes B L, Angyalossy V Comparative bark anatomy of root and stem in *Styrax camporum* (Styracaceae). IAWA J, 2005, 26(4), 477-487;
13. Jaradat N, Al-Masri M, Zaid A N, Hussein F, Shadid K A, Al-Rimawi F, Shayeb K, Sbeih A, Eid A. Assessment of the antimicrobial and free radical scavenging activities of *Moluccella spinosa*, *Helichrysum sanguineum*, and *Styrax officinalis* folkloric medicinal plants from Palestine. Orient Pharm Exp Med, 2018; 18: 107-114 https://doi.org/10.1007/s13596-018-0316-z
14. Sass JE Botanical Microtechnique, 3rd Edition. London: Constable and Company LTD; 1958
15. Roeser KR Die nadel der schwarzkiefer-massenprodukt und kunstwerk der natur. Mikrokosmos (Stuttg), 1972; v. 61: 33–36;
16. Johansen DA Plant Microtechnique. New York: McGraw-Hill; 1940
17. Jensen WA Botanical histochemistry. San Francisco: Freeman; 1962
18. Ventrella MC, Almeida AL, Nery LA, Coelho VP Métodos histoquímicos aplicados às sementes. Viçosa (MG): Universidade Federal de Viçosa; 2013.
19. Pearse AG, Everson. Histochemistry: Preparative and optical technology v.1: Theoretical and applied. 4th ed. London: Churchill Livingstone; 1980. Vol. 1.
20. Garcia de Fátima J, Armstrong L, da Rocha L T, Dos Anjos CA, Fladzinski K A, Miguel MD., Miguel O G, Montrucchio D P, Dias, JDG Morphoanatomy and Histochemistry of *Symplocos tetrandra* (Mart.) Mart. (Symplocaceae). Microsc Res Tech; 2025 88(4): 976-985. https://doi.org/10.1002/jemt.24745;
21. Arambarri AM, Freire SE, Colares MN, Bayón ND, Novoa MC, Monti C, Stenglein SA. Leaf anatomy of medicinal shrubs and trees from Misiones Forest of the Paranaense Province (Argentina). Part 2. Bol. Soc. Argent. Bot 2008; 43(1-2): 31-60;
22. Schadel WE, Dickison WC Schadel, WE, Dickison, WC. Leaf anatomy and venation patterns of the Styracaceae. J Arnold Arbor 1979; 60(1): 8-37;
23. Habermann G, Machado SR, Guimarães VF, Rodrigues JD. Leaf heliotropism in *Styrax camporum* Pohl from the Brazilian cerrado: distinct gas exchange and leaf structure, but similar leaf temperature and water relations. Braz J Plant Physiol 2008; 20: 71-83. https://doi.org/10.1590/S1677-04202008000100008;
24. Machado CD, Santos VLPdos, Novak RS, Koch MS, Arcaro G, Raman V, Franco CRC, Farago PV, Budel JM. Contributions of trichome micromorphology to the characterization of species traded as “Boldo”. Flora 2021; 279: 151827. https://doi.org/10.1016/j.flora.2021.151827;
25. Monchak, IT, Santos MC, Koetz M, Raman V, Apel MA, Henriques AT, Manfron J. Trichome Microscopy as a Diagnostic Tool for Species of the *Solidago* Complex: *S. chilensis* and *S. microglossa.* Braz Arch Biol Technol 2023; 66 (spe). https://doi.org/10.1590/1678-4324-ssbfar-2023230070;
26. Antunes KA, Raman V, Perera WH, Heiden G, Pontarolo R, Farago PV, Khan IA, Manfron J. Authentication and Quality Control of the Brazilian Traditional Herb ‘Carquejas’ (*Baccharis* Species) Using Morpho-Anatomy and Microscopy. Plants 2024; 13(21): 3030. https://doi.org/10.3390/plants13213030;
27. Wang X, Shen C, Meng P, Tan G, Lv, L. Analysis and review of trichomes in plants. BMC Plant Biol 2021*;* 21(1): 70. https://doi.org/10.1186/s12870-021-02840-x
28. Wosch L, Imig DC, Cervi AC, Moura BB, Budel JM, Santos CAM. Comparative study of *Passiflora* taxa leaves: I. A morpho-anatomic profile. Rev Bras Farmacogn 2015; 25 (4): 328-343. https://doi.org/10.1016/j.bjp.2015.06.004;
29. Antunes KA, Monteiro, LM, Almeida, VP de, Baldiserra LJ, Raman V, Manfron J. Comparative Anatomy of *Ilex paraguariensis* "Erva-Mate" and its Adulterant *Citronella gongonha* "Falso-Mate”. Braz Arch Biol Technol 2023; 66 (spe). https://doi.org/10.1590/1678-4324-ssbfar-2023230040;
30. Franceschi VR, Nakata PA. Calcium oxalate in plants: formation and function. Annu. Rev. Plant Biol. 2005; 56(1): 41-71. https://doi.org/10.1146/annurev.arplant.56.032604.144106;

31. Raeski PA, Heiden G, Novatski A, Raman V, Khan IA, Manfron J. Calcium oxalate crystal macropattern and its usefulness in the taxonomy of *Baccharis* (Asteraceae). Microsc Res Tech 2023; 86(7): 862-881. https://doi.org/10.1002/jemt.24363;
32. He H, Veneklaas E J, Kuo J, Lambers H. Physiological and ecological significance of biomineralization in plants. Trends Plant Sci 2014; 19(3):166-74. doi: 10.1016/j.tplants.2013.11.002. Epub 2013 Nov 28. PMID: 24291440.
33. Khan, MI, Pandith, SA, Shah, MA, Reshi, ZA. Calcium oxalate crystals, the plant 'Gemstones': insights into their synthesis and physiological implications in plants. Plant Cell Physiol 2023; 64(10): 1124-1138. https://doi.org/10.1093/pcp/pcad081
34. de Almeida V P, Monchak IT, da Costa Batista J V, Grazi M, Ramm H, Raman V, Baumgartner S, Holandino C and Manfron J. Investigations on the morpho-anatomy and histochemistry of the European mistletoe: *Viscum album* L. subsp. *album*. Sci Rep 2023; 13(1): 4604. https://doi.org/10.1038/s41598-023-29799-z;
35. Ménard D, Blaschek L, Kriechbaum K, Lee CC, Serk H, Zhu C, Lyubartsev A, Nuoendagula, Bacsik Z, Bergström L, Mathew A, Kajita S, Pesquet E. Plant biomechanics and resilience to environmental changes are controlled by specific lignin chemistries in each vascular cell type and morphotype. Plant Cell 2022; 34(12): 4877–4896. https://doi.org/10.1093/plcell/koac284
36. Pauletti PM, Teles HL, Silva DH, Araújo ÂR, Bolzani VS. The Styracaceae. Rev Bras Farmacogn 2006; 16: 576-590. https://doi.org/10.1590/S0102-695X2006000400023
37. Lattanzio V. Phenolic compounds: introduction 50. Nat. Prod 2013; 1543-1580. DOI 10.1007/978-3-642-22144-6_57;
38. Hovaneissian M, Archier P, Mathe C, Culioli G, Vieillescazes C. Analytical investigation of styrax and benzoin balsams by HPLC-PAD-fluorimetry and GC-MS. Phytochem Anal 2008; 19(4): 301-310. https://doi.org/10.1002/pca.1048
39. Machado SR, Rodella RA, Angyalossy V, Marcati CR. Structural variations in root and stem wood of *Styrax* (Styracaceae) from Brazilian forest and cerrado. IAWA J 2007; 28(2): 173-188.
40. Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Lins-Neto EMF, Souza JP. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: a quantitative approach. J Ethnopharmacol 2007; 114(3): 325–354.
41. Cherubim DJL, Martins CVB, Fariña LO, Lucca RAS. Polyphenols as natural antioxidants in cosmetics applications**.** J Cosmet Dermatol 2020; 19(1): 33-37.
42. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods and nutraceuticals: Antioxidant activity and health effects - A review. J Funct Foods 2015; 18: 820–897 http://dx.doi.org/10.1016/j.jff.2015.06.018
43. Taiz L, Zeiger E, Møller I M, Murphy A. Plant Physiology and Development 6th ed. Sunderland (MA): Sinauer Associates; 2015. ISBN 978-1-60535-255-8;
44. Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 2003; 64(1): 3-19. https://doi.org/10.1016/S0031-9422(03)00300-5.
45. Langenheim JH. Plant resins: chemistry, evolution, ecology, and ethnobotany. Portland (OR): Timber Press; 2003.
46. He Q, Sun Y, Chen X, Feng J, Liu Y. Benzoin resin: an overview on its production process, phytochemistry, traditional use and quality control. Plants 2023; 12: 1976. https://doi.org/10.3390/plants12101976

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