



ISSN: 2525-815X

Journal of Environmental Analysis and Progress

Journal homepage: www.jeap.ufrpe.br/

10.24221/jeap.6.3.2021.3969.268-277



Anatomical and histochemical characterization of leaves of *Spondias purpurea* L.

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ARTICLE INFO

Received 25 Nov 2020

Accepted 20 Apr 2021

Published 16 Sep 2021

ABSTRACT

The Anacardiaceae family has species-rich in secondary metabolites. They are widely used in popular medicine. Among them, *Spondias purpurea* L. stands out for containing several secondary metabolites with important biological activities. A precise identification of the species was the objective of this study, to perform an anatomical and histochemical characterization of leaves of *S. purpurea*. Microscope slides containing cross-sections of the petiolule and leaflets, in addition to paradermal sections of the leaflets, were prepared and analyzed in an optical and polarized microscope. Histochemical tests were performed on fresh leaflets. The microscopic analysis identified the anatomical structures to a diagnosis of the studied species, such as petiolule with concave-convex shape, non-glandular and glandular trichomes, druses in phloem; leaflets amphistomatic, with non-glandular trichomes on the adaxial face and glandular trichomes on the abaxial face, midrib with concave-convex shape, two collateral vascular bundles, one layer of palisade parenchyma, druses in phloem and mesophyll. The histochemical analysis evaluated phenolic compounds, alkaloids, tannins, triterpenes, steroids, lipophilic compounds, essential oils, lignin, starch, and calcium oxalate crystals, evidenced in the leaflets. The results are important for the quality control of plant material and expand the knowledge about the species.

Keywords: Anacardiaceae, pharmacobotany, quality control.

Introduction

Spondias purpurea L. is a fruit tree about three to five meters in height, a native of Central America. It can be commonly found in the northeastern region of Brazil (Lorenzi, Lacerda & Bacher, 2015; Fortuny-Fernández, Ferrer & Ruenes-Morales, 2017). Depending on the location, it may be known as red mombin, purple mombin, ciruela, seriguela, ciriguela, and jocote (Silva et al., 2014; Lorenzi, Lacerda & Bacher, 2015; Marisco & Pungartnik, 2015; Mitchell & Daly, 2015).

The plant belongs to the family Anacardiaceae, a tropical and subtropical distribution, including about 80 genera and 873 species (Pell et al., 2011; APG IV, 2016). In Brazil, there are 14 genera and 53 species (Silva-Luz & Pirani, 2015). Among them, several fruits of economic importance stand out, such as the cashew tree (*Anacardium occidentale* L.), the mango tree

(*Mangifera indica* L.), the group of ambarella (*Spondias* spp.), and pistachio (*Pistacia vera* L.) (Lorenzi, Lacerda & Bacher, 2015).

From a phytochemical point of view, species of the Anacardiaceae family are rich in secondary metabolites, particularly phenolic compounds, with interesting biological activities. Therefore, many species of this family have also been used as medicinal plants to treat infectious diseases and as an anti-inflammatory (Silva et al., 2014; Schulze-Kaysers, Feuereisen & Schieber, 2015; Sameh et al., 2018).

In Brazil, the leaves of *S. purpurea* have traditionally been used to treat gastritis (Borges & Moreira, 2016; Castro et al., 2011), hypertension (Silva et al., 2012; Marisco et al., 2017b) and diarrhea (Franco & Barros, 2006; Oliveira, Barros & Moita Neto, 2010; Freitas et al., 2012). Pharmacological studies have demonstrated its antimicrobial (Alencar et al., 2015; Miranda-Cruz

et al., 2012; Marisco et al., 2017a; Santos, Santos & Silva, 2017), antitumor (Santos, Santos & Silva, 2017), antioxidant and antiulcer potential (Almeida et al., 2017).

In a literature review, Silva et al. (2014) showed that the most studied species of *Spondias* are *S. mombin* L., *S. pinnata* (L.f.) Kurz, and *S. tuberosa* Arruda, mainly in the areas of ethnobotany and pharmacology. There are also in the literature some anatomical and histochemical studies of *Spondias* species that highlight the importance of knowing the microscopic characteristics of these medicinal species, which can aid in taxonomy and quality control of plant material (Nascimento-Silva & Paiva, 2007; Nascimento-Silva, Chinalia & Paiva, 2008; Norfaizal & Latiff, 2013; Chisom, Chukwu & Okeke, 2014; Vasconcelos, Vasconcelos & Randau, 2016). However, no data have been found in the literature about the microscopic identification of *S. purpurea*. Therefore, the objective of the present study was to perform an anatomical and histochemical characterization of leaves of *S. purpurea*.

Material and Methods

The plant material was collected in the city of Vitória de Santo Antão, Pernambuco, Brazil. A voucher specimen n° 91797 was deposited at the Herbarium Dárdano de Andrade Lima of the Instituto Agrônomo de Pernambuco.

For anatomical characterization, several expanded leaves, collected in April 2018 between the third and fifth nodes from three specimens, were fixed in FAA50 (formaldehyde, acetic acid, and ethyl alcohol 50%, 1:1:18 v/v) (Johansen, 1940). Cross-sections at the middle region of the petiolule and leaflets were obtained by freehand, using a common razor blade and the medulla of *Cecropia* sp. petiole as a support material. For the leaflets, paradermal sections were also performed on the adaxial and abaxial faces. Subsequently, the sections were submitted to a sodium hypochlorite solution (50%) for discoloration (Kraus & Arduin, 1997). After washing in distilled water, the cross-sections were stained according to a technique described by Bukatsch (1972), using safranin and Astra blue. The paradermal sections were stained with methylene blue (Krauter, 1985). All sections were mounted on semipermanent slides following usual procedures in plant anatomy (Johansen, 1940; Sass, 1951). A light and polarization

microscope (Leica DM750M), coupled with a digital camera (Leica ICC50W), was used to analyze the semipermanent histological slides. The images were processed in the software LAS EZ.

Histochemical tests were performed in cross-sections at the middle region of fresh leaflets collected from three specimens between the third and fifth nodes. The following reagents were used to indicate the presence of metabolites: potassium dichromate (10%) for phenolic compounds (Johansen, 1940), Dragendorff's reagent for alkaloids (Yoder & Mahlberg, 1976), vanillin chloridric for tannins (Mace & Howell, 1974), antimony trichloride for triterpenes and steroids (Mace, Bell & Stipanovic, 1974), Sudan III for lipophilic compounds (Sass, 1951), Nadi reagent for essential oils (David & Carde, 1964), Lugol for starch (Johansen, 1940), phloroglucinol for lignin (Johansen, 1940), and hydrochloric acid (10%) to establish the nature of the crystals (Jensen, 1962). Controls were performed parallel to histochemical tests, and semipermanent slides were prepared to contain cross-sections of the material (Johansen, 1940; Sass, 1951). The analysis of the images was conducted using the Toup View Image software. The images were obtained by a digital camera coupled to an optical light microscope (Alltion).

Results and Discussion

The *S. purpurea* petiolule has a concave-convex shape in the transversal section, with two small lateral projections on the adaxial side (Figure 1A). The *S. mombin* petiolule showed a convex symmetry with undulation (Vasconcelos, Vasconcelos & Randau, 2016). The epidermis is uniseriate and covered by a thin layer of the cuticle (Figure 1A). There are non-glandular and glandular trichomes, both being uniseriate and multicellular (Figure 1B). Only non-glandular trichomes were observed in *S. mombin* (Vasconcelos, Vasconcelos & Randau, 2016). Parenchyma fills the entire petiolule (Figure 1A). There are collateral vascular bundles arranged in a ring shape in the central region (Figure 1A). In the phloem, there are secretory cavities and druses (Figure 1CD). The presence of secretory structures is a characteristic of the Anacardiaceae family (Metcalf & Chalk, 1950; Dantas et al., 2019; Sá et al., 2019). Druses have also been found in the petiolule of *S. mombin*, but are located close to the epidermis (Vasconcelos, Vasconcelos & Randau, 2016).

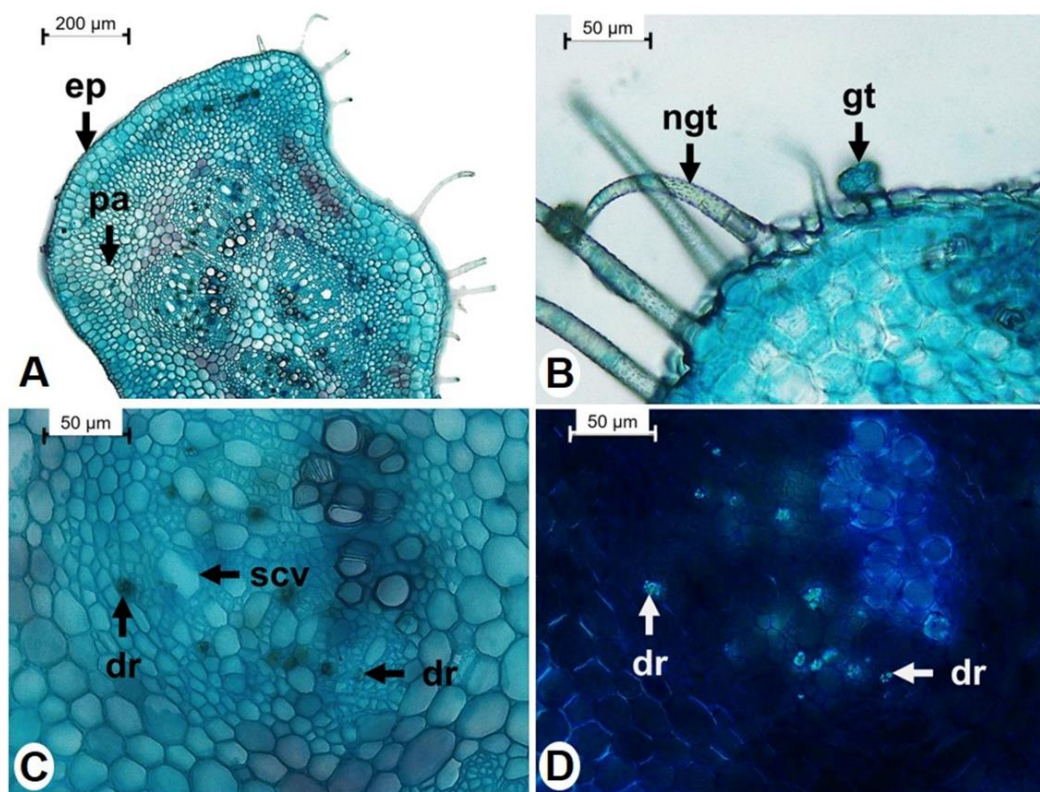


Figure 1. Cross-sections of the petiولة of *Spondias purpurea*. A-C. optical microscopy; D. polarized microscopy; A. detail of epidermis (ep) and parenchyma (pa); B. detail of non-glandular trichome (ngt) and glandular trichome (gt); C,D. secretory cavity (scv) and druses (dr) in the phloem. Font: Santos et al. (2020).

There is a controversy in the literature for the leaflets of *S. mombin*. In the frontal view, the leaflets present cells of straight or slightly sinuous contour in both faces (Figure 2AB). It is classified as amphistomatic, with anomocytic stomata in both faces (Figure. 2AB). *Spondias tuberosa* and *S. dulcis* presented hypostomatic leaflets (Nascimento-Silva & Paiva, 2007; Lima, Magalhães & Randau, 2020). Chisom, Chukwu & Okeke (2014) classified it as amphistomatic, and Vasconcelos, Vasconcelos & Randau (2016) classified it as hypostomatic. There are non-glandular trichomes on the adaxial face (Figure 2A) and glandular trichomes on the abaxial face (Figure 2C). *Spondias mombin* had only non-glandular trichomes on both faces while *S. dulcis* had non-glandular trichomes on the abaxial face (Vasconcelos, Vasconcelos & Randau, 2016; Lima, Magalhães & Randau, 2020).

The midrib has a concave-convex contour and uniseriate epidermis in cross-section, covered by a cuticle (Figure 2D). The same midrib contour was observed in *S. dulcis* Parkinson (Lima, Magalhães & Randau, 2020). *Spondias mombin* presented a biconvex contour midrib (Vasconcelos, Vasconcelos & Randau, 2016). The palisade parenchyma fills in the midrib on the adaxial side (Figure 2D). The abaxial side is filled with parenchyma (Figure 2D). There are two collateral

vascular bundles in the central region, one of which is larger and faces the abaxial side. The other bundle is smaller and faces the adaxial side (Figure 2D). Four collateral vascular bundles were visualized in *S. dulcis* and three in *S. mombin*, being one bundle is larger, and two bundles are accessories (Vasconcelos, Vasconcelos & Randau, 2016; Lima, Magalhães & Randau, 2020). As in the petiولة, secretory cavities and druses were found in the phloem (Figure 2EF). In *S. cytherea* (synonym of *S. dulcis*) and *S. pinnata*, the druses are located in the phloem and the midrib parenchyma (Norfaizal & Latiff, 2013). In *S. mombin*, the druses are located only in the midrib parenchyma (Vasconcelos, Vasconcelos & Randau, 2016).

The mesophyll is dorsiventral, with one layer of palisade parenchyma and about six layers of spongy parenchyma, with few intercellular spaces (Figure 2G). *Spondias cytherea* and *S. pinnata* have only one layer of palisade parenchyma (Norfaizal & Latiff, 2013), while *S. mombin* presented two layers of palisade parenchyma near the midrib and one layer near the margin of the leaflets (Vasconcelos, Vasconcelos & Randau, 2016). Druses are present in the mesophyll (Figure 2GH). Lima, Magalhães & Randau (2020) also found druses in the mesophyll of *S. dulcis*.

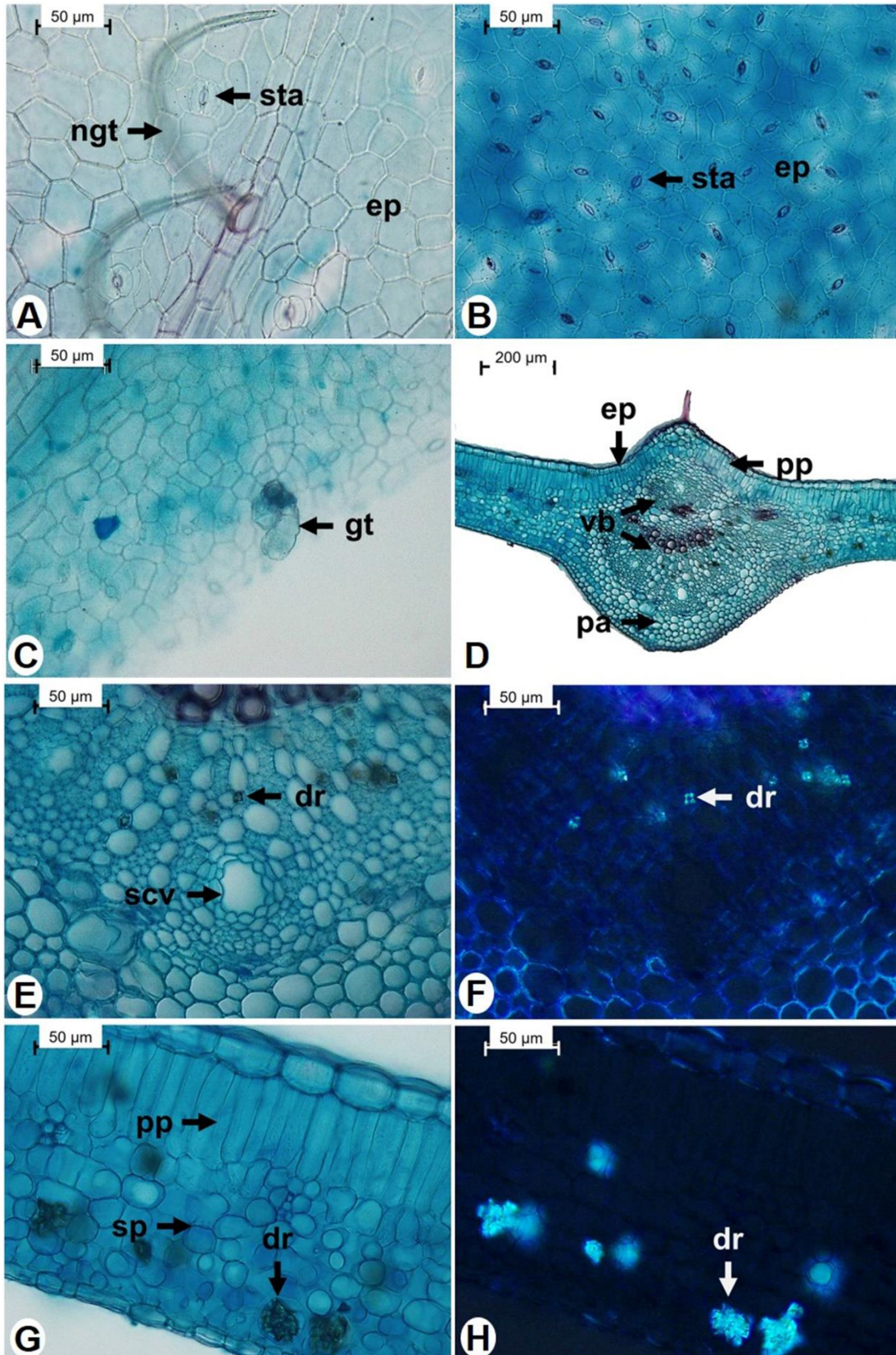


Figure 2. Paradermal and cross-sections of the leaflets of *Spondias purpurea*. A-E,G. optical microscopy; F,H. polarized microscopy; A. adaxial face, showing epidermis (ep), stomata (sta), and non-glandular trichome (ngt); B,C. abaxial face, showing epidermis (ep), stomata (st), and glandular trichome (gt); D-F. midrib showing epidermis (ep), palisade parenchyma (pp), parenchyma (pa) and vascular bundles (vb); G,H. mesophyll showing palisade parenchyma (pp), spongy parenchyma (sp), and druses (dr). Font: Santos et al. (2020).

Figure 3AB shows the leaflets, in cross-section, without any treatment. Phenolic compounds were found in the epidermis (Figure 3C), spongy parenchyma (Figure 3C), palisade parenchyma (Figure 3D), and midrib parenchyma (Figure 3D). In *S. mombin*, phenolic compounds were found only in the palisade parenchyma (Vasconcelos, Vasconcelos & Randau, 2016). In *S. tuberosa* they were found in the trichomes and the parenchyma sheath (Nascimento-Silva, Chinalia & Paiva, 2008). Phenolic compounds have been identified in the leaves of *S. purpurea*, such as quercetin 3-O-rutinoside (Pereira et al., 2015), caffeic acid, and epigallocatechin (Almeida et al., 2017). The latter two compounds were correlated with antioxidant and anti-ulcer properties of the species' leaves (Almeida et al. 2017).

Alkaloids were identified in the central vein parenchyma (Figure 3E). In *S. tuberosa*, alkaloids were also identified in the parenchyma (Nascimento-Silva, Chinalia & Paiva, 2008). The test to identify alkaloids was negative in *S. mombin* (Vasconcelos, Vasconcelos & Randau, 2016). Tannins were observed in the midrib parenchyma (Figure 3F), in the cells around the secretory cavities (Figure 3G), in the palisade parenchyma (Figure 3H), and the spongy parenchyma (Figure 3I). In *S. tuberosa* the tannins were observed in the parenchymal sheath (Nascimento-Silva, Chinalia & Paiva, 2008). In *S. mombin*, these compounds were not identified (Vasconcelos, Vasconcelos & Randau, 2016).

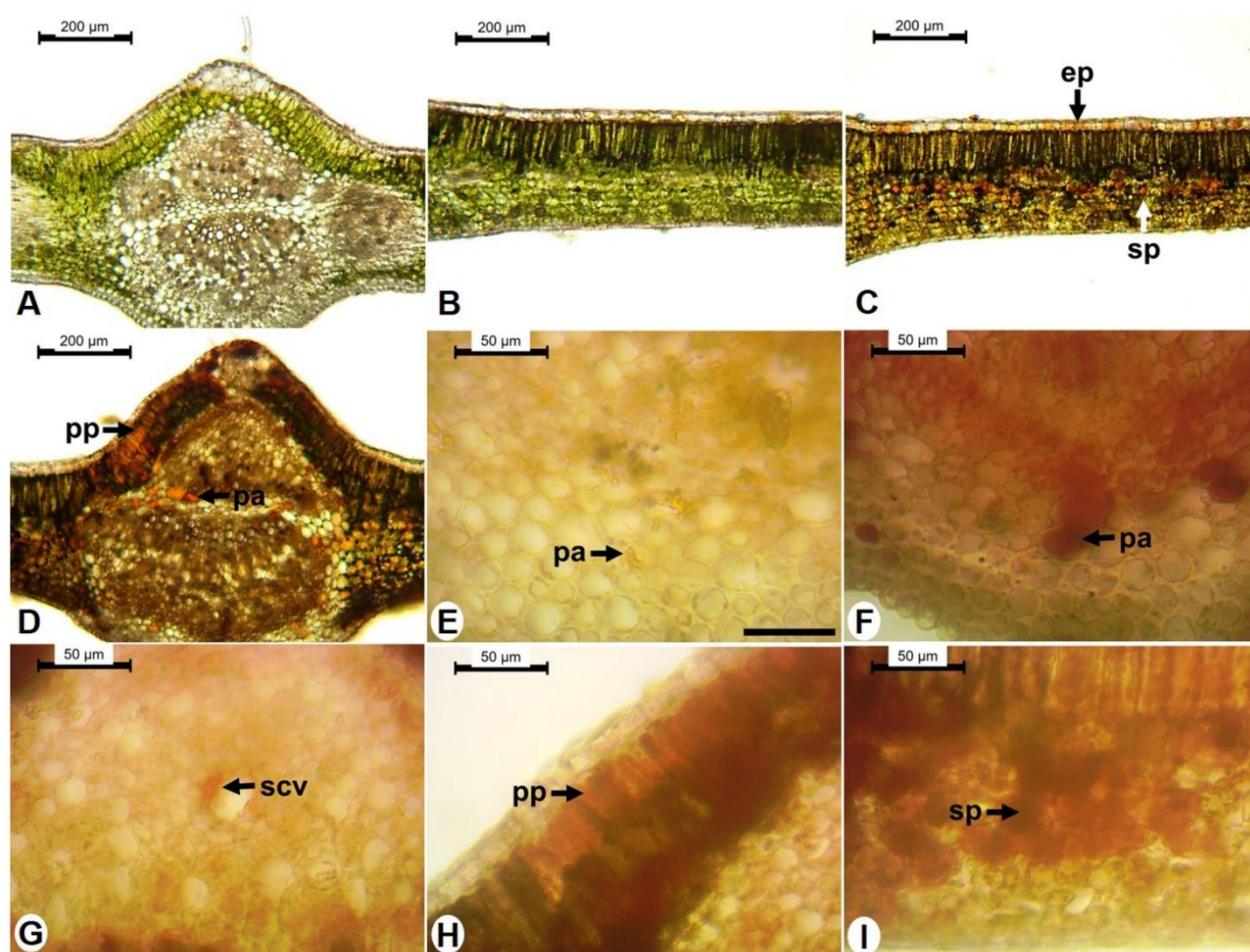


Figure 3. Histochemistry of the leaflets of *Spondias purpurea*. A,B. control; C,D. Potassium dichromate (10%) - phenolic compounds in the epidermis (ep), palisade parenchyma (pp), spongy parenchyma (sp), and parenchyma (pa); E. Dragendorff's reagent - alkaloids in the parenchyma (pa); F-I. Vanillin chloridric - tannins in the parenchyma (pa), in the cells around the secretory cavity (scv), in palisade parenchyma (pp), and spongy parenchyma (sp). Font: Santos et al. (2020).

Triterpenes and steroids were visualized in the midrib parenchyma (Figure 4A) and the spongy parenchyma (Figure 4B). Marisco et al. (2017a) identified some terpenes in the leaves of *S. purpurea*, such as spathulenol, linolenic acid,

transcaryophyllene, and alpha-muurolene. The fraction containing these terpenes showed antifungal activity against *Moniliophthora pernicioso*. Lipophilic compounds were evident in the cuticle (Figure 4C). Essential oils were found

in the secretory cavities (Figure 4D), in the midrib parenchyma (Figure 4D), and the palisade parenchyma (Figure 4E). Lemos et al. (1995) identified β -caryophyllene and δ -cadinol as major components of the essential oil of leaves of this species. However, Lima, Oliveira & Brito (2016) found the content of only 2.35% de β -caryophyllene, with heptacosano (28.80%) as the major constituent of the essential oil of the leaves of *S. purpurea*.

Lignin was evidenced in the conductive cell wall, xylem fibers (Figure 4F), and starch grains observed in the midrib parenchyma (Figure

4G). Vasconcelos, Vasconcelos & Randau (2016) also evidenced starch grains in the central vein parenchyma of *S. mombin*, while Nascimento-Silva, Chinalia & Paiva (2008) evidenced starch grains in the mesophyll of *S. tuberosa*. The test with hydrochloric acid (10%) showed that the druses are of calcium oxalate, after their dissolution, without forming bubbles (Figure 4HI). Histochemical tests with *S. mombin* (Vasconcelos, Vasconcelos & Randau, 2016) and *S. tuberosa* (Nascimento-Silva, Chinalia & Paiva, 2008) also confirmed that the chemical composition of the crystals present in the species is calcium oxalate.

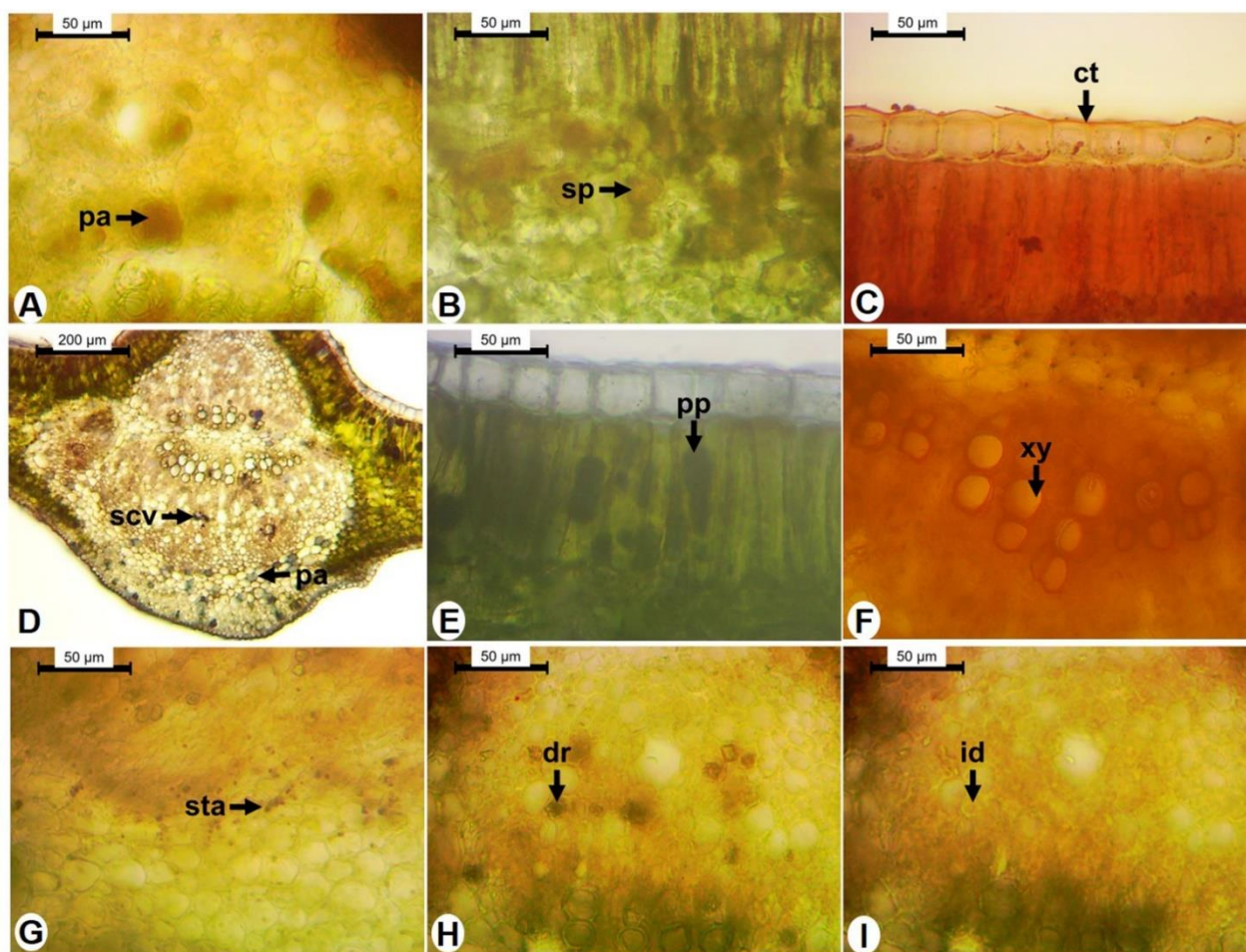


Figure 4. Histochemistry of the leaflets of *Spondias purpurea*. A,B. Antimony trichloride - triterpenes and steroids in the parenchyma (pa) and spongy parenchyma (sp); C. Sudan III - lipophilic compounds in the cuticle (ct); D,E. Nadi reagent - essential oils in parenchyma (pa), secretory cavities (scv) and palisade parenchyma (pp); F. Phloroglucinol - lignin in xylem (xy); G. Lugol - starch (sta) in parenchyma; H,I. Hydrochloric acid (10%) - druse (dr) and idioblast (id) that contained druse. Font: Santos et al. (2020).

Table 1 and Table 2 summarize the main results, comparing with literature data about other species of *Spondias*.

Table 1. Anatomical characters of *Spondias* species. ¹Lima, Magalhães & Randau (2020); ²Norfaizal & Latiff (2013); ³⁰Vasconcelos, Vasconcelos & Randau (2016); ⁴Chisom, Chukwu & Okeke (2014); ⁵Nascimento-Silva & Paiva (2007). Font: Santos et al. (2020).

Characters	<i>Spondias cytherea</i> (<i>S. dulcis</i>)	<i>Spondias mombin</i>	<i>Spondias pinnata</i>	<i>Spondias purpurea</i>	<i>Spondias tuberosa</i>
petiolule	shape	--	convex with undulation ³	--	concave-convex with two small lateral projections on the adaxial side
	trichomes	--	non-glandular ³	--	non-glandular and glandular
	position of stomas	--	located close to the epidermis ³	--	located in phloem
	position of stomas	hypostomatic ¹	amphistomatic ⁴ hypostomatic ³	--	amphistomatic
leaflet	trichomes	non-glandular on the abaxial face ¹	non-glandular on both faces ³	--	non-glandular on the adaxial face and glandular on the abaxial face
	midrib	concave-convex ¹	biconvex ³	--	concave-convex
	stomata	four ¹	three ³	--	two
	druses	mesophyll ¹ ; midrib parenchyma and phloem ²	midrib parenchyma ³	phloem and midrib parenchyma ²	phloem and mesophyll
palisade parenchyma	one layer ²	two layers near the midrib and one layer near the margin of the leaf blade ³	one layer ²	one layer	--

Table 2. Histochemical characters of *Spondias* species. ¹Vasconcelos, Vasconcelos & Randau (2016); ²Nascimento-Silva, Chinalia & Paiva (2008). Fonte: Santos et al. (2020).

Characters	<i>Spondias mombin</i>	<i>Spondias purpurea</i>	<i>Spondias tuberosa</i>
phenolic compounds	palisade parenchyma ¹	epidermis, spongy parenchyma, palisade parenchyma, and midrib parenchyma	trichomes and parenchyma sheath ²
alkaloids	negative ¹	midrib parenchyma	parenchyma ²
tannins	negative ¹	midrib parenchyma, cells around the secretory cavities, palisade parenchyma, and spongy parenchyma	parenchyma sheath ²
triterpenes and steroids	--	midrib parenchyma and spongy parenchyma	--
lipophilic compounds	--	cuticle	--
essential oils	--	secretory cavities, midrib parenchyma, and palisade parenchyma	--
lignin	--	xylem	--
starch grains	midrib parenchyma ¹	midrib parenchyma	mesophyll ²

chemical composition of the crystals	calcium oxalate ¹	calcium oxalate	calcium oxalate ²
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Conclusion

This study provides the main characters for the identification of the species, such as petiolule with concave-convex shape, non-glandular and glandular trichomes, druses in phloem; leaflets amphistomatic, with non-glandular trichomes on the adaxial face and glandular trichomes on the abaxial face, midrib with concave-convex shape, two collateral vascular bundles, one layer of palisade parenchyma, druses in phloem and mesophyll. Moreover, it was possible to demonstrate the accumulation site of the different metabolites present in the species, contributing to the taxonomy of the genus *Spondias*.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research funding (409452/016-5).

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