Diversitas Journal

ISSN 2525-5215
DOI: 10.17648/diversitas-journal-v6i1-1571

Volume 6, Número 1 (jan./mar. 2021) pp: 620-633.
https://periodicos.ifal.edu.br/diversitas_journal/

Anatomy and histochemistry of the leaf blade of *Syzygium cumini* (L.) Skeels

Anatomia e histoquímica da lâmina foliar de *Syzygium cumini* (L.) Skeels

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Recebido em: 19 de agosto de 2020; Aceito em: 07 de janeiro de 2021; publicado em 31 de 01 de 2021. Copyright© Autor, 2021.

RESUMO: Diabetes é uma Doença Crônica Não-Transmissível responsável por cerca de 1,6 milhões de mortes no mundo em 2016. O uso de plantas medicinais é comumente relatado na literatura como adjuvante no tratamento de diabetes. *Syzygium cumini* (L.) Skeels, popularmente conhecida como azeitona preta ou jambolão, é uma importante espécie medicinal da família Myrtaceae, popularmente usada para diabetes. Este trabalho teve como objetivo realizar um estudo anatômico e histoquímico da lâmina foliar de *S. cumini*. Lâminas histológicas semipermanentes foram preparadas para análise da lâmina foliar em microscopia óptica, de polarização e microscopia eletrônica de varredura. Testes histoquímicos foram realizados em secções transversais de lâminas foliares frescas, utilizando reagentes específicos para cada grupo de metabólitos. A análise microscópica permitiu identificar elementos importantes no diagnóstico da espécie. Os testes histoquímicos evidenciaram a presença de compostos fenólicos, taninos, triterpenos e esteroides, alcaloides, óleos essenciais, compostos lipofílicos, cristais de amido, lignina e oxalato de cálcio. Os resultados apresentados contribuem para a padronização farmacobotânica da espécie.
PALAVRAS-CHAVE: farmacobotânica, microscopia, Myrtaceae.

ABSTRACT: Diabetes is a noncommunicable disease responsible for about 1.6 million deaths in 2016 worldwide. The use of medicinal plants is commonly reported in the literature as an adjuvant in the treatment of diabetes. *Syzygium cumini* (L.) Skeels, popularly known as azeitona preta or jambolão, is an important medicinal species of the Myrtaceae family used for diabetes. This work aimed to conduct an anatomical and histochemical study of the leaf blade of *S. cumini*. Semipermanent histological slides were prepared for analysis of the leaf blade in optical microscopy, polarization and scanning electron microscopy. Histochemical tests were performed in cross-sections of fresh leaf slides, using specific reagents for each group of metabolites. Microscopic analysis allowed the identification of important elements in the diagnosis of the species. The histochemical tests evidenced the presence of phenolic compounds, tannins, triterpenes and steroids, alkaloids, essential oils, lipophilic compounds, starch, lignin and calcium oxalate crystals. The results presented contribute to the pharmacobotanical standardization of the species.
KEYWORDS: pharmacobotany, microscopy, Myrtaceae.

INTRODUÇÃO

The Myrtaceae family is represented by approximately 3.500 species, distributed in about 140 genera (APG IV, 2016). The genus *Syzygium* comprises fruitful species and is mainly found in the tropical and subtropical regions of the world (AYYANAR; SUBASH-BABU, 2012). One of its representatives is *Syzygium cumini* (L.) Skeels, a dense crown tree, 12-20 m in height. In Brazil, it is found in the North, Northeast, South and Southeast regions (LORENZI et al., 2015; SOBRAL et al., 2015).

It is popularly known as azeitona roxa (RODRIGUES; ANDRADE, 2014), azeitona preta (LOZANO et al., 2014), jamelão (ALMEIDA et al., 2014) and jambolão (FEIJÓ et al., 2012). The leaves of the species have been widely used by the population for the treatment of diabetes (VENDRUSCOLO; MENTZ, 2006; ALBUQUERQUE et al., 2007; AGRA et al., 2008; SANTOS; LIMA, 2008; FEIJÓ et al., 2012; LOZANO et al., 2014). Diabetes is a noncommunicable disease (NCD) responsible for about 1.6 million deaths in 2016 worldwide and is an important risk factor and comorbidity for other more serious conditions, such as cardiovascular diseases, renal failure and blindness (WHO, 2018).

In Brazil, drug therapy for diabetes has been used by the population served in the health units of the Unified Health System, including the availability of free drugs (MALTA et al., 2011; MALTA; SILVA JUNIOR, 2013). The use of medicinal plants is commonly reported in the literature as an adjuvant in the treatment of diabetes (FEIJÓ et al., 2012; SANTOS et al., 2012). For there to be a safe use of these species, pharmacobotanical studies are necessary. Some anatomical data for *S. cumini* are already found in the literature, however, there are still no histochemical studies with the species. Therefore, the aim of the study was to perform anatomical and histochemical characterization of the leaf blade of *S. cumini*.

THEORETICAL REFERENCE

Noncommunicable diseases constitute the biggest global health problem, generating a high number of premature deaths. According to the World Health Organization (WHO), of the 57 million deaths that occurred worldwide in 2016, about 41 million were due to NCDs (WHO, 2018). Individuals belonging to vulnerable

groups, such as the elderly and those with low education and income, are the most severely affected (MALTA et al., 2019). About 78% of all NCDs deaths in 2016 occurred in low and middle income countries (WHO, 2018).

In Brazil, NCDs are also the main cause of mortality (DUNCAN et al., 2012; HIRSCHMANN *et al.*, 2020). In 2016, of a total of 1,320,000 deaths, 975,400 of them were attributed to NCDs, corresponding to 74% of the total. Cardiovascular diseases, cancers, respiratory diseases and diabetes accounted for the vast majority of these deaths (WHO, 2018).

Medicinal plants are used as an adjunct in the treatment of NCDs (FEIJÓ et al., 2012; ROSA; BARCELOS; BAMPI, 2012; SANTOS; NUNES; MARTINS, 2012; FARIAS et al., 2016; BERKE et al., 2019; LUCENA; GUEDES, 2020). Some of these vegetables used have medicinal action proven through scientific studies, such as *S. cumini* (SRIVASTAVA; CHANDRA, 2013; CHAGAS et al., 2015). Literature review studies regarding the antidiabetic properties of leaves of *S. cumini* have shown that this activity is mainly related to the presence of flavonoids (AYYANAR et al. 2013; SRIVASTAVA; CHANDRA, 2013, CHAGAS et al., 2015).

The use of infusion of the plant leaves was one of the most cited practices in a study carried out with elderly people assisted in a Basic Health Unit in Pelotas, Rio Grande do Sul, about the medicinal plants used as complementary therapy in the treatment of diabetes symptoms (FEIJÓ et al., 2012). Additionally, there are also reports of use of leaf decoction (AGRA et al., 2008; SANTOS; LIMA, 2008; RODRIGUES; ANDRADE, 2014).

METHODOLOGICAL PROCEDURE

Plant material

The material studied was collected in 2012 in Aldeia, Camaragibe, Pernambuco, Brazil. The voucher specimen was deposited in the Herbarium Dárdano de Andrade Lima, of the Instituto Agronômico de Pernambuco (IPA), under registration number 88151.

Anatomical characterization

The anatomical study was performed using leaf blades obtained between the third and fifth nodes, fixed in FAA_{50} (JOHANSEN, 1940). Various cross-sections were obtained by hand, using a common razor blade, at the middle region of the leaf blades. Paradermal sections were also performed on the adaxial and abaxial faces. All sections were clarified in 50% sodium hypochlorite solution (KRAUS; ARDUIN, 1997). Semipermanent histological slides were prepared containing the cross-sections, stained with safranin and Astra blue (BUKATSCH, 1972), and the paradermal sections, stained with 1% methylene blue (KRAUTER, 1985), following usual plant anatomy procedures (JOHANSEN, 1940; SASS, 1951). The analysis of the semipermanent histological slides were conducted on images in software (LAS EZ), obtained by digital camera (Leica ICC50 W) coupled to an optical and polarized microscope (Leica DM750M). Measurements of crystal diameter were determined using the LAS EZ program and the mean and standard deviation were calculated.

For the anatomical characterization in Scanning Electron Microscopy (SEM), samples of fresh leaf blades were fixed in 2.5% glutaraldehyde (buffered with 0.1M sodium cacodylate) and post-fixed using 2% osmium tetroxide solution (buffered with 0.1M sodium cacodylate). The material was submitted to dehydration in ethanol series and to critical point drying (Bal-Tec CPD 030), mounted onto SEM stubs, using double-sided adhesive tape and sputter-coated with gold (Leica EM SCD 500) (HADDAD et al., 1998). The samples were examined with a scanning electron microscope (Quanta 200 FEG) in the Centro de Tecnologias Estratégicas do Nordeste (CETENE).

Histochemical characterization

Histochemical tests were made on cross-sections of fresh leaf blades obtained by the same method used in anatomical study (JOHANSEN, 1940). The specific reagents used were: potassium dichromate (10%) for phenolic compounds (GABE, 1968), vanillin chloridric for tannins (MACE; HOWELL, 1974), antimony trichloride for triterpenes and steroids (MACE et al., 1974), Dragendorff's reagent for alkaloids (YODER;

MAHLBERG, 1976), Nadi for essential oils (DAVID; CARDE, 1964), Sudan III for lipophilic substances (SASS, 1951), lugol's iodine reagent for starch (JOHANSEN, 1940), phloroglucinol for lignin (JOHANSEN, 1940) and hydrochloric acid (10%) to establish the nature of the crystals (JENSEN, 1962). Cross-sections without any treatment performed in parallel with the tests. Semipermanent histological slides were prepared containing the cross-sections and were analyzed in optical microscope (Alltion).

RESULTS AND DISCUSSION

In frontal view, under optical microscopy, the leaf blade of *S. cumini* presents epidermal cells with sinuous walls in the adaxial face (Fig. 1a) and cells with straight or slightly wavy walls on the abaxial face (Fig. 1a). It is also verified the presence of secretory cavities, which occur on both sides (Fig. 1a-b). Under SEM, it is seen that the epidermal cells are covered by smooth cuticle (Fig. 1c-d).

The leaf blade is hypoestomatic (Fig. 1a-b), corroborating other studies with the species (RUGGIERO, 2004; KANTACHOT et al., 2007; SIQUEIRA-NUNES; MARTINS, 2010). However, Alberton et al. (2001) described it as amphistomatic.

There is a divergence in the literature regarding the types of stomata found in the leaf blade of the species. In the present study were identified anomocytic and anisocytic stomata (Fig. 1b). According to Metcalfe and Chalk (1950), stomata are usually anomocytic in the Myrtaceae family. This was the only type described by Alberton et al. (2001) and Ruggiero (2004) for *S. cumini*, while Siqueira-Nunes and Martins (2010) cited only paracytic stomata and Kantachot et al. (2007) found anisocytic and paracytic stomata.

According to Kantachot et al. (2007), of seventeen species of *Syzygium* analyzed, only *S. cumini*, *S. laetum* subsp. *jugorum* and *S. ripicola* presented anisocytic and paracytic stomata. Considering the others, five showed only anomocytic stomata and nine showed only paracytic stomata.

Trichomes were not visualized on the leaf blade of *S. cumini*. Despite the occurrence of trichomes be reported for species of the Myrtaceae family (METCALFE;

CHALK, 1950), in a study with seventeen species of the genus *Syzygium* in Thailand, none presented trichomes (KANTACHOT et al., 2007), including *S. cumini*.

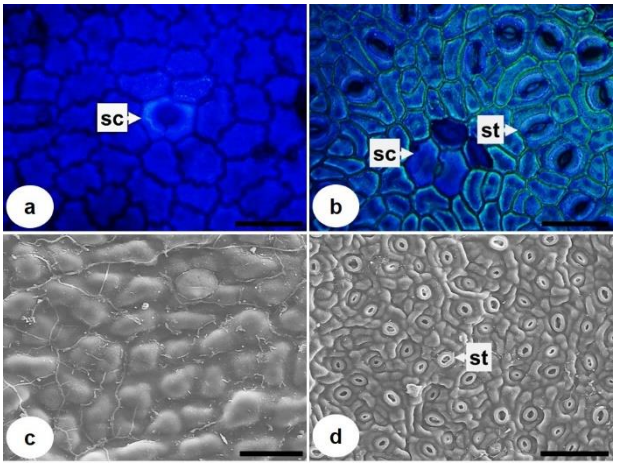

Figure 1. Frontal view of the leaf blade of *Syzygium cumini* (L.) Skeels. a and b: optical microscopy; c and d: scanning electron microscopy. a and c: adaxial face; b and d: abaxial face. Abbreviations: sc = secretory cavity; st = stomata. Bars: a, b and c = 50 μm; d = 100 μm.

In cross-section, the midrib is slightly concave on the adaxial face and slightly convex on the abaxial face (Fig. 2a). Ruggiero (2004) also described the shape of the midrib of the species as concave-convex, while Siqueira-Nunes and Martins (2010) described as plane-convex. The epidermis is uniseriate, formed by rounded cells, covered by thick cuticle (Fig. 2a).

In the central parenchyma region of the midrib is located a bicollateral vascular bundle, which is continuously surrounded by a sclerenchyma (Fig. 2a). The bicollateral type of vascular bundle is common in the family Myrtaceae (METCALFE; CHALK, 1950) and has been mentioned for this species (RUGGIERO, 2004; KANTACHOT et al., 2007; SIQUEIRA-NUNES; MARTINS, 2010). Druses are found in the parenchyma and phloem (Fig. 2b-c). The druses of the parenchyma have a mean diameter of 19.82 μm ± 1.86 and the druses of the phloem have a mean diameter of 10.22 μm ± 1.33. Secretory cavities are observed in the midrib and mesophyll (Fig. d-e). According to Metcalfe and Chalk (1950), the secretory cavities are generally present in the Myrtaceae family and secrete oily substances.

The mesophyll is isobilateral (Fig. 2e). This is another predominant feature in the family Myrtaceae (METCALFE; CHALK, 1950; KANTACHOT et al., 2007). The

palisade parenchyma is located below both sides of the epidermis, consisting of two layers on the adaxial face and one layer on the abaxial face. The spongy parenchyma consists of seven to nine layers of irregular cells (Fig. 2e). In this last tissue occur druses, which have a mean diameter of 22.82 μm ± 4.02 (Fig. 2e-f). Soh and Parnell (2011) studied 81 species of *Syzygium* from Southeast Asia and the South West Pacific area and described, with respect to crystals, that in some species druses predominate and prismatic crystals are rare; in others, prismatic crystals predominate and druses are rare; and also that there are species where the occurrence of both types of crystals is the same.

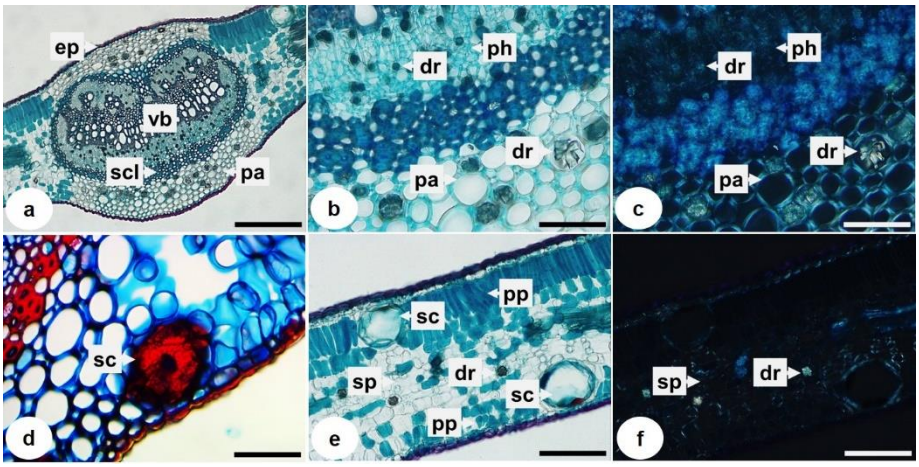

Figure 2. Cross-sections of the leaf blade of *Syzygium cumini* (L.) Skeels. a, b, d and e: optical microscopy; c and f: polarization microscopy. a, b, c and d: midrib; e and f: mesophyll. Abbreviations: dr = druse; ep = epidermis; pa = parenchyma; ph = phloem; pp = palisade parenchyma; sc = secretory cavity; scl = sclerenchyma; sp = spongy parenchyma; vb = vascular bundle. Bars: a = 200 μm; b, c and d = 50 μm; e and f = 100 μm.

The Figure 3a shows the cross-section of the leaf blade of *S. cumini* without addition of any reagent. Phenolic compounds were evidenced in the epidermis (Fig. 3b), in the parenchyma of the midrib (Fig. 3b), in the palisade parenchyma and spongy parenchyma (Fig. 3c). Tannins were found in the same places where the phenolic compounds were observed, besides being also visualized in the phloem and in the secretory cavities (Fig. 3d). Triterpenes and steroids were identified in the secretory cavities (Fig. 3e), in the collenchyma (Fig. 3f), in the parenchyma of the midrib (Fig. 3f) and in the palisade parenchyma (Fig. 3g).

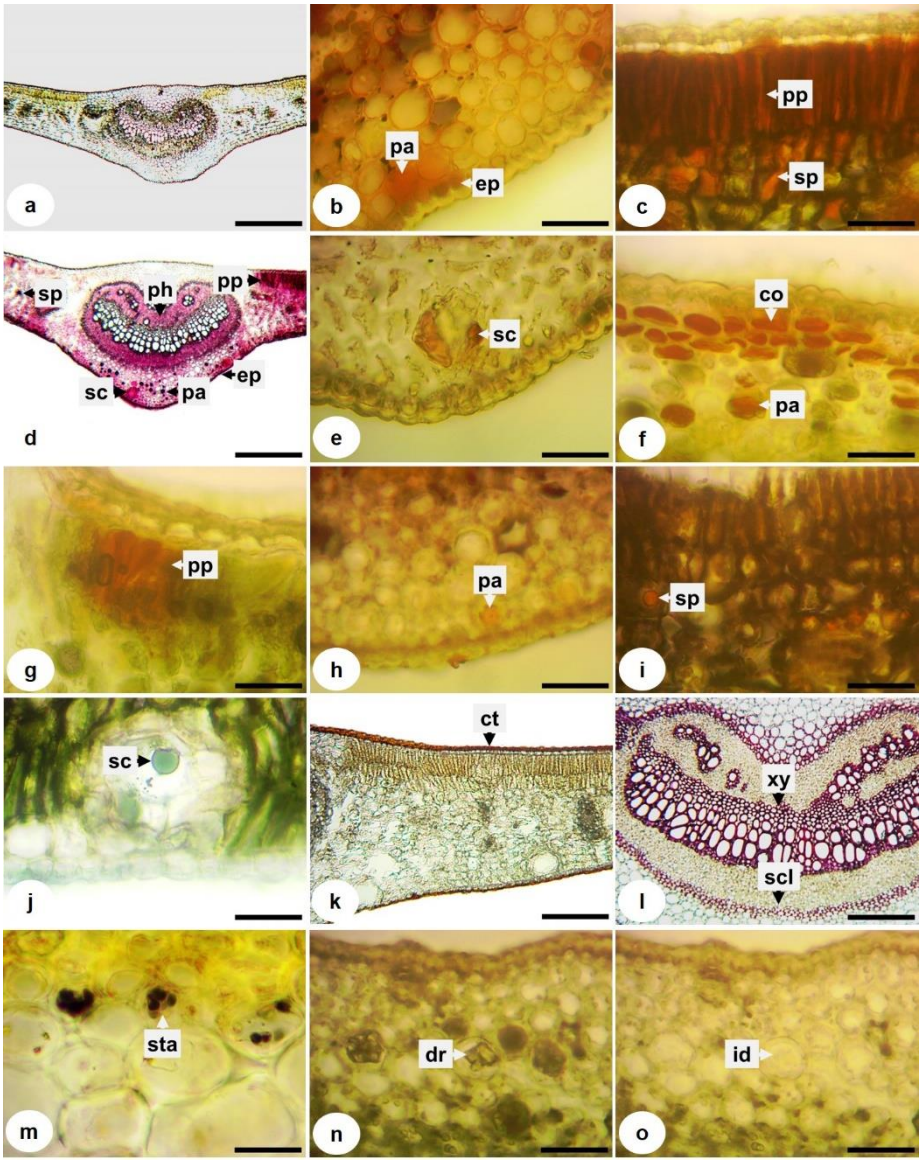

Figure 3. Histochemistry of the leaf blade of *Syzygium cumini* (L.) Skeels. a: control; b and c: potassium dichromate (10%); d: vanillin chloridric; e, f and g: antimony trichloride; h and i: Dragendorff; j: Nadi; k: Sudan III; l: phloroglucinol; m: lugol's iodine reagent; n and o: hydrochloric acid (10%). Abbreviations: co = collenchyma; ct = cuticle; dr = druse; ep = epidermis; id = idioblast; pa = parenchyma; ph = phloem; pp = palisade parenchyma; sc = secretory cavity; scl = sclerenchyma; sp = spongy parenchyma; sta = starch; xy = xylem. Bars: a and d = 500 µm; b, c, e, f, g, h, i, j, n and o = 50 µm; k and l = 200 µm; m = 20 µm.

Alkaloids were visualized in the parenchyma of the midrib (Fig. 3h) and in the spongy parenchyma (Fig. 3i). Essential oils were found in the secretory cavities (Fig. 3j). Lipophilic compounds were evidenced in the cuticle (Fig. 3k) and lignin was evidenced

in xylem and sclerenchyma (Fig. 3l). The presence of starch was demonstrated in the parenchyma of the midrib (Fig. 3m). The Figure 3n shows the presence of druses in the leaf blade of *S. cumini* and the Figure 3o shows the dissolution of the druses with the test of hydrochloric acid 10%, indicating that they are of calcium oxalate.

The presence of phenolic compounds, tannins, triterpenes, steroids and alkaloids corroborate phytochemical investigations of the literature (RUGGIERO, 2004; ITANKAR et al. 2015; VEBER et al., 2015; SANCHES et al., 2016, RAMOS; BANDIOLA, 2017). Ruggiero (2004) analyzed the essential oil of the leaves of the species collected in São Paulo and found α-pinene (36.54%), α-terpineol (12.85%) and β-pinene (12.74%) as major constituents.

CONCLUSION

The present study provided anatomical information useful for the correct identification of *S. cumini*. The histochemical tests evidenced the presence of phenolic compounds, tannins, triterpenes and steroids, alkaloids, essential oils, lipophilic compounds, starch and lignin. Besides that, the study showed the chemical nature of the crystals and contributes with the taxonomy of the genus.

ACKNOWLEDGMENTS

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support in the form of fellowship awards. They also thank to Fundação de Amparo à Ciência e Tecnologia (FACEPE) (APQ-0220-4.03/15) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (409452/016-5) for research funding and to CETENE for the analysis in SEM.

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