BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902019000117227

Article

Pharmacobotanical study of *Manilkara zapota* (L.) P.Royen (Sapotaceae)

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Manilkara zapota (L.) P. Royen, popularly known as sapoti or sapota (sapodilla), is a tree bearing an important fruit, in addition to different parts of the plant being widely used in folk medicine in the management of inflammation, pain, fevers, coughs, diarrhea, dysentery, among other ailments. This study aimed to conduct a pharmacobotany standardization study of *M. zapota*. Semi-permanent slides, containing transversal sections of stem, petiole, leaf blade and fruit; and paradermic sections of leaf blade were prepared, and analyzed by light microscopy. Histochemical tests were also performed in cross-sections of the leaf blade. Microscopic analysis allowed the identification of important elements in the diagnosis of the species; while the use of histochemical techniques on the leaf blade showed evidence of the presence of phenolic compounds, tannins, triterpenes and steroids, lipophilic compounds, starch, lignin and calcium oxalate crystals. The results presented contributed to characterization of the species.

Keywords: Anatomy. Histochemistry. Sapota. Sapoti. *Manilkara*/drug effects. Sapotaceae/drug effects.

INTRODUCTION

The Sapotaceae family contains 58 genera and approximately 1250 species that occur in neotropical regions of the world (Pennington, 1991; Govaerts, Frodin, Pennington, 2001; APG IV, 2016). In Brazil, 233 species are registered, grouped into 12 genera, which can be found in the Amazon, Atlantic Forest, Caatinga, Cerrado, Pampas and Pantanal (Alves Araújo, Alves, 2013; Flora do Brasil 2020, 2017).

Manilkara zapota (L.) P.Royen, native to Mexico and Central America, is the most known fruit tree species of Sapotaceae (Silva Jr. *et al*., 2014). At present, it is distributed in pantropical regions and is cultivated for its fruit, timber and latex (Lorenzi, Lacerda, Bacher, 2015; Milind, Preeti, 2015). Its common English name is sapodilla (Lim, 2013), while in Brazil it is popularly known as sapoti or sapota, depending on the format in which its fruits are presented (Miranda *et al*., 2002).

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Various parts of the plant are used in folk medicine in the management of inflammation, pain, fevers, coughs, diarrhea, dysentery, because they present diuretic and tonic properties and prevent formation of kidney and bladder stones; in addition to the fruit being useful due to its high nutritional content (Lim, 2013; Milind, Preeti, 2015).

Scientific studies have demonstrated analgesic (Manirujjaman *et al*., 2014); anti-arthritic (Singh *et al*., 2011); antidiarrhoeal (Manirujjaman *et al*., 2013); anti-inflammatory and anti-pyretic (Hossain *et al*., 2012; Ganguly *et al*., 2013); antimicrobial (Islam *et al*., 2013; Priya *et al*., 2014); antioxidant (Kaneria, Chanda, 2012; Fayek *et al*., 2012; Priya *et al*., 2014); antitumor (Khalek *et al*., 2015); hypoglycemic and hypocholesterolemic effects (Fayek *et al*., 2012; Paul, Hakim, 2015).

The species also presents a diversity of chemical compounds, such as phenolic compounds, terpenes, steroids, saponins, fixed oils, hydrocarbons, carbohydrates, amino acids, minerals and vitamins (Ahmed, Ifzal, Zaidi, 1982; Selvaraj, Pal, 1984; Carvalho Filho *et al*., 2012; Fayek *et al*., 2012).

However, there are few studies related to anatomical aspects and to the histolocalization of metabolites in the plant, which could be used as diagnostic features

for the species. Therefore, this study aimed to perform an anatomical and histochemical characterization of *M. zapota*.

MATERIAL AND METHODS

The material studied was collected at a site in Jaguarana, located in Paulista in the state of Pernambuco, Brazil. The voucher specimen was deposited in the Herbarium Dárdano de Andrade Lima, of the Instituto Agronômico de Pernambuco (IPA), under registration number 90.643.

The anatomical study was performed using stems and leaves obtained between the third and fifth nodes, and mature fruits obtained at the crown periphery. The samples were fixed in FAA_{50} (Johansen, 1940). Various cross-sections were obtained by hand, using a common razor blade, in the middle region of the stem, petiole, leaf blade and fruit. For the leaf blade, paradermal sections were also performed on the adaxial and abaxial surfaces. All sections were clarified in 50% sodium hypochlorite solution (Kraus, Arduin, 1997). Semi-permanent 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).

Histochemical tests were made on cross-sections of fresh leaf blades obtained by the same method as that used in the anatomical study (Johansen, 1940). The specific reagents used were: 10% potassium dichromate for phenolic compounds (Gabe, 1968), vanillin hydrochloric acid for tannins (Mace, Howell, 1974), antimony trichloride for triterpenes and steroids (Mace, Bell, Stipanovic, 1974), Sudan III for lipophilic substances (Sass, 1951), Lugol's iodine reagent for starch (Johansen, 1940), phloroglucinol for lignin (Johansen, 1940) and 10% hydrochloric acid to establish the nature of the crystals (Jensen, 1962). Cross-sections without any treatment were used as analytical white control.

The semi-permanent histological slides prepared for anatomical and histochemical characterization were analyzed in images captured by digital camera coupled to a light microscope (Alltion), by using a software program (Toup View Image).

RESULTS

The stem, in cross-section, presented a uniseriate epidermis covered with thick cuticle and lenticels in primary growth (Figures 1A and B). In secondary growth, the development of periderm was observed (Figure 1C).

The cortical region was composed of three to five layers of angular collenchyma, and approximately ten layers of parenchyma (Figures 1A and B), whereas, in secondary growth, the cortical region of the stem was formed of parenchyma only (Figure 1C).

In the cortical region of the stem, in both primary and secondary growth, laticifers, lignified cells and prismatic crystals (Figures 1A and C) were found. In the pith of the stem, in both primary and secondary growth, only laticifers and starch grains occurred (Figures 1E and F). Starch grains were also visualized in the endodermis (Figure 1D).

The vascular system of the stem in primary and secondary growth was collateral. Sclerenchyma fibers were located externally to the bundles and between the layers of phloem (Figures 1A and D).

The petiole, in cross-section, had concave-convex shape, with two more prominent regions on the adaxial surface (Figures 2A and B). The epidermis was composed of a single layer of cells and covered with a thick cuticle (Figures 2A and B). The angular collenchyma was composed of three to six layers of cells and was also present in the ribs on the adaxial surface (Figures 2A and B). The most internal layers were formed of parenchyma (Figures 2A and B).

In the cortical region of the petiole, there was presence of isolated sclereids and groups of fibers (Figure 2A), as well as laticifers, lignified cells, prismatic crystals and starch grains (Figures 2B and C). In the medullary region, laticifers and a nucleus of phloem were found, accompanied by lignified cells (Figure 2D).

A collateral vascular bundle of concave-convex conformation was found in the central region of the petiole (Figure 2D). Sclerenchymatic fibers surrounded the vascular bundle and were also located in the ribs (Figures 2A and D).

In front view, the leaf blade showed epidermal cells with a strongly sinuous contour on the adaxial side (Figure 3A) and cells that had straight or slightly sinuous walls on the adaxial surface (Figure 3B). The leaf blade was hypostomatic (Figures 3A and B) and had anomocytic stomata on the abaxial surface, located on the same level as the epidermal cells (Figure 3B).

In cross-section, the leaf blade had uniseriate epidermis coated with a thick cuticle (Figure 4A). There were unicellular 2-armed trichomes on both sides of the leaf blade (Figure 4C).

The midrib had concave-convex shape (Figures 4A and B). The angular collenchyma was arranged in three to six layers of cells, being more developed in the abaxial region (Figures 4B and C). The ground parenchyma

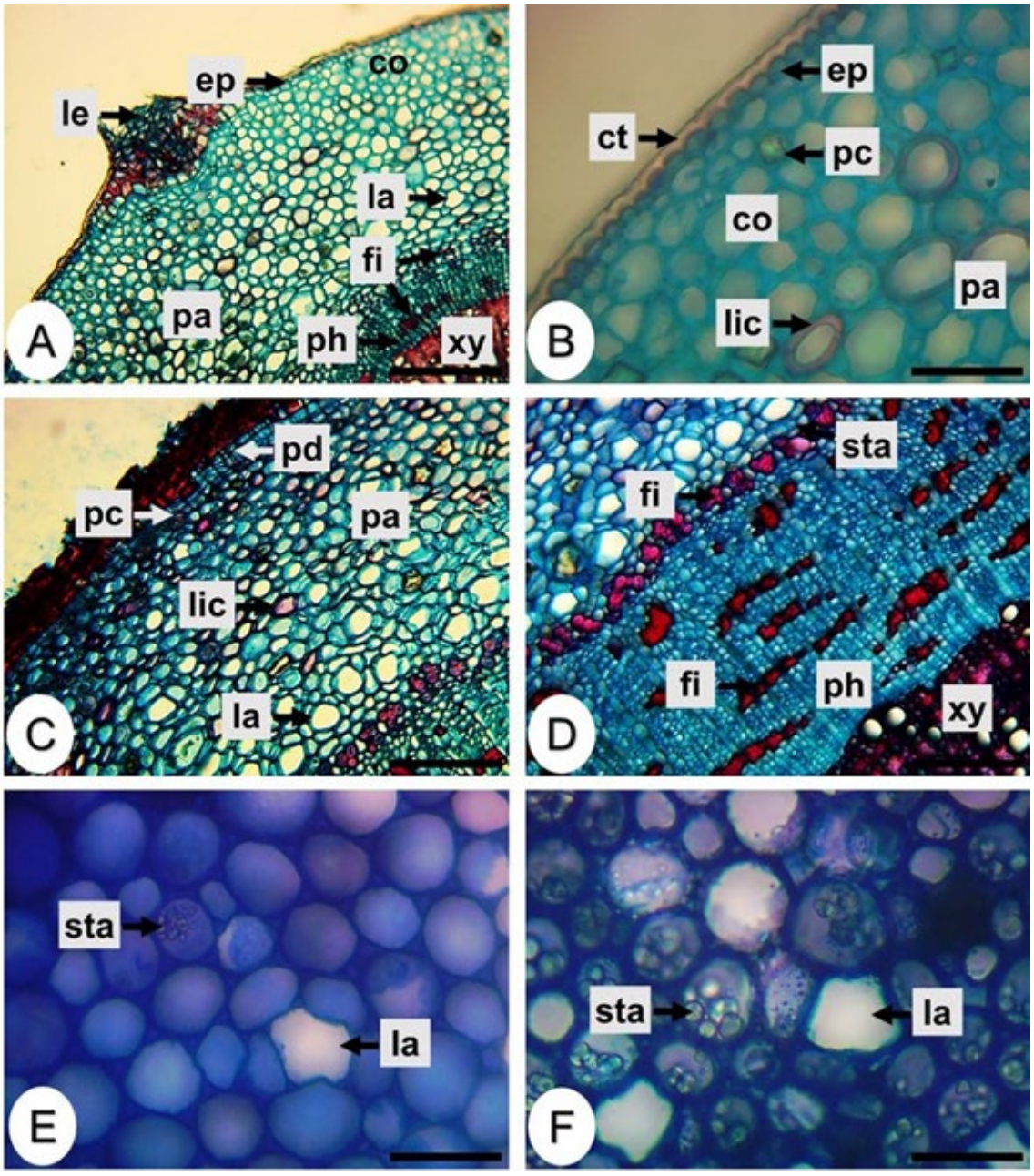

FIGURE 1 - Cross-sections of the stem of *Manilkara zapota* (L.) P.Royen (Sapotaceae). A, B, E: primary growth. C, D, F: secondary growth. Abbreviations: co = collenchyma; ct = cuticle; ep = epidermis; fi = fiber; la = laticifer; le = lenticel; lic = lignified cell; pa = parenchyma; pc = prismatic crystal; pd = periderm; ph = phloem; sta = starch; xy = xylem. Bars: A, D, E = 200 µm; B, C, F = 50 µm.

occupied the central region of the midrib, interrupted by sclerenchymatic fibers that surrounded the vascular system (Figures 4A and B). The vascular system was disposed in the same way as that observed in the petiole (Figures 4A and B). Also similar to that found in the petiole, there was the presence of laticifers, lignified cells, prismatic crystals and starch grains in the cortical region (Figures 4A and B). However, starch grains were also found in the medullary region, in addition to laticifers and a nucleus of phloem accompanied by lignified cells (Figures 4A, B and E). Stone cells were visualized next to the vascular system (Figures 4A and F).

On the adaxial surface of the leaf blade, a subepidermal layer composed of large and rounded cells was found (Figure 4G). The mesophyll was dorsiventral, presenting one to two layers of palisade parenchyma and four to six layers of spongy parenchyma (Figure 4G). There were sclereids of diverse sizes and formats (Figures 4G and H), in addition to several prismatic crystals (Figures 4G and H) and vascular bundles, which were

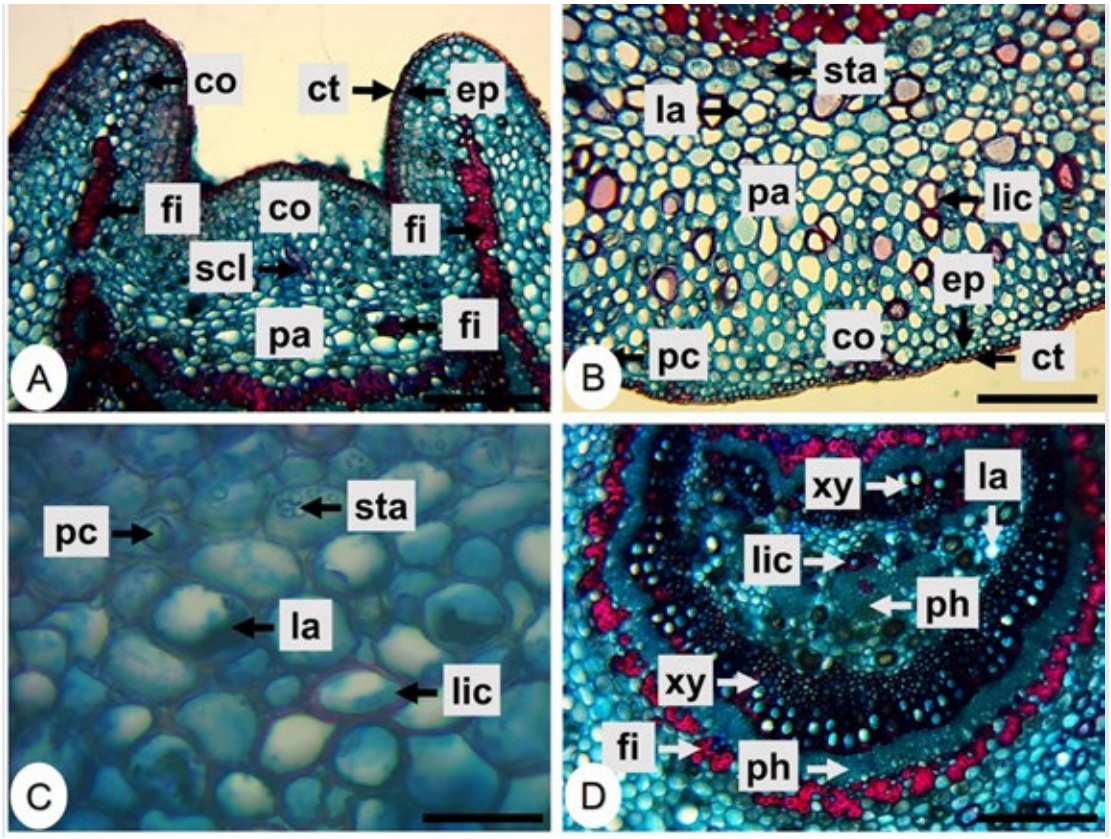

FIGURE 2 - Cross-sections of the petiole of *Manilkara zapota* (L.) P.Royen (Sapotaceae). A, B, C: general organization of the structures. D: detail of the cortical region. Abbreviations: co = collenchyma; ct = cuticle; ep = epidermis; fi = fiber; la = laticifer; lic = lignified cell; pa = parenchyma; pc = prismatic crystal; ph = phloem; scl = sclereid; sta = starch; xy = xylem. Bars: A, B, D = 200 µm; C = 50 µm.

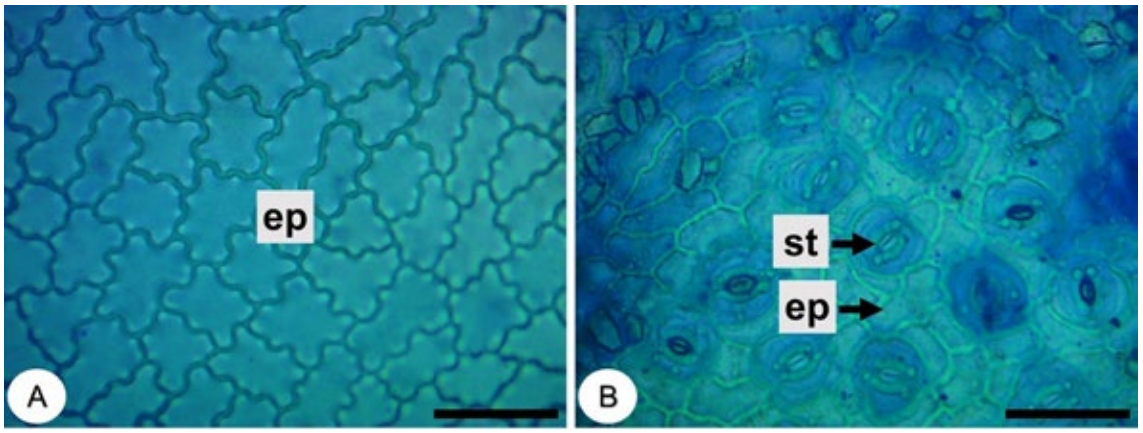

FIGURE 3 - Frontal view of the leaf blade of *Manilkara zapota* (L.) P.Royen (Sapotaceae). A: Adaxial surface. B: Abaxial surface. Abbreviations: ep = epidermis; st = stomata. Bars: A, B = 50 µm.

surrounded by sclerenchymatic fibers (Figure 4G).

In the cross-sections of the fruit, it was observed to be coated by a periderm (Figure 5A). The mesocarp consisted of parenchyma, and laticifers, groups of large stone cells (Figure 5A), vascular bundles (Figure 5B) and idioblasts containing raphides were visualized (Figure 5C).

Figures 6A and 6B correspond to the controls. The Figure 6A shows the central region of the midrib, and the Figure 6B shows the mesophyll. The phenolic compounds were identified by the presence of red coloring in the adaxial subepidermal layer and in the palisade parenchyma (Figure 6C).

Tannins were also seen in the adaxial subepidermal

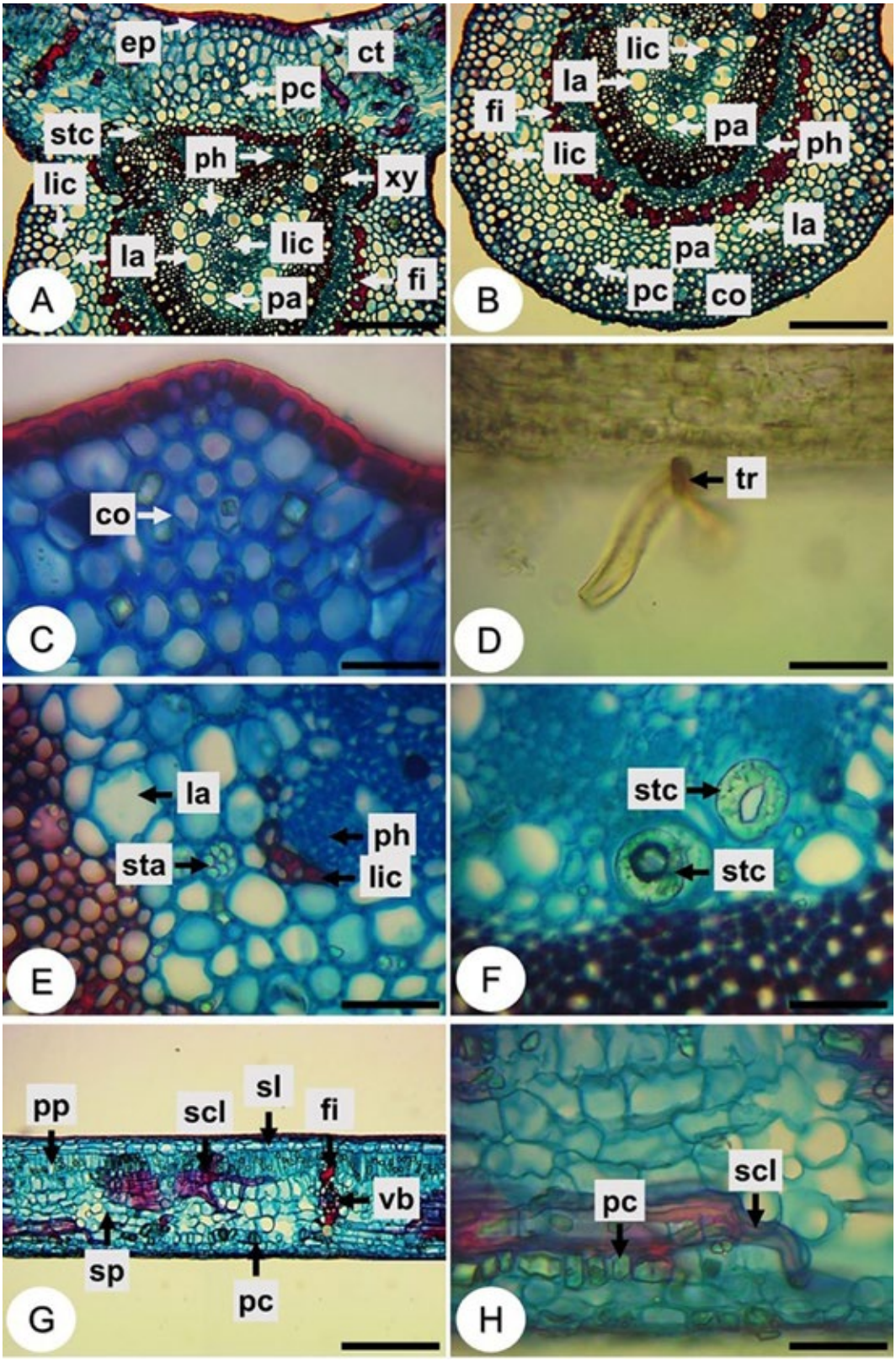

FIGURE 4 - Cross-sections of the leaf blade of *Manilkara zapota* (L.) P.Royen (Sapotaceae). A, B, C, E, F: midrib. D, G, H: mesophyll. Abbreviations: co = collenchyma; ct = cuticle; ep = epidermis; fi = fiber; la = laticifer; lic = lignified cell; pa = parenchyma; pc = prismatic crystal; ph = phloem; pp = palisade parenchyma; scl = sclereid; sl = subepidermal layer; sp = spongy parenchyma; sta = starch; stc = stone cell; tr = trichome; vb = vascular bundle; xy = xylem. Bars: A, B, G = 200 µm; C, D, E, F, H = 50 µm.

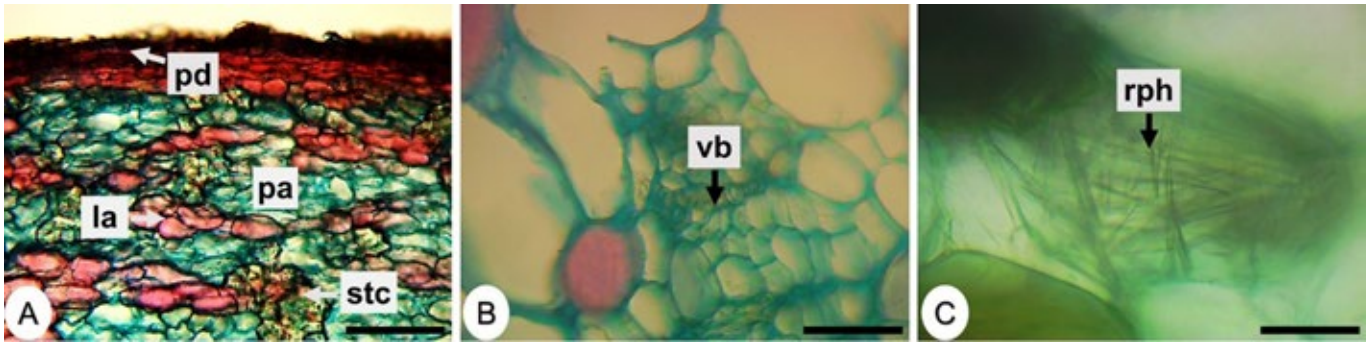

FIGURE 5 - Cross-sections of the fruit of *Manilkara zapota* (L.) P.Royen (Sapotaceae). A, B: general organization of the structures. C: detail of raphides. Abbreviations: la = laticifer; pa = parenchyma; pd = periderm; rph = raphide; stc = stone cell; vb = vascular bundle. Bars: A = 200 µm; B, C = 20 µm.

layer, as well as in the palisade and spongy parenchyma (Figure 6D). Triterpenes and steroids were visualized in the epidermal cells (Figure 6E).

Lipophilic compounds were observed in cell inclusions and in the cuticle that covered the epidermis (Figure 6F). In the midrib, the presence of starch grains was revealed in the parenchymatic cells (Figure 6G); lignin in the xylem, in the lignified cells and in the sclerenchymatic fibers (Figure 6H). The test to establish the nature of the crystals demonstrated their dissolution, confirming that they consisted of calcium oxalate (Figures 6I and J).

DISCUSSION

The presence of laticifers was the main diagnostic characteristic of the Sapotaceae family (Monteiro, Andreata, Neves, 2007). According to Solereder (1908), in Sapotaceae they are of the articulated type and may be situated in all organs of the plant. In *M. zapota*, they are found in all analyzed parts. In the stem and petiole, laticifers are present in the cortex and in the medullar region. In the stem of *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn. and *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell, they were found only in the cortex (Ayensu, 1972; Silva, 2008).

In the case of the petiole, in *Pouteria grandiflora* (A.DC.) Baehni (Palazzo, Monteiro, 2010) and in another 13 species of *Manilkara* studied by Almeida Jr. *et al.* (2013), laticifers were also verified in the cortex and medulla, while in *Sideroxylon obtusifolium* they were present only in the cortex (Silva, 2008). In the leaf blade of *M. zapota* the laticifers occurred only in the midrib, a characteristic also described by Almeida Jr. *et al.* (2013) for other species of *Manilkara*. However, the presence of laticifers in the mesophyll in Sapotaceae has also been reported in the literature, as in *Synsepalum dulcificum*, *Pouteria bangii* (Rusby) T.D. Penn., *P. caimito* (Ruiz & Pav.) Radlk., *P. gardneriana* (A.DC.) Radlk., *P. grandiflora*, *P. procera* (Mart.) T.D. Penn., *P. salicifolia* (Spreng.) Radlk. and *P. venosa* T.D. Penn. (Ayensu, 1972; Monteiro, Neves, Andreata, 2007).

Other structures characteristic of the Sapotaceae family are the trichomes (Solereder, 1908; Monteiro, Andreata, Neves, 2007). According to Metcalfe, Chalk (1950) they are commonly unicellular, 2-armed, but one of the arms is sometimes reduced or absent. In the present study, these trichomes were only visualized in the leaf blade of *M. zapota*. However, there have also been reports of trichomes in the stem, petiole and leaf blade of *Sideroxylon obtusifolium* (Silva, 2008), in the petiole and leaf blade of *Synsepalum dulcificum* (Ayensu, 1972) and species of *Pouteria* (Monteiro, Neves, Andreata, 2007), and in petioles of species of *Manilkara* (Almeida Jr. *et al.*, 2013).

The occurrence of trichomes in the leaf blade of *M. zapota* is divergent in the literature. Jorge *et al.* (2005) reported the absence of trichomes, while Nagani, Kaneria, Chanda (2012) described the presence of unicellular trichomes. In this study, as was also found by Nagani, Kaneria, Chanda (2012), unicellular trichomes were visualized, however, it is worth noting that it was difficult to observe them in the slides after the discoloration procedure with sodium hypochlorite and staining with safranin and Astra blue. It was only possible to obtain images of the trichomes in cross-sections of fresh leaves that were not submitted to the previously mentioned methods.

With respect to the crystals, two types were identified in the studied organs of *M. zapota*: prismatic crystals were present in stem, petiole and leaf blade, while raphides were present in the fruits. According to Metcalfe, Chalk (1950), in Sapotaceae, the crystals may occur in solitary, clustered, or in crystal-sand form. Almeida Jr. *et al.* (2013)

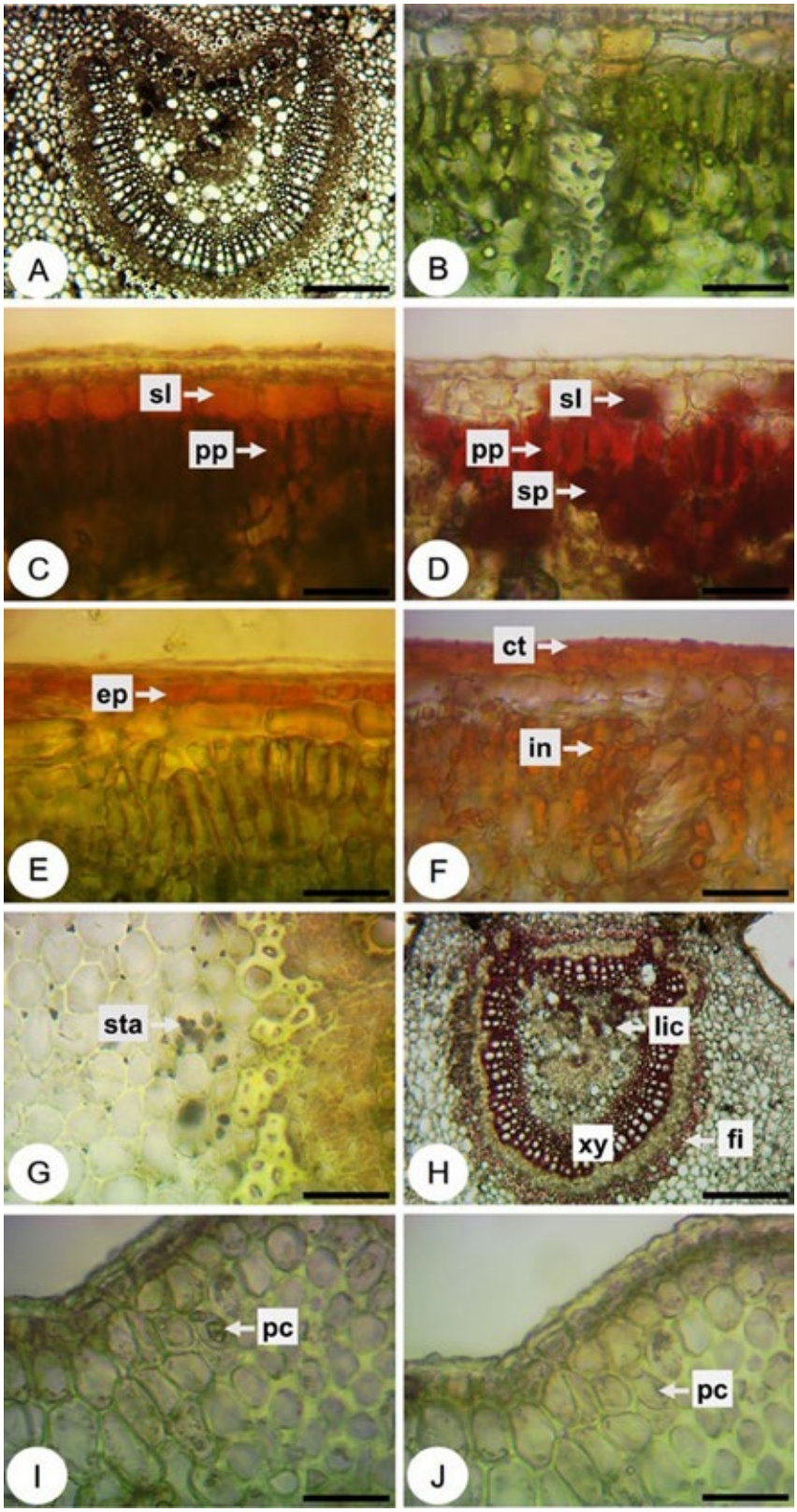

FIGURE 6 - Cross-sections of the leaf blade of *Manilkara zapota* (L.) P.Royen (Sapotaceae) – histochemistry. A: midrib. B: mesophyll. C: potassium dichromate (10%). D: vanillin hydrochloric acid. E: antimony trichloride. F: Sudan III. G: Lugol's iodine reagent. H: phloroglucinol. I, J: hydrochloric acid (10%). Abbreviations: ct = cuticle; ep = epidermis; fi = fiber; in = inclusion; lic = lignified cell; pc = prismatic crystal; pp = palisade parenchyma; sl = subepidermal layer; sp = spongy parenchyma; sta = starch; xy = xylem. Bars: A, H = 200 µm; B, C, D, E, F, G, I, J = 50 µm.

cited that the presence of prismatic crystals in the petiole and leaf blade was a character for differentiation of the species of *Manilkara*, since, of the 13 species studied, only 4 showed prismatic crystals. Silva (2008) reported druses in the petiole of *Sideroxylon obtusifolium*. Relative to the location of the crystals, in the stem and petiole of *M. zapota*, they appeared only in the cortex, while in the stem of *Synsepalum dulcificum* and species of *Madhuca*, *Pouteria* and *Sideroxylon* they occurred in the cortex and medulla (Metcalfe, Chalk, 1950; Ayensu, 1972).

In the stem of species of Sapotaceae, the secondary phloem usually contained fibers (Metcalfe, Chalk, 1950), as found in this study of *M. zapota,* and by Ayensu (1972) and Silva (2008) for *Synsepalum dulcificum* and *Sideroxylon obtusifolium*, respectively.

Almeida Jr. *et al.* (2013) demonstrated that in common, the petioles of species of *Manilkara* presented a thick cuticle, uniseriate epidermis, sclerenchymatic fibers surrounding the vascular bundle and laticifers present in the cortical and medullary regions. In contrast, the shape of the petiole and the petiole vascular bundle conformation were characteristics that presented variations among the species of *Manilkara*. The presence of a nucleus of phloem in the medullary region was also described in *Pouteria grandiflora* by Palazzo, Monteiro (2010).

Anomocytic stomata, thick cuticle and dorsiventral mesophyll are common features in the leaf blades of species of *Manilkara* (Metcalfe, Chalk, 1950; Almeida Jr. *et al.*, 2013). With respect to the adaxial surface of the leaf blade, some species of the genus have only one epidermal layer, while others may be biseriated, as in the case of *Manilkara zapota*, *M. salzmannii* (A. DC.) H.J. Lam, *M. dardanoi* Ducke and *M. rufula* (Miq.) H.J. Lam (Almeida Jr. *et al.*, 2013). Metcalfe, Chalk (1950) described this subepidermal stratum as a hypodermis.

According to Monteiro, Neves, Andreata (2007), the occurrence of biseriated palisade parenchyma associated with the presence of a subepidermal layer and thick cuticle refers to species with xeromorphic characteristics. This may be related to the fact that the species of the genus *Manilkara* are distributed in different types of vegetation, with greater representativeness in areas of the Atlantic Forest and "restinga" (Almeida Jr., 2010).

Esau (1997) stated that some fruits developed suber from a phellogen of subepidermal origin and this phenomenon always occurred in some species; while in other species, this depended on the environmental conditions. Considering that the development of phellogen in the coating of fruits is rare (Jorge *et al*., 2005), the presence of the periderm in the fruit of *M. zapota* makes it an important diagnostic element.

Few histochemical studies with leaves of Sapotaceae species were found in the literature. Silva (2008) also observed the presence of lignin in the xylem and in sclerenchymatic fibers; tannins in the palisade and spongy parenchyma, and lipophilic compounds in the cuticle of the leaf blade of *Sideroxylon obtusifolium*. However, the cited authors did not identify starch grains and demonstrated the presence of phenolic compounds in both parenchyma and in the phloem, differing from the data found here for *M. zapota*.

Some phenolic compounds and terpenes have previously been isolated and identified from leaves of *M. zapota* cultivated in Egypt. Extracts from these leaves exhibited antihyperglycemic, hypocholesterolemic and antioxidant activities (Fayek *et al*., 2012).

CONCLUSION

Through the microscopic analysis, common characteristics were observed in the Sapotaceae; and anatomical characters useful in the diagnosis of *M. zapota* were found, such as the occurrence of laticifers in the cortex and in the medullary region of stem and petiole; presence of prismatic crystals in the stem, petiole and leaf blade, and raphides in fruits; presence of unicellular trichomes and subepidermal layer in the leaf blade, and periderm in the fruit. The histochemical study showed the location of phenolic compounds, tannins, triterpenes, steroids, lipophilic compounds, starch grains, lignin and calcium oxalate crystals in the leaf blade. The information found here helped with the pharmacobotanical standardization of the species studied.

ACKNOWLEDGMENT

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

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Received for publication on 18th April 2017
Accepted for publication on 28th May 2018