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Morpho-anatomical evaluation of hypoglycaemic medicinal plants

Avaliação morfoanatômica de plantas medicinais hipoglicemiantes

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This study aimed to morpho-anatomical characterize leaves of plants with hypoglycemic effect used by the Maracanã community of São Luís-MA. It was selected two vegetal species with lack of morpho-anatomical studies, which were *Cissus verticillata* (L.) Nicolson & C. E. Jarvis (Vitaceae) and *Caesalpinia ferrea* Mart. ex. Tul. var. *leiostachya* Benth (Leguminoceae). The botanic material was collected in the Dom Delgado University City, UFMA. It was realized paradermic and transverse sections free hand with blade support, using astra blue and basic fuchsin as colorants. The laminas were observed and photographed in optic microscopic. *Cissus verticillata* presented simple and opposite leaves, with membranous consistency, serrated margin, and acute top and cordate base. The mesophyll is dorsiventral composed by palisade and spongy parenchyma. Central rib with presence of reserve parenchyma and angular chollenchyma with some points of ring deposition. *Caesalpinia ferrea* presented smooth and simple leaves, uniseriate epidermis, curled cuticles and presence of stomata on both sides. Central rib with fundamental parenchyma composed by spherical cells and angular chollenchyma. The reported morphoanatomic characters of *C. verticillata* and *C. ferrea* leaves contribute to the identification and characterization of the quality of these vegetables, improving knowledge verification of plant species authenticity.

Key-words: Hypoglycemic medicinal plants, morpho-anatomical.

Este estudo objetivou caracterizar morfoanatomicamente folhas de plantas com efeito hipoglicemiante utilizadas pela população do bairro Maracanã de São Luís-MA. Selecionaram-se duas espécies vegetais com escassez de estudos morfoanatômicos, as quais foram *Cissus verticillata* (L.) Nicolson & C. E. Jarvis (Vitaceae) e *Caesalpinia ferrea* Mart. ex. Tul. var. *leiostachya* Benth (Leguminoceae). O material botânico foi coletado na Cidade Universitária Dom Delgado, UFMA. Realizaram-se secções paradérmicas e transversais a mão livre com auxílio de lâmina, utilizando-se azul de astra e fucsina básica como corantes. As lâminas foram observadas e fotografadas em microscópio óptico. *Cissus verticillata* apresentou folhas simples, opostas, com consistência membranácea, margem dentada, ápice agudo e base cordada. O mesofilo é dorsiventral constituído por parênquima paliçádico e lacunoso. Possui nervura central com presença de parênquima de reserva e colênquima angular com alguns pontos de deposição anular. *Caesalpinia ferrea* apresentou folhas lisas, simples, epiderme unisseriada, cutículas onduladas e presença de estômatos em ambas as faces. Nervura central com parênquima fundamental constituído de células esféricas e colênquima angular. Os caracteres morfoanatômicos das folhas de C. *verticillata* e C. *ferrea* relatados contribuem para a identificação e caracterização da qualidade destes vegetais, aprimorando o conhecimento da verificação de autenticidade de espécies vegetais.

Palavras-chave: Plantas medicinais hipoglicemiantes, morfoanatomia.

1. INTRODUCTION

Since ancient times, plants are therapeutic resources within the reach of human beings. For millennia, man empirically deepened their knowledge aimed at improving the supply conditions and cure their illnesses, demonstrating a close interrelationship between the use of plants and their evolution, and the knowledge passed down from generation to generation [1].

The interest in plants with hypoglycemic properties has evolved with broad prospects and the first concern relates to quality/authenticity of such plants, in order to avoid tampering, frequent

falsifications and toxicity. Only through adequate control of the plant drug quality it is possible to ensure the necessary effectiveness and safety of pharmaceuticals, cosmetics and related prepared from herbal drugs. However, not all herbal drugs have established parameters that can contribute to the quality control [2, 3].

The authenticity of plant material has usually been evaluated by the botanical identity parameters, by macro and microscopic tests, based on the comparison with an authentic standard sample, pharmacopoeia descriptions and/or specialized literature. Also, by the presence of active and/or characteristic chemical constituents of the species [4].

In the macroscopic evaluation there are reference compendiums to assist the qualified professional in comparisons, based on the characteristic elements identification of species under study. It is essential to define structures that allow differentiation of medicinal plant species from those most frequently found as adulterants [3, 4]. Microscopic evaluation can be based on structures investigation of epidermis (hairs, glands, stomata), parenchyma (fill, xylem, phloem), crystals, vessels, fibers, starch, and among others. In addition, can be performed histochemical reactions [4].

Diabetes Mellitus (DM) is the term used to describe a metabolic disorder with multiple etiologies, characterized by chronic hyperglycemia resulting from disturbances in the metabolism of carbohydrates, lipids and proteins, which are result of impaired secretion and/or action of insulin, or both [5].

In a study conducted by our research group on the ethnobotanical survey of hypoglycemic medicinal plants in Maracanã neighborhood, São Luís - Maranhão, Brazil, were mentioned 27 plant species used with hypoglycemic purpose, and the medicinal plants most cited by respondents were pata-de-vaca (*Bauhinia forficata* Link), corresponding to 19.3%, vegetable insulin (*Cissus verticillata*) with 15.3% and noni (*Morinda citrifolia* L.) with 9.6%, still the stick iron (*Caesalpinia ferrea*) had 3.4% of citations [6].

In some of plant species already exist *in vivo* and *in vitro* studies showing the ability of those present in hypoglycemic action and pharmacobotany characterization studies of their pharmacogens. However, morphological and anatomical data for some of the above species *Cissus verticillata* (L.) Nicolson & C. E. and *Caesalpinia ferrea* Mart. ex. Tul. var. *leiostachya* Benth are still scarce, although the pharmacological importance of each is well defined.

Cissus verticillata (L.) Nicolson & C. E. Jarvis is a known popularly as “anil trepador”, “uva brava”, “cipó-pucá” or “insulina vegetal” in Brazil [7]. Its leaves are used externally against rheumatism, abscesses and infusions of leaves and stems are used in muscle inflammation, hypotension and activating the blood circulation. The species in reference has been widely used by the population for diabetes treatment, and it is known as “vegetable insulin”, giving reason for botanical studies, chemical and pharmacological in Brazil and abroad [7, 8, 9, 10, 11].

Caesalpinia ferrea Mart. ex. Tul. var. *leiostachya* Benth, popularly known as “pau-ferro” in Brazil, it is a tree 10-20 m tall, straight trunks, dark and irregular white spots, very used for afforestation of parks and squares. Its fruits are pods of black-red color, fleshy and indehiscent [12]. The fruits has antidiarrheal, anticatarral and healing properties and the roots are antipyretic [13]. It has been used for diabetes treatment [14]. Other therapeutic properties of this plant include anti-inflammatory, antiulcer [15, 16], analgesic [17], anticancer [18], antibacterial [19] and antihypertensive [20]. Given its importance etnomedicinal, the Ministry of Health in Brazil included this species in the National List of Medicinal Plants important for the Unified Health System [20].

Based on the above and considering the leaves of *C. verticillata* (Insulina vegetal) and *C. ferrea* (Pau ferro) has abundant materials referenced by its various properties, including hypoglycemic, it demonstrated the necessity of conducting a morphoanatomic study their leaves, in order to increase knowledge on these plant species used by the Maracanã neighborhood population, São Luís, Maranhão, Brazil.

2. BOTANICAL IDENTIFICATION, MORPHOLOGICAL AND ANATOMICAL STUDIES

The *Cissus verticillata* and *Caesalpinia ferrea* species were selected by the lack of morphoanatomics studies in these species. The samples were collected and used for botanical identification, morphological and anatomical studies. The species were collected in the Horto Medicinal Berta Langes de Morretes of Dom Delgado University City, Federal University of Maranhão (UFMA) and identified in the Herbarium Atticus Seabra (SAH) from the same institution. The herbarium specimens are deposited in SAH under number 00834 for *C. ferrea* and number 01251 for *C. verticillata*. synonyms in the botanical nomenclature of each species were surveyed in Tropicos site [21], linked to the Missouri Botanical Garden.

For external morphological study, four leaves of each species were observed as to phyllotaxy, composition, color, consistency, the leaf surface, size, shape, apex, base, margin and veining system [22]. For the analysis of the internal organization of the adult leaf tissues were carried out cross-sections of the middle region of the leaf, including the midrib, and paradermic sections of both sides obtained a free hand with the aid of cutting blades. The sections were clarified with sodium hypochlorite solution 50%, washed with distilled water, stained with astra blue and basic fuchsin, both at 0.5%. Then mounted between slide and cover slip with 50% glycerin. Descriptions and photomicrographs were taken in an optical microscope Olympus CH30 coupled to digital camera Samsung SDC - 415.

3. RESULTS AND DISCUSSION

The leaves of *Cissus verticillata* are simple, opposite, with membranous consistency, ovate shape limbo, toothed margin, acute apex and cordate base (Figure 1A). These macroscopic characteristics corroborate the studies by Lorenzi & Matos (2002) [23] and Souza & Lorenzi (2005) [24]. Also, showed simple tendril with cylindrical format, and a green color (Figure 1B). This structure is the result of total or partial modification of leaf, stem or root. These tendrils allow launch and assisting in the plant support [25].

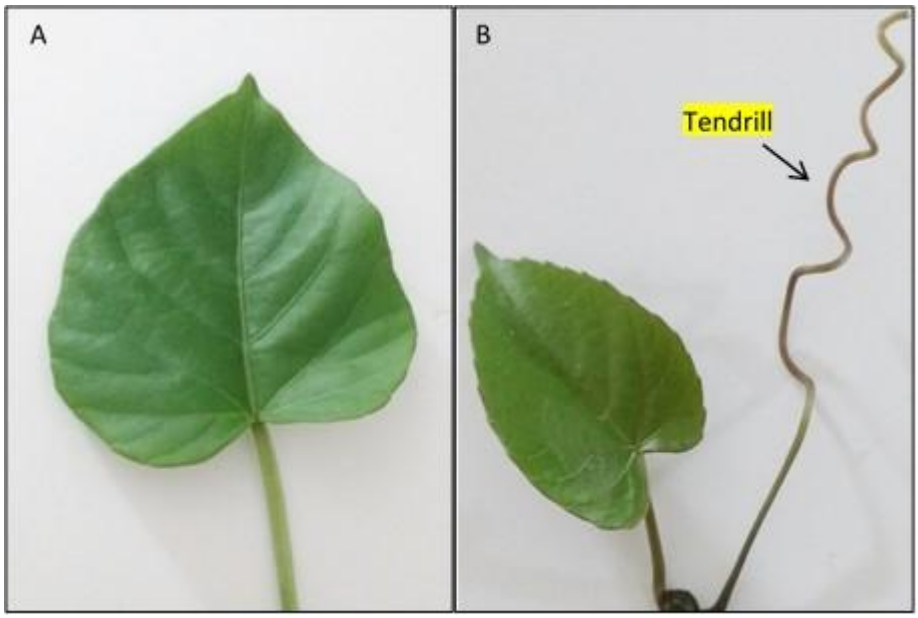

Figure 1: Cissus verticillata (Vitaceae). A: Leaf Blade, B: General view of the leaf tendril branch.

In cross section, the species *C. verticillata* showed the leaf blade with uniseriate epidermis, and presence of cuticles and absence of trichomes (Figure 2A). In morphoanatomic study of some species of *Cissus*, the presence of trichomes was evident [26]. The cuticles reduce water loss through transpiration, present an additional barrier when in contact with the dirty air inlet

and pathogens into the interior of leaves, representing a major adaptation developed for terrestrial plants [27, 28].

In the central rib *C. verticillata* we observed the presence of reservation parenchyma (aerenchyma) and angular collenchyma with some points of annular deposition (Figure 2A). According Seago et al. (2005) [29], the aerenchyma formation occurs by expanding intercellular spaces of gaps, division and cell expansion or, less often, by destroying or lysing the cell. The chollenchyma of void type develops from the angle, the latter being the most common [40].

The mesophyll is dorsiventral, which consists of a palisade layer, followed by four to five layers of spongy parenchyma (Figure 2B). In the middle of parenchyma occurs idioblasts (Figure 2B), in presence of calcium oxalate crystals in the form of drusen or raphides. According to Fahn (1990) [30], idioblasts can secrete various substances, being tannins, mucilages, oils, essences, and among others. The identification of idioblasts as local secretion of these compounds, responsible for potential activity of the plant, is very important for the future of bioprospecting research on this species.

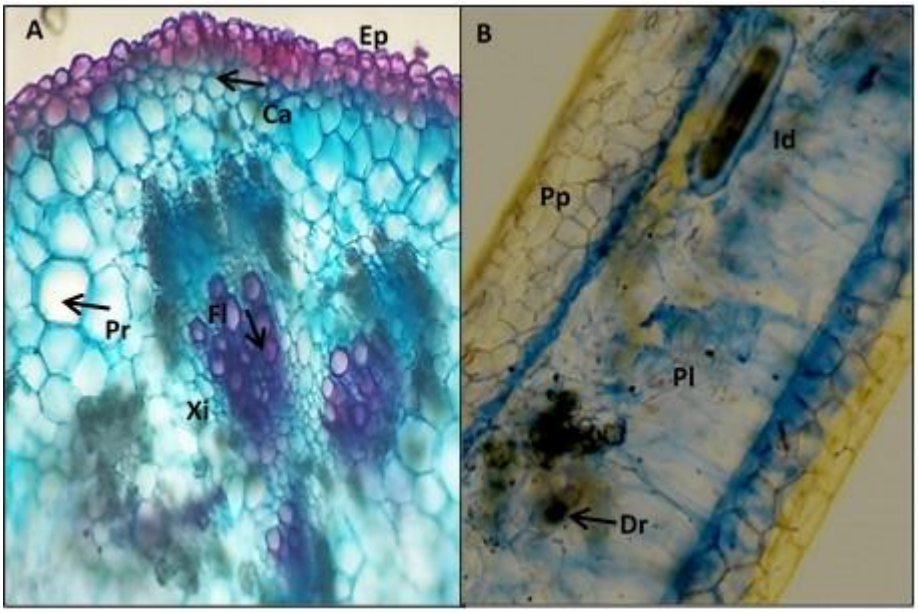

Figure 2: Cross section of leaf of Cissus verticillata. A: Central Vein B: Mesophyll. Ca: Angular Chollenchyma; Dr: Druze; Ep: Epidermis; Fl: Phloem; Id: idioblast with raphides; Pr: Reserve Parenchyma (aerenchyma); Pp: Palisade Parenchyma; Pl: Spongy Parenchyma; Xi: Xylem.

The leaves of *C. verticillata* is amphistomatic with stomata of anomocytic type (Figure 3). The epidermis of abaxial surface (Figure 3A) has a higher concentration of stomata in relation to adaxial (Figure 3B). In the study by Greulach (1973) [31], it was reported that amphistomatic leaves usually have higher amount of stomata in the epidermis of abaxial face, but in some species the stomata appear in the same proportions. However, it is noteworthy that such a characteristic is greatly influenced by environmental issues, where plants grow in full sunlight have more stomata than those growing in the shade and the presence of the highest concentration in the abaxial face can be a way to avoid losing excessive water [32], and increasing the photosynthetic efficiency [28].

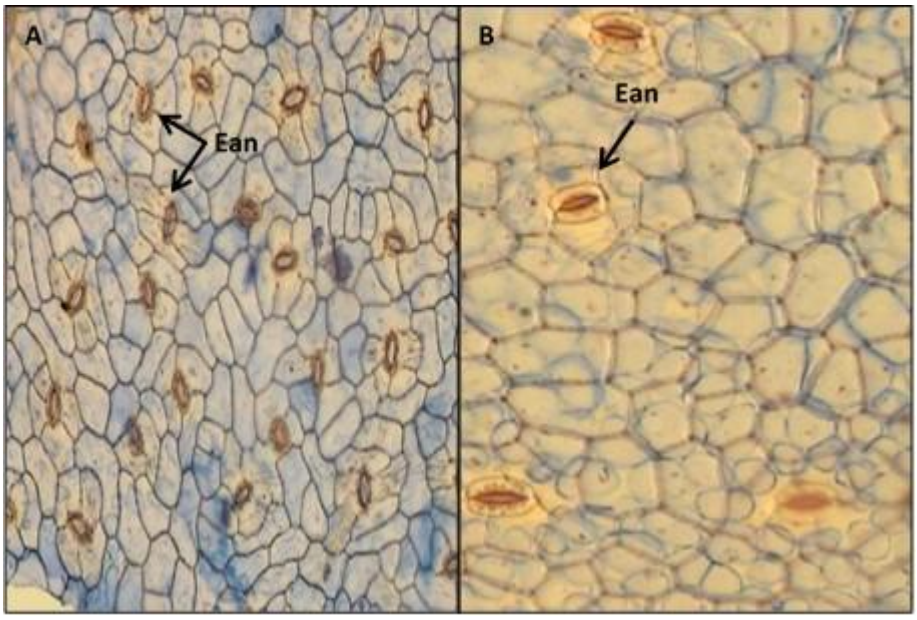

Figure 3: Cross section of the leaves of Cissus verticillata. A: abaxial face, B: adaxial side. Ean: anomocytic stomata.

The leaves of *Caesalpinia ferrea* are simple, smooth, opposite or alternate, opaque, and discolores. Have symmetrical based asymmetric, rounded to cuneate and rounded apex, with entire flat edge (Figure 4). They presented uniseriate epidermis, with cuticles in wavy aspect. These aspects were also observed in studies of Bortoluzzi et al. (2007) [33].

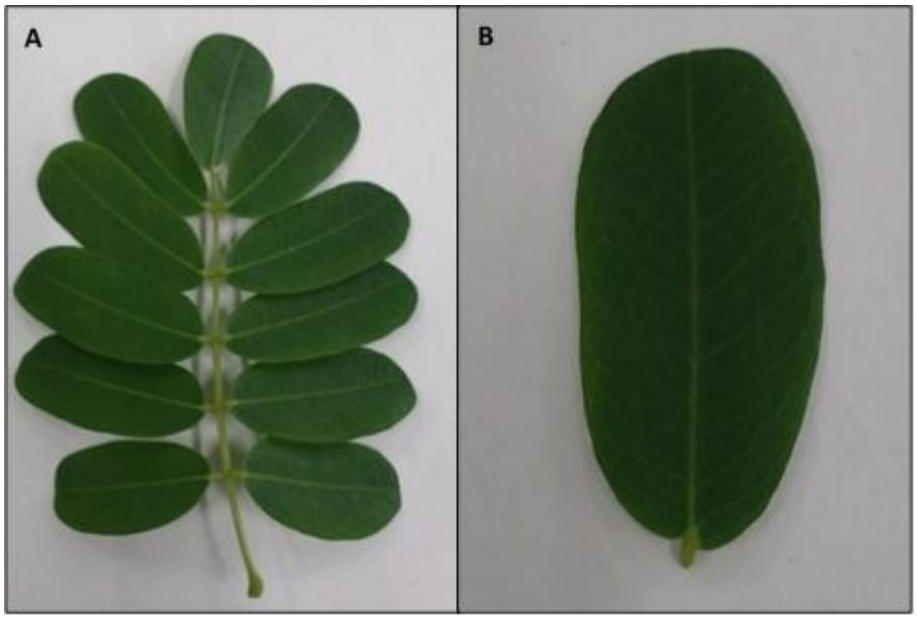

Figure 4: Caesalpinia ferrea (Leguminosae). A: general appearance of the branches, B: leaf blade.

In transverse sections, the *C. ferrea* leaves have basic parenchyma or fill cells with spherical and angular collenchyma (Figure 5A). There is the presence of trichomes (Figure 5B) which are common in the genus, and the species can present simple trichomes, glandular, peltate or branched [34]. The trichomes form, usually a dense covering, may serve as a mechanical barrier against various external factors, such as herbivores and pathogens, ultraviolet radiation, extreme heat and excessive water loss. While the glandular trichomes are involved with chemical protection by release of lipophilic substances [35].

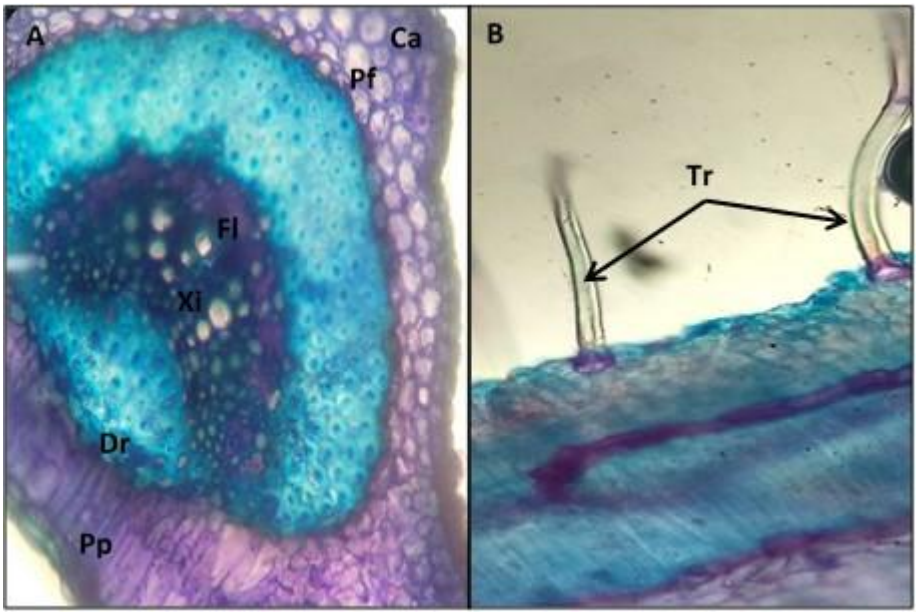

Figure 5: Cross section of leaf of Caesalpinia ferrea. A: Central rib B: mesophyll. Ca: angular chollenchyma; Dr: Druze; Ep: epidermis; Fl: phloem; Mp: fundamental parenchyma with spherical cells; Pp: palisade; Pl: spongy parenchyma; Tr: trichomes; Xi: xylem.

The mesophyll of *C. ferrea* is dorsiventral, compact, has a typical palisade parenchyma located along the adaxial epidermis (Figure 6). The parenchymal cells facing the abaxial epidermis with small intercellular spaces are similar to the palisade and also have a sclerenchymatous sheath. According to Metcalfe & Chalk (1950) [36] and Watson (1981) [37], the mesophyll in Caesalpinioideae is generally dorsiventral, proven characteristic by Cuttis et al. (1996) [38]. The palisade parenchyma allows better penetration of light in chloroplasts. Thus, it is believed that leaves exposed to sun, with the presence of palisade parenchyma well developed, has an efficient structure in terms of photosynthesis [39]. Another environmental factor that may relate to structural changes in the mesophyll is the availability of water. Plants of dry environment (xerophytes) have palisade parenchyma more developed than spongy. Already hydromorphic leaves appear as large common character intercellular spaces and mesophilic feature combinations of these two environments [40].

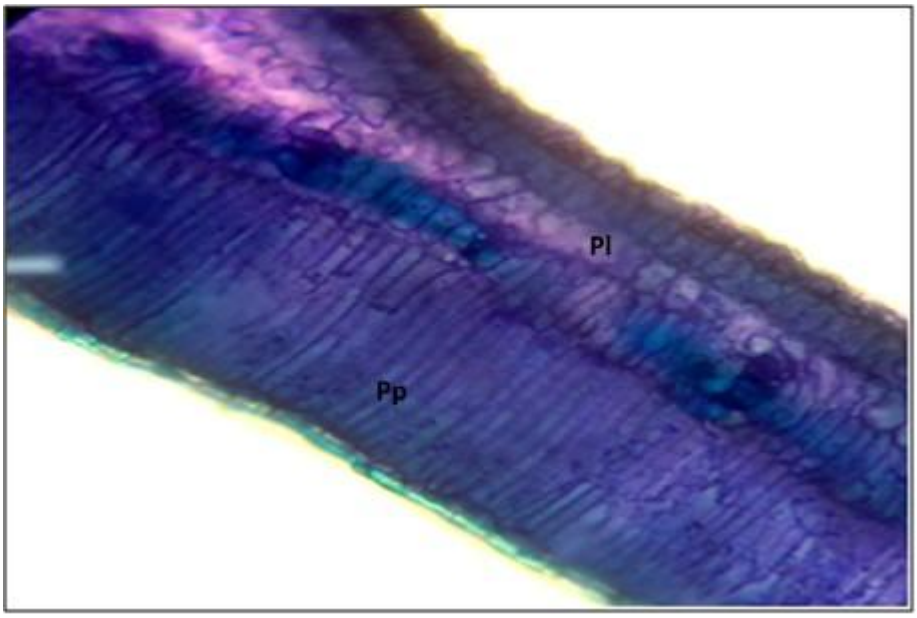

Figure 6: Cross section of mesophyll region of Caesalpinia ferrea. Pp: palisade parenchyma; Pl: spongy parenchyma.

The paradermic cut of *C. ferrea* indicated the stomata presence of paracitic type (Figure 7) on leaves both sides and the abaxial surface (Figure 7A) is displayed in a larger quantity, classified as amphistomatic. The paracytic stomata of *C. ferrea* are typical of his family [41]. In the most of Caesalpinioideae, the stomata are mostly found in abaxial face, and in the adaxial face are scarce and restricted to areas adjacent to ribs [36, 37].

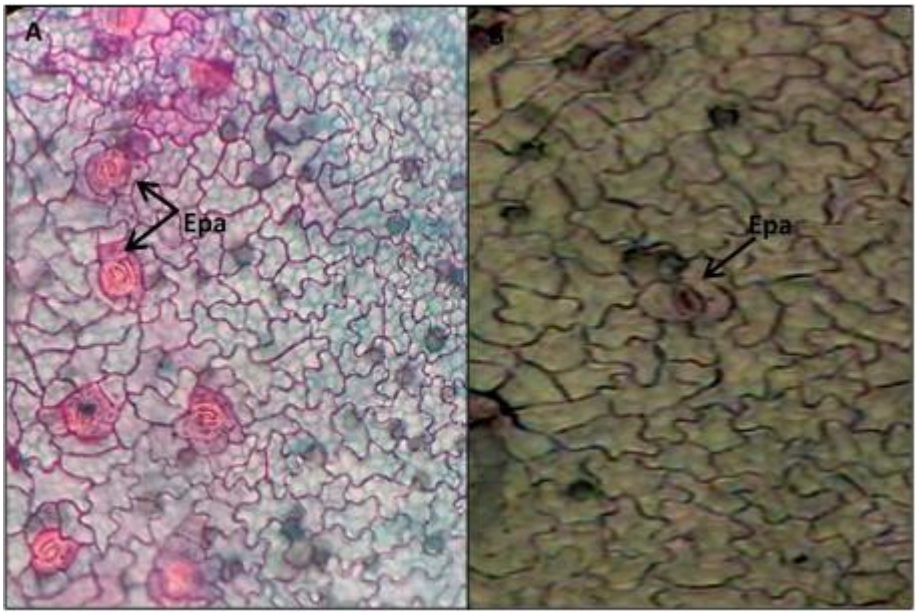

Figure 7: Cross section of the leaves of Caesalpinia ferrea. A: abaxial side, B: adaxial side. Epa: paracytic stomata.

4. CONCLUSION

Morphological and anatomical features of the leaves of *C. verticillata* and *C. ferrea* reported contribute to the identification and characterization of vegetables quality, improving the knowledge of these species in the area of pharmacobotany. Become necessary studies to ensure the quality of plant and herbal drug, as well as and the application of methods to assess the authenticity, since macroscopic and microscopic characteristics are taken into account as authenticity parameter. Given the above, the importance of knowledge pharmacobotanic is associated with three factors: authenticity check of plant species; elaboration of monographs for incorporation species on official books, such as Pharmacopoeias, and quality control of commercial samples.

5. ACKNOWLEDGMENTS

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