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ANATOMICAL CHARACTERIZATION AND MICROCHEMISTRY OF *PEPEROMIA PELLUCIDA* (L.) H.B.K. (PIPERACEAE)

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ABSTRACT: *Peperomia pellucida* L. (H.B.K.) is an arboreal species occurring in the secondary vegetation in Amazonia. This study aims to perform anatomy analysis by light microscopy and microchemistry analysis of leaves and petiole of *P. pellucida*. It was observed that the cells, on the adaxial surface, are juxtaposed with irregular shape and heterodimensional and straight walls and, the abaxial surface is formed by juxtaposed and irregularly shaped cells with thin and heterodimensional walls. The microchemical tests showed the presence of secondary metabolites, mainly in the palisade tissue, such as alkaloids, flavonoids and starch. Such anatomical and microchemical features are fundamental in the characterization of the species.

INTRODUCTION: About ten genus are included in the family Piperaceae but *Piper* and *Peperomia* are the main ones in Brazilian flora¹, and there are approximately one thousand and four hundred species in this family. This family has a pantropical distribution whose representatives are predominantly herbaceous.

Piperaceae species have always been of ornamental interest due to its gaudy foliage.

Besides ornamental interest, the species of this family also arouse curiosity because they have characteristics of both Magnoliopsida and Liliopsida², such as the tetracytic stomata^{3, 4} and the stem with a monostelic vascular system².

Several species of the genus *Peperomia* representing more than half of the family have leaves with water reservoir specialized tissue, with a high degree of succulence, which changes considerably the leaf morphology⁵.

It is a tissue that also works as a light filter⁶, conveying to the mesophyll only 70% of the light falling on the leaf⁷.

Once it is an epiphytic species, it has the ability to store water^{8, 9}.

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The species *Peperomia pellucida* L. (H.B.K.) is an herbaceous plant found in many South American and Asian countries. It is used in Belém-Pará in folk medicine as an anti-inflammatory, antimicrobial and diuretic. However, there is a lack of information about the anatomy of its vegetative organs. According to the current legislation, obtaining any medicinal plant-derived, a herbal medicine, for example; quality control is required since cultivation, management, collection of the plant species, manufacturing of the intermediate products and achievement of the formulation¹⁰. Thus, this paper aims to study the anatomy and microchemistry of vegetative organs of *P. pellucida* to establish salient features to assist pharmacobotanical studies.

MATERIALS AND METHODS:

Collection and botanical identification of plant material: The plant material was obtained from the Associations of Herb Vendors Ver-o-Peso Market (Ver-as-Ervas[®]), metropolitan area of Belém-Pará,

Icoaraci district. Species identification was performed by the trusteeship of Paraense Emílio Goeldi Museum and Herbarium, where a voucher specimen is deposited under the MG registration number: 191457.

Light microscopy: Plant material was fixed in FAA₇₀¹¹ for 48 hours and preserved in alcohol 70⁰ GL. For sections, hydration was processed with gradual reduction of alcohol concentration (70%, 50%, 30%, and distilled water) at intervals of 30 minutes for two hours. The sections were stained with basic fuchsin and Astra blue¹². Sections of 8 µm thick were made with a Jung rotating microtome to make permanent slides. To obtain images of the structures of the material, a photomicroscope, model XSZ-150Ai (Medlux[®]) was used.

Microchemical tests: Free-hand cross sections were made of the leaf blade to verify the presence of substances using the reagents described in **Table 1**¹³.

TABLE 1: MICROCHEMICAL TESTS OF THE LEAVES OF PEPEROMIA PELLUCIDA L. (H.B.K.)

SUBSTANCES	REAGENTS	AUTHORS
Phenolic Compounds	10% Ferric Chloride	14
Alkaloids	Dragendorff	15
Reducing Sugars	Fehling	16
Anthraquinones	5% KOH	15
Starch	Lugol	17

RESULTS AND DISCUSSION: In front view, the cells on the adaxial surface are juxtaposed with irregular shape and heterodimensional and straight walls, in this view the cells are not divided into costal and intercostals, except the midrib (**Figure 1: A and B**), in cross-section, the epidermis is uniseriate with heterodimensional quadrangular cells with straight periclinal and slightly sinuous anticlinal walls (**Figure 1: C**). The abaxial surface is formed by juxtaposed irregularly shaped cells with sinuous heterodimensional walls divided into costal and intercostal zones. Intercostal cells are juxtaposed in a rectangular shape, in cross-section the heterodimensional uniseriate epidermis and cells of irregular shape, and the smooth walls can be observed (**Figure 1: C and D**).

The mesophyll has a palisade tissue layer consisting of juxtaposed cells with oval shape and uniform size, as in the spongy tissue is formed by two or three layers of heterodimensional irregularly

shaped cells with slightly sinuous walls, where there are bulky intercellular spaces (**Figure 1: C**).

The mesophyll has a palisade tissue layer consisting of juxtaposed cells with oval shape and uniform size, as in the spongy tissue is formed by two or three layers of heterodimensional irregularly shaped cells with slightly sinuous walls, occurring bulky intercellular spaces (**Figure 1: C**).

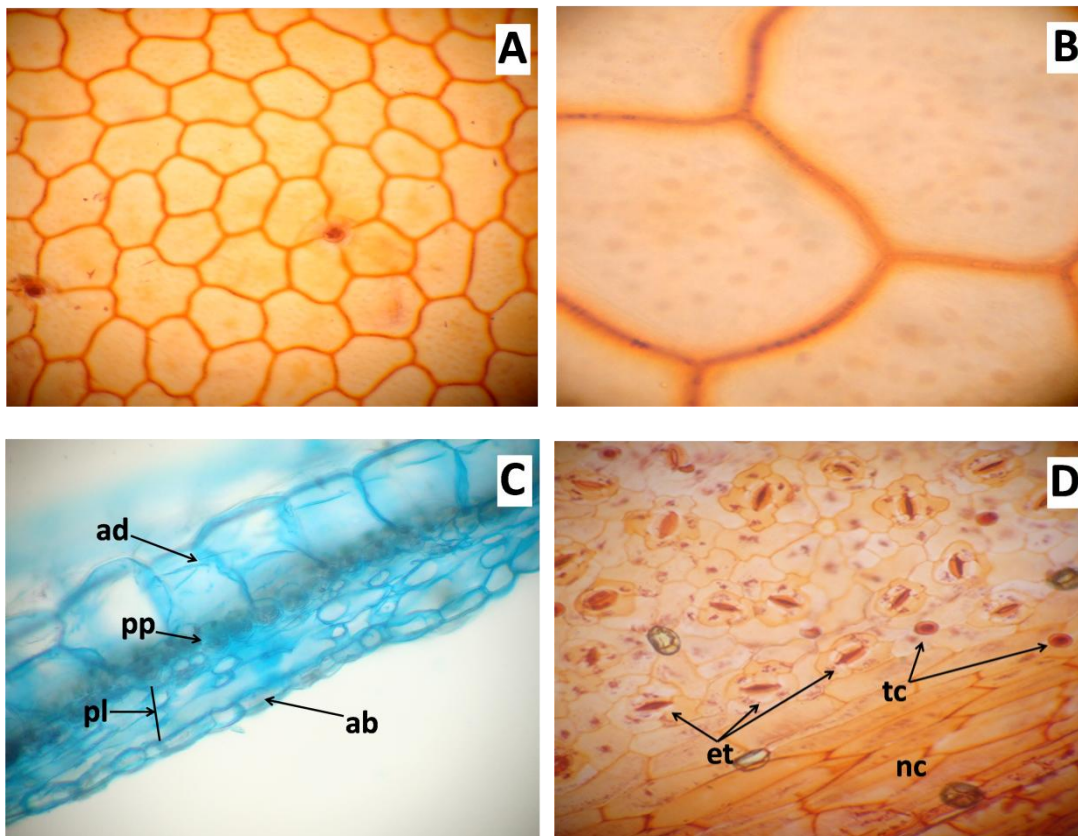
The midrib is abaxially crested and comprises colenquimatic cells. The epidermis in this surface is constituted by a set of colenquimatic cells with a circular shape followed by two or three layers of parenchymatous cells with a flat wall and small intercellular spaces. The vascular tissue is collateral with phloem towards the abaxial surface and xylem towards the adaxial one and we found the occurrence of secretory ducts inside the phloematic tissue (**Figure 1: E and F**).

The leaves are hypostomatic with stomata homogeneously distributed in the leaf in the intercostal areas and on the midrib, the stomatal complex is anomocytic with three or four subsidiary cells, the guard cells are positioned at a higher level than other cells of the leaf (**Figure 2 : A and B**). Presence of glandular trichomes with pearl shape formed by a cell, unicellular, and these are evenly distributed on the adaxial and abaxial surface and on the costal and intercostal zones, occurring in greater numbers on the abaxial surface (**Figure 2: C-F**). Groups of dense crystals of calcium oxalate with rhombic shape occur in primary, secondary and tertiary ribs (**Figure 2: G and H**).

The petiole is half-plane and convex with two ridges of colenquimatic cells, the cortex is composed, from the convex region to the vascular tissue, for four or five layers of colenquimatic cells with a rhomboid shape, smooth and hetero-dimensional walls, and reduced intercellular

spaces. From the vascular to the half-plane tissue, the cortex comprises the parenchyma cells which are juxtaposed, rhomboid with heterodimensional and smooth walls (**Figure 3: A**), and, generally, occur in the convex region and have similar glandular trichomes to those described for the leaf blade (**Figure 3: B and C**).

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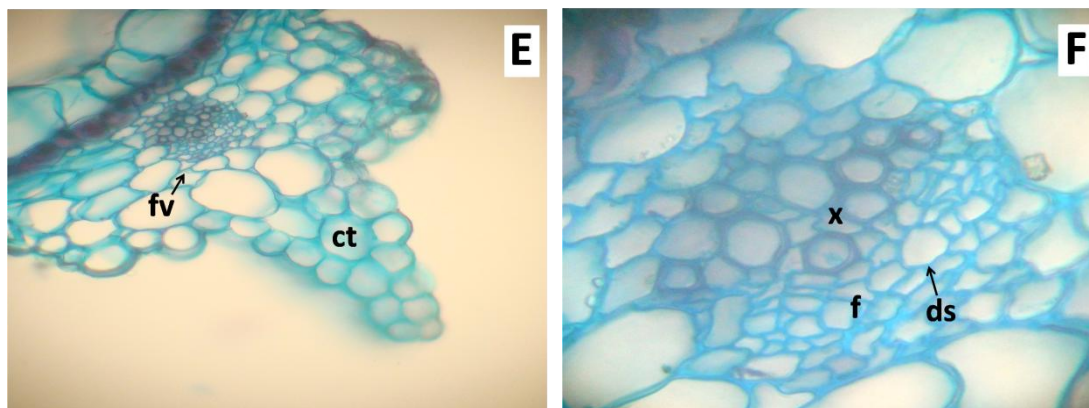


FIGURE 1: **A:** Overview of the adaxial surface; **B:** Detail of the cell walls on the adaxial surface; **C:** Overview of the mesophyll; **D:** Overview of the abaxial surface; **E:** Overview of the midrib; **F:** Detail of the vascular bundle. **ab:** abaxial; **ad:** adaxial; **ct:** crest; **ds:** secretory duct; **et:** stomata; **f:** phloem; **fv:** vascular bundle; **nc:** midrib; **pl:** spongy parenchyma; **pp:** palisade parenchyma; **tc:** trichome.

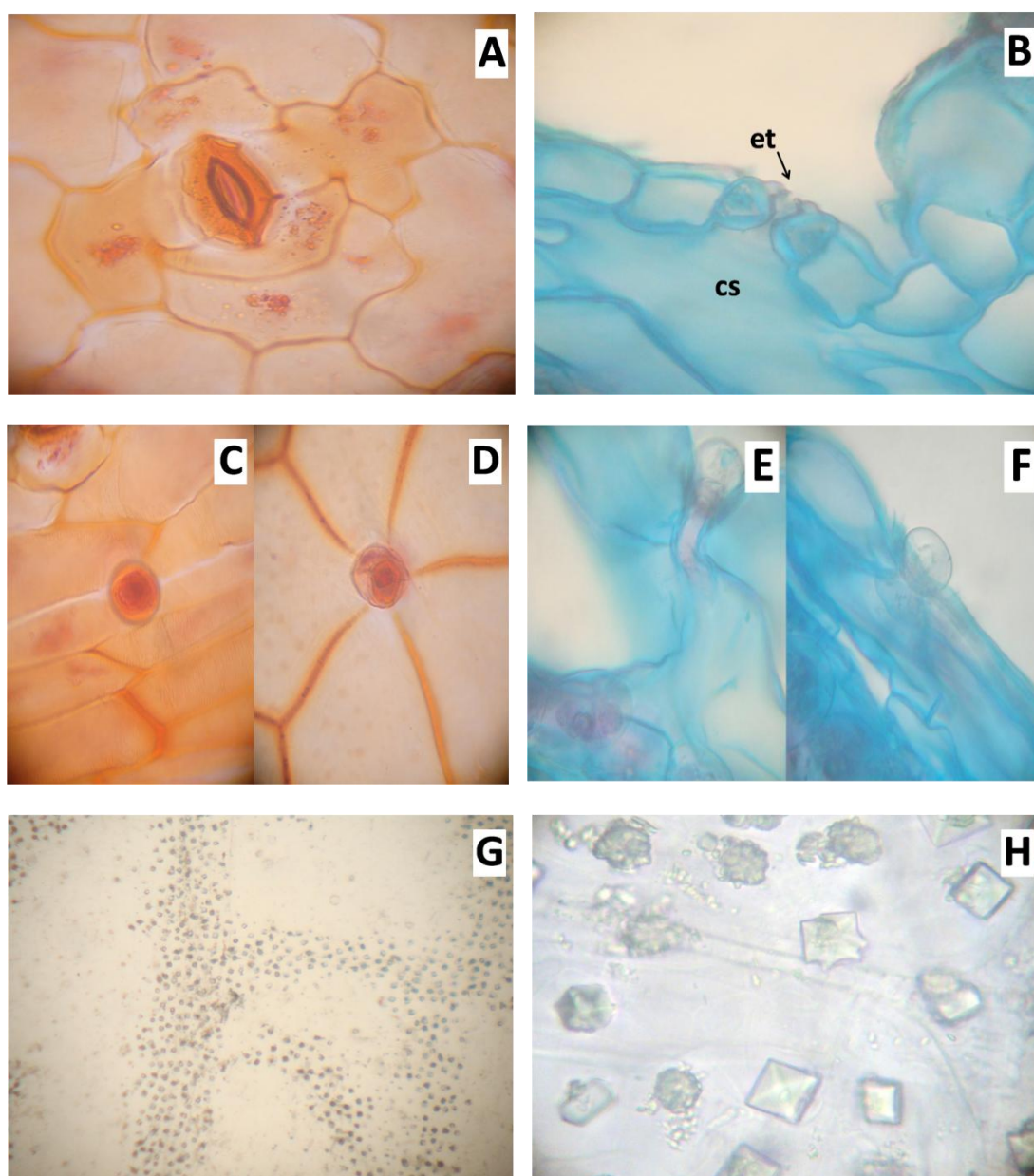


FIGURE 2: **A:** Detail of the stomata (front view); **B:** Detail of the stomata (cross-section); Figures **C-F:** Detail of the trichome; **C and D:** Front view; **C:** Abaxial surface, on the midrib; **D:** Abaxial surface; **E and F:** Detail of the trichome (cross-section); **E:** Adaxial surface; **F:** Abaxial surface; **G:** Overview highlighting the distribution of calcium oxalate crystals; **H:** Detail of the rhombic crystal shape; **cs:** Substomatal chambers; **et:** Stomata.

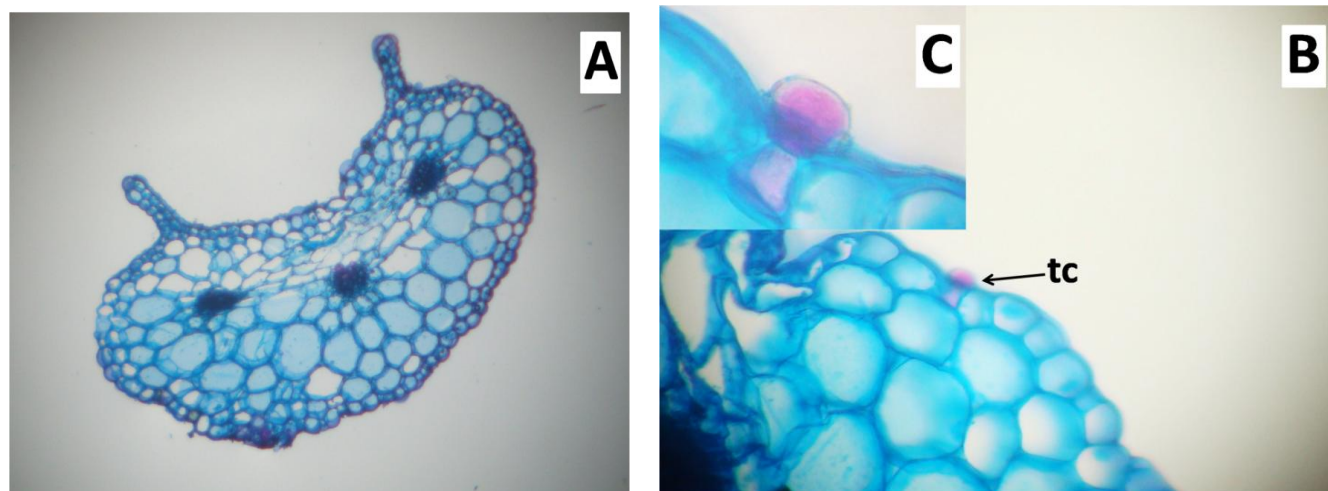


FIGURE 3: A-B: Petiole; **A:** Overview of the cross-section of the petiole; **B:** Highlighting the occurrence of trichome; **C:** Detail of the glandular trichomes; **tc:** trichome.

The microchemical tests showed the presence of products of the secondary metabolism, mainly, in the palisade tissue. Some of these substances were the following: alkaloids, flavonoids and starch (Table 2 e Figure 4: A-F).

TABLE 2: PRODUCTS OF SECONDARY METABOLISM OF *PEPEROMIA PELLUCIDA* L. (H.B.K.), OBSERVED THROUGH THE MICRO-CHEMICAL TESTS

Reagents	Substances	Condition
Ferric Chloride	Phenolic Compounds	Absent
Dragendorff	Alkaloids	Present
Fehling	Reducing Sugars	Absent
KOH	Flavonoids	Present
	Anthraquinones	Absent
Lugol	Starch	Present
Sudan IV	Fatty Substance	Absent

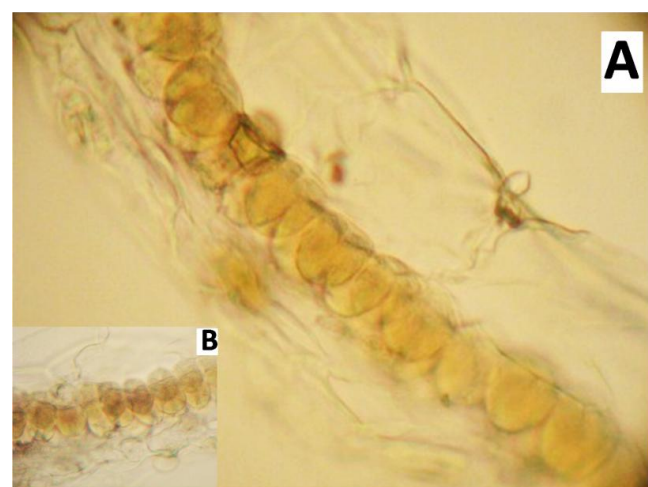
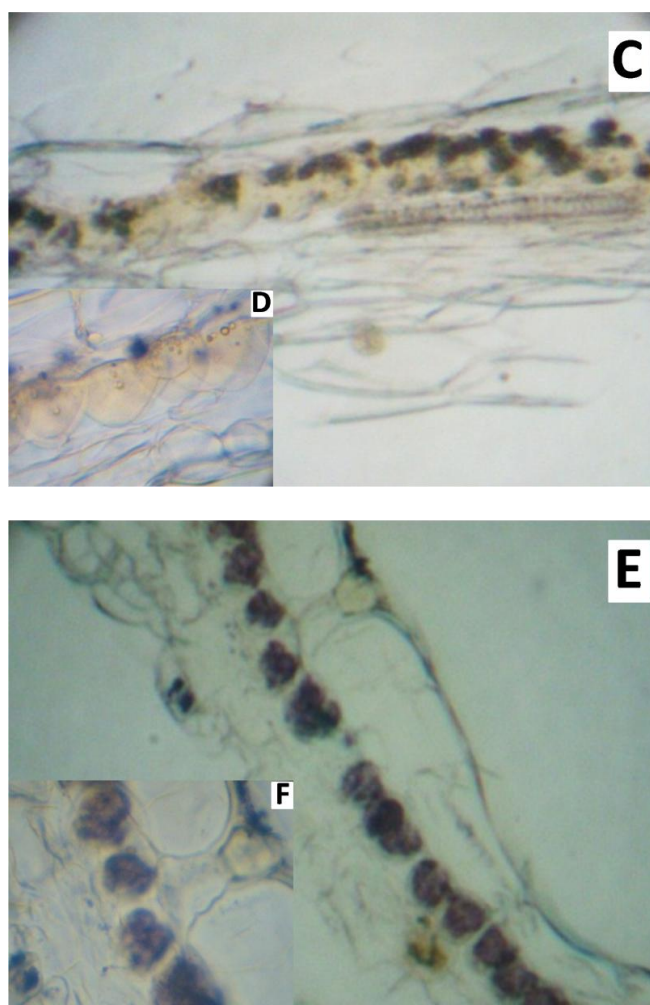


Figure 4: A-F: Microchemical tests; **A and B:** Alkaloids; **C and D:** Flavonoids; **E and F:** Starch.

CONCLUSION: The anatomical characteristics of the leaves and petioles of *P. pellucida* are relevant to determine the authenticity of various compounds of this species responsible for different biological activities.

Microscopically, the presence of different structural formations are features that, when analyzed together, help to control the botanical quality of the plant studied as a pharmaceutical ingredient.

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