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Leaf Epidermal Features for Differentiating *Cecropia pachystachya* Trécul from its Adulterant *Tetrapanax papyrifer* (Hook.) K. Koch

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HIGHLIGHTS

- Microscopy was important in identification and quality control of *C. pachystachya*.
- Epidermal features helped to differentiate between *C. pachystachya* and *T. papyrifer*.
- Trichomes are the main anatomical marker in the differentiation.

Abstract: In the quality control of herbal drugs, analysis of microscopy structures is crucial to detect any adulterants or substitutes. Microscopic analysis is considered one of the first pharmacopeial parameters of pharmacognostic identification. *Cecropia pachystachya* Trécul (Urticaceae), commonly known as “embaúba” or Ambay pumpwood, is a broadleaved tree species native to South America, from Brazil to Argentina. *Cecropia pachystachya* is used in traditional medicine as a diuretic, antiasthmatic, antidiabetic, anti-inflammatory, and anti-hypertensive. Due to the malpractice of adulterating botanicals with other species having similar morphology and therapeutic uses, an unrelated plant *Tetrapanax papyrifer* (Hook.) K. Koch (Araliaceae) is commonly used as an adulteration of *C. pachystachya*. *Tetrapanax papyrifer* was introduced into Brazil and utilized in folk medicine as an antidiarrheal, antitussive, anti-inflammatory, and expectorant. This work aimed to identify and differentiate between the two species using microscopic features of the leaf

epidermis. The presence or absence and the morphotype of trichomes are sufficient to identify and differentiate between these species, in whole or pulverized form.

Keywords: Araliaceae; adulteration; quality control; embaúba; micromorphology; Urticaceae.

INTRODUCTION

Botanicals are commonly adulterated or substituted with other species having similar appearances uses, and common names. In Brazil, the commercialization of adulterated herbal drugs is not uncommon. In this context, detailed studies on the pharmacobotany of medicinal species are essential in the quality control of botanical raw materials. Adulterating or substituting medicinal plants can negatively affect their safety and therapeutic effectiveness [1].

Firstly, for quality control, microscopic analysis is a main parameter to the correct botany identification of medicinal plants, and this procedure allows to identification of the anatomical features of the plant. In this context, quality control is imperative and microscopic analysis is the main parameter to identify the botanical material. Microscopic techniques are considered reasonably fast, cheap and efficient and can be applied in different samples, such as fresh, dried, fragmented or powdered forms [1,2]. For this technique, transversal and longitudinal sections of the plant material can be submitted to dye or exposed to specific reagents to evidence anatomical markers or chemical groups [1,3].

Cecropia pachystachya Trécul is a tree up to 12 m high, with stems measuring 15-25 cm in diameter. The leaves are entire, palmed in form and regularly separated into 9-13 finger-like segments. The leaf blades are rough, hairy, from papery to slightly leathery. It has a grayish-green petiole. It is popularly known as “embaúba” and the leaves are widely used as antitussive, expectorant, antiasthmatic, hypoglycemic, anti-hypertensive, diuretic, anti-inflammatory, cardiotonic, sedative, antioxidant and neuroprotective [4,5]. *Cecropia pachystachya* is often adulterated by some species with the same folk name, morphological similarities and therapeutic uses, such as *Macaranga gigantea* (Rchb.f. & Zoll.) Müll. Arg. (Euphorbiaceae), *Cecropia glaziovii* Snethl and *Tetrapanax papyrifer* (Hook.) K. Koch (Araliaceae) [5,6].

Tetrapanax papyrifer was introduced in the Southern states of Brazil [7] and is known as “embaúba” and rice paper plant. It is a shrub reaching up to 6 m in height. The stem is slightly slender. The leaves are palmed reaching over a 40-60 cm in diameter, are 5-11 lobes, deeply veined, and bright green with a lighter underside. The petiole is long [8]. The leaves and stems are used in traditional medicine as antitussive and antiasthmatic [9]. Pharmacological studies also have reported antithrombotic, antihepatotoxic [10], anticancer [11], analgesic [12], antioxidant [13], diuretic [8], antimicrobial, antiaging and skin whitening properties [14]. However, studies about the microscopic characteristics of *T. papyrifer* are considered scarce.

Considering that *C. pachystachya* and *T. papyrifer* are commonly known as “embaúba”, have similarities in leaf morphology, and both species are commercialized in South America, the present study aimed to provide botanical microscopic markers of leaf epidermis by light and field emission scanning electron microscopy for quality control of the raw material.

MATERIAL AND METHODS

Plant material

Cecropia pachystachya (Figure 1A, C) leaves were collected in Tuneiras do Oeste (latitude 23º 52’ 14” S and longitude 52º 52’ 34” W), Paraná, Brazil, along the road BR-487 (Estrada Boiadeira), in April 2016.*Tetrapanax papyrifer* (Figure 1B, D) leaves were collected in the medicinal garden at the State University of Ponta Grossa (UEPG) (latitude 25°5'40"S and longitude 50°6'5"W), Paraná, Brazil, in July 2022. The specimens were identified by Taxonomist Dr. Inês Janete Mattozo and stored at the Herbarium of the UEPG under the numbers HUPG 22306 (*C. pachystachya*) and HUPG 2376 (*T. papyrifer*). The access to botanical materials was authorized and licensed by the Genetic Heritage Administration Council (CGEN/SISGEN) according to codes A18F30A and AE93A56.

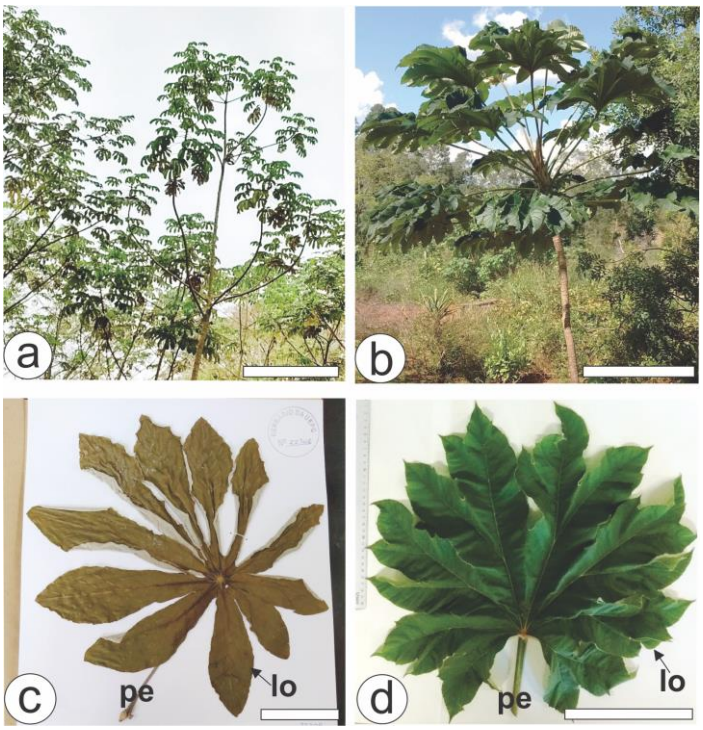

Figure 1. Plant habit and leaves of *Cecropia pachystachya* and *Tetrapanax papyrifer*. a, c: *C. pachystachya*. b,d: *T. papyrifer*. (lo: foliar lobe; pe: petiole). Scale bars: a = 8 m, b = 4 m, c = 27 cm, d = 45 cm.

Anatomical studies

The leaf samples of *C. pachystachya* and *T. papyrifer* were fixed in FAA solution (formalin, acetic acid and alcohol 70%; 5:5:90) for five days [15]. Posteriorly, the material was washed with distilled water and stored in 70% ethanol [16].

The leaf fragments were cleared using sodium hypochlorite 50% for the epidermal study. After total depigmentation of leaves, the material was neutralized with acetic acid (5%, washed with distilled water (6x), and stained in safranin (1%) [17]. The semipermanent anatomical slides were visualized and imaged using an Olympus CX31 optical microscope coupled with an Olympus C-7070 digital camera in the Pharmacognosy Laboratory.

Field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDS)

The samples stored in 70% ethanol were dehydrated by passing them through 80%, 90% and 100% ethanol and dried in a Balzers CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein) supplied with liquid CO_2. Then, the samples were mounted on aluminum stubs and covered with gold using a sputter coater (Quorum, SC7620). The analysis was made by field emission scanning electron microscopy (Mira 3 Tescan – Oxford Instruments, Oxford, UK). During the FESEM procedure, energy dispersive x-ray spectroscopy (EDS) was performed to obtain the chemical composition of the crystals. This analysis was randomly made for the crystals as well as the cells devoid of crystals as a control, using an EDS detector on the same variable pressure microscope at 15 kV. Both FESEM and EDS were performed at the Multiuser Laboratory Complex (C-Labmu) at UEPG.

RESULTS AND DISCUSSION

Cecropia pachystachya leaves, in frontal view, show epidermal cells with straight anticlinal walls on adaxial side (Figure 2 A) and sinuous on the abaxial side (Figure 3 A); anomocytic stomata are observed only on the face, characterizing the leaves as hypostomatic (Figure 3 A, C); non-glandular trichomes with short apex and broad base are found on the face (Figure 2 A, B, C); unicellular non-glandular trichomes with or without broad base are present on both leaf surfaces (Figures 2 A, D, E, F, 3 B); filariform and contorted non-glandular trichomes are observed numerously on abaxial side (Figure 3 B, C); glandular trichomes or pearl bodies with 1-2 celled stalk and a multicellular head are found on abaxial side (Figure 3 D); prismatic crystals, formed by calcium oxalate are present on the face (Figures 2 G, 3 C).

Tetrapanax papyrifer, in frontal view, presents straight anticlinal cell walls on the adaxial surface (Figures 2 H-M) and sinuous ones on the abaxial surface (Figure 3 E, F); anomocytic and anisocytic stomata are found on both sides, yet are rare on adaxial side (hipoamphistomatic leaf) (Figures 2 I, J, K, 3 E, F); calcium oxalate drusen-like crystals are also found on adaxial face (Figure 2 L); non-glandular stellate trichomes are rare on the adaxial face, where they are numerous on the abaxial face (Figures 2 M, 3 G, H).

The present study revealed that the stellate trichomes are present in *T. papyrifer*, but absent in *C. pachystachya*, therefore, based on the analysis of the anatomical characteristics of the "embaúba" species, it is possible to differentiate the two studied species by observing the presence or absence and type of trichomes (Table 1).

The identification of trichomes is vital for quality control, as it is considered an anatomical marker that helps to authenticate and identify plant species [3,18,19]. This identification and characterization can also be observed in a study responsible for differentiating three species of "boldo" (*Plectranthus barbatus* Andrews, *Plectranthus neochilus* Schltr. and *Peumus boldus* Molina), just by observing the micromorphology of the trichomes [20]. In addition, other anatomical markers have been evidenced in order to characterize and differentiate species, such as crystal morphotypes [21,22].

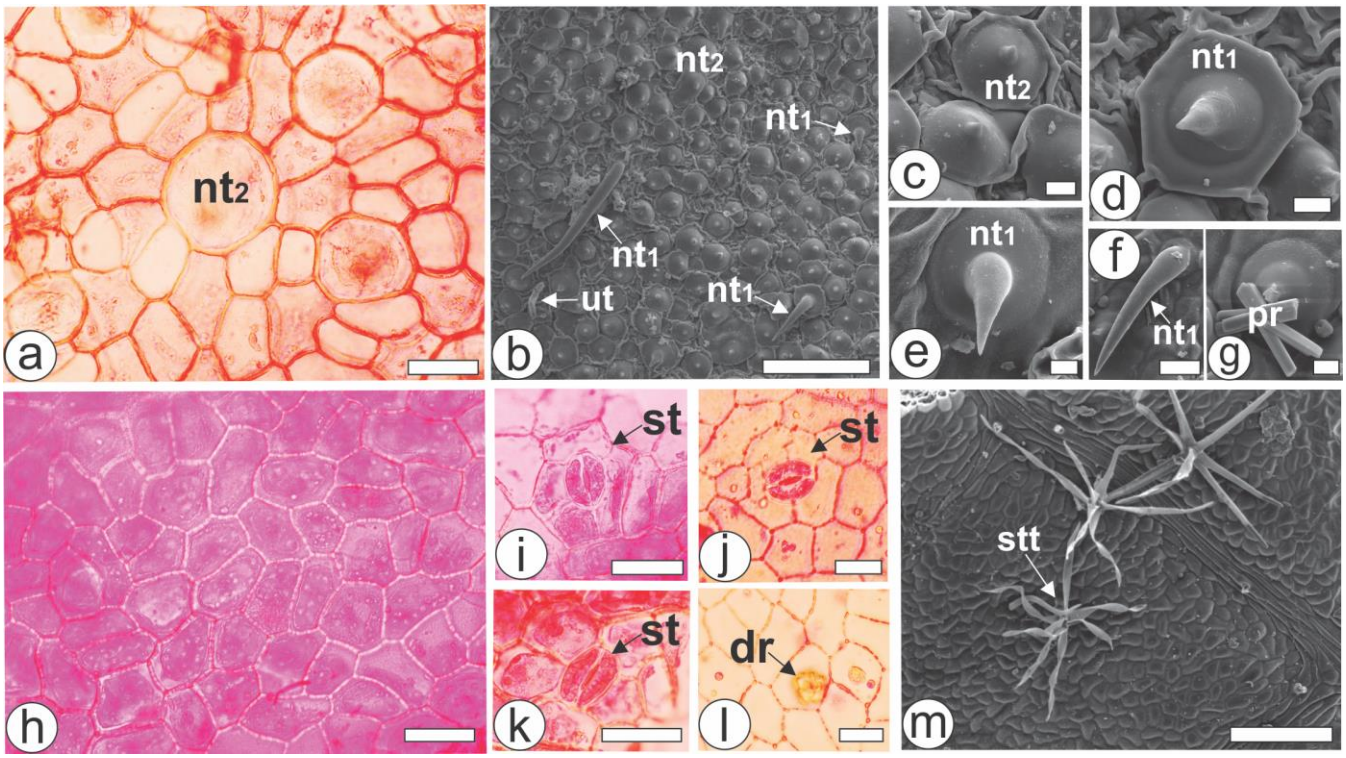

Figure 2. Surface view of the adaxial face of the leaves of *Cecropia pachystachya* (a – g) and *Tetrapanax papyrifer* (h – m). (a, h – l: light microscopy; b – g, m: FESEM). [nt_2: short apex non-glandular trichome; nt_1: long apex non-glandular trichome; ut: uniseriate trichome; pr: prismatic crystal; st: stomata; dr: druse; stt: stellate trichome]. Scale bars: a, f, h – l = 50 µm, b = 100 µm, c – e, g = 20 µm, m = 200 µm.

Table 1. Leaf epidermal features of the species of "embaúba"

Epidermal features	*C. pachystachya*	*T. papyrifer*
Anticlinal walls	Straight (adaxial) and sinuous (abaxial)	Straight (adaxial) and sinuous (abaxial)
Stomata type	Anomocytic	Anomocytic and anisocytic
Occurrence of stomata	hypostomatic	amphistomatic
Non-glandular trichomes	+	-
Filariform trichomes	+	-
Stellate trichomes	-	+
Glandular trichomes	+	-
Crystals on the leaf surface	+	+

[1]Acronyms: (+) present or (-) absent of anatomy elements.

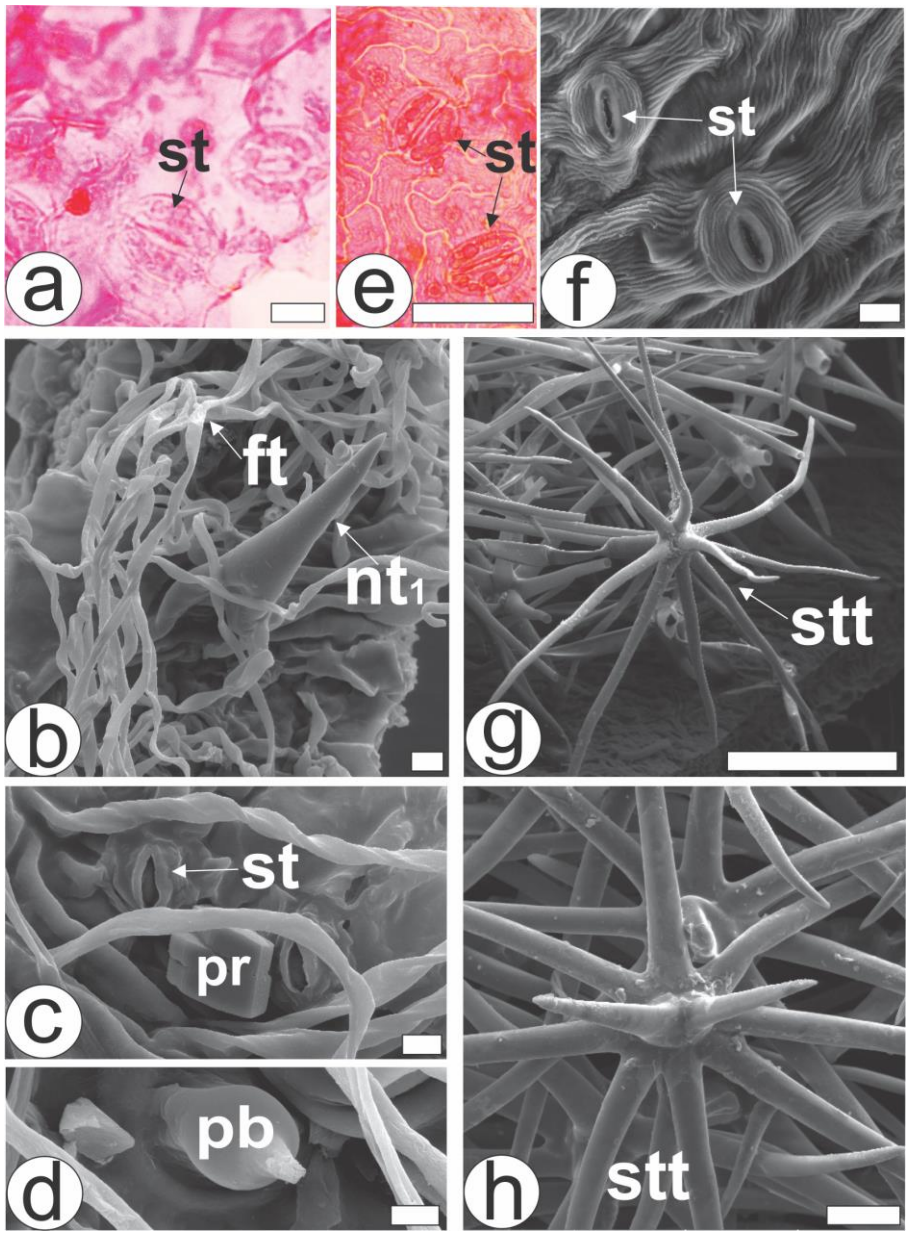

Figure 3. Surface view of the abaxial face of the leaves *of Cecropia pachystachya* (a – d) and *Tetrapanax papyrifer* (e – h). (a, e: light microscopy; b – h: FESEM). [st: stomata; ft: filariform trichome; nt_1: long apex non-glandular trichome; stt: stellate trichome; pr: prismatic crystal; pb: pearl body]. Scale bars: a, e, h = 50 µm, b, c, f = 20 µm, d = 10 µm, g = 100 µm.

CONCLUSION

The anatomical features of the leaf epidermis identified in this study are sufficient to differentiate between the two species of “embaúba”, *C. pachystachya* and *T. papyrifer*. The micromorphology of the trichomes is a key diagnostic feature that can be used to authenticate the commercial botanical raw drug in the entire, cut and sifted, or pulverized form. In addition to microscopy, histochemical tests can help distinguish morphologically similar species.

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Conflicts of Interest: The authors declare no conflict of interest.

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