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Pharmacobotanical study of *Psychotria carthagenensis* Jacq. (Rubiaceae), a species known as toxic and medicinal

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Psychotria carthagenensis (Rubiaceae), popularly known as "erva-de-rato", is known as poisonous, and its leaves are used as a constituent of "ayahuasca". An anatomical study of its leaves was carried out to define parameters to support its taxonomy and quality control of ethnodrugs. Transverse and paradermic sections of petioles and leaf blades were made following the usual techniques of botanical anatomy. All semi-permanent laminas were then analyzed and photographed by light microscopy. Leaf blades were also studied with the support of the scanning electron microscope. *Psychotria carthagenensis* has hypostomatic epidermis, 1-2-stratified, in cross section, with three types of stomata (paracytic, anisocytic and anomocytic), and predominantly straight anticlinal cell walls, in frontal view, and glandular-peltate trichomes on both surfaces, the cuticle is smooth and thickened. The leaf edges are acute, mesophyll dorsiventral with the palisade parenchyma 1-seriate, and spongy parenchyma 4-seriate. The midrib is flat-convex with 4 bundles; petiole is semicircular with lateral projections with 9 vascular bundles. Midrib and petiole have collateral vascular system. Anisocytic and anomocytic stomata, glandular-peltate trichomes, petiole with the central bundle invaginate at the end, the syntopism of epicuticular waxes are distinctive parameters for *P. carthagenensis,* which support the standardization of its leaves used as medicines, in addition to contributing to the systematic of the *Psychotria* genus.

Keywords: Ethnobotany, Leaf anatomy, Medicinal plant, *Palicourea*, Psycotrieae, Quality control

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Rubiaceae Juss. belongs to the order Gentianales and comprises approximately 630 genera and 13,000 species subordinated to three subfamilies: Cinchonoideae, Ixoroideae, and Rubioideae[1]. It is one of largest group of angiosperms, which occurs around the world, mainly in the Neotropical regions. The main centers of diversity are Central and South America, in different biomes. In Brazil, species of Rubiaceae occur in different phytogeographic domains: Amazon Rainforest, Caatinga, Central Brazilian Savanna, Atlantic Rainforest, Pampa, and Pantanal[2]. Rubiaceae comprises some economically very important species, including *Coffea arabica*, and numerous species of Rubiaceae are used as medicinal, ornamental, and food resources[3].

The genus *Psychotria* L. belongs to the tribe Psycotrieae Chamisso & Schlechtendal and shows the greatest diversity and taxonomic complexity among angiosperm species[2-4]. The genus has traditionally been divided into three subgenera[1], based on morphological characters and geographical distributions. About 236 species are found in Brazil, of these 137 are endemic[2].

Recent studies (supported by molecular analyses) have modified the infra-generic positions of several species of the genus, transferring them from the subgenus *Heteropsychotria* to the subgenus *Palicourea* Aublet[5]. Species of *Psychotria* and *Palicourea* show significant morphological affinities and can be easily confused when in their vegetative stages[5], so that characteristics of their inflorescences (such as color and flower shape), as well as elements associated with their reproduction (such as pollinator types) are essential to distinguishing them. The molecular phylogenies indicate that *Psychotria* comprises at least 1600 species, and many others surely have yet to be described[6].

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Psychotria species are popularly used as medicines against several diseases. Different parts of the plant are used as infusion or decoction, like flowers and fruits, as was recorded for *Psychotria colorata* (Willd. ex Schult.) Müll. Arg[7,8]. Many species of the genus are toxic and poisonous already reported for *Psychotria bracteocardia* (DC.) Müll. Arg. and *Psychotria rigida* Kunth (= *Palicourea rigida* Kunth subsp. *rigida*)[9]. In addition, other ethnomedicinal uses, as depurative and against rheumatism, were referred by Sanz-Biset[10] to four species of *Psychotria* (*P. alba* Ruiz & Pav. *P. carthagenensis* Jacq., *P. ernestii* K. Krause and *P. viridis* Ruiz & Pav.), and also used in the treatment of inflammations, respiratory diseases, and for treating hallucinations[11].

Secondary metabolites compounds of *Psychotria* species have been widely studied, mainly alkaloids[7,8,12]. A complete review of the chemical constituents of *Psychotria* was performed by Yang[8], which reported 159 compounds isolated from 41 species of *Psychotria*. The compounds found in *Psychotria* species showed potent bioactivities, such as bioactive on the cardiovascular system as well as antitumor[13], antioxidant[14,15], and against neurodegenerative diseases that affect the central nervous system[16], anti-mutagenic property[14], as analgesic[17], anti-inflammatory[18], antimicrobial[19], antiviral[20], and as antiparasitic[9,16] among other activities. A study performed with some species of *Psychotria* showed the highest antioxidant activity (79.1%±3.70%) by *P carthagenensis*[15], which also showed flavonoids, condensed tannins, and flavonol.

Psychotria carthagenensis Jacq. is a Neotropical species widely distributed in Brazil, and occurs in all of the phytogeographic domains of that country[21]. It is popularly known as “cafeeiro-do-mato", "erva-de-rato-branca", and "carne-de-vaca", and its leaves are used for medicinal purposes in the Amazon and as a component of “ayahuasca” – a hallucinogenic beverage used in rituals[22]. *Psychotria carthagenensis* is popularly known as "Yaku-bushiklla" and "Chakruna" in Peru, and its leaf decoction is used as depurative (purifying blood) and also against rheumatism[12]. In addition, the authors refer to the uses of leaf decoction always associated with parts of other plant species from different families.

According to Dickison[23], anatomical characters have systematic importance in different taxa, as well as epidermal characters and related structures (such as cuticle, epicuticular waxes, trichomes and stomata), the mesophyll (types, cell shapes, number of layers), the presence of secretory structures and crystals, venation patterns, and the arrangements of vascular strands in the leaves and petioles. Micro-morphological studies have been performed as an efficient device for plant systematics[24]. Anatomical characters can contribute to hypotheses of phylogenetic relationships, especially those observed in vegetative organs such as leaves (which are anatomically variable, but may show specific patterns in sections, genera or even families)[25].

Anatomical studies were performed to support the quality control of species of ethnomedicinal uses, as already demonstrated for *Solanum* species[25], *Cissampelos*[26], for distinguish species of medicinal uses of the genera *Psychotria*[27], *Bauhinia* and *Schnella*[28], *Byrsonima sericea*[29], among others. Moreover, these studies have been shown to have great taxonomic value in many families[30], and can provide useful characters for both infra-generic and interspecific delimitations, as seen in *Solanum*[25,31-33].

Due to the ethnomedicinal importance of *P. carthagenensis,* a leaf anatomical characterization was carried out in order to find parameters that can contribute to testing samples of its ethnodrugs to distinguish *P. carthagenensis* from other species of *Psychotria* with similar features, since *Psychotria* is a taxonomically complex genus.

Materials and Methods

Psychotria carthagenensis specimens were obtained from Mata do Pau-Ferro Ecological Reserve, located in an altitudinal forest, at municipality of Areia, Paraíba State, Brazil (6° 58'12' S; 35° 42'15 ' W), at elevations of 400 to 600 m. The regional landscape consists of valleys, hills and slopes, and the mean rainfall rate of 1400 mm.

The collected material was treated according to the usual taxonomic procedures, and a voucher specimen (*Agra et al. 7899*) was deposited in the Prof. Jayme Coelho de Moraes Herbarium (EAN), with duplicates in the Prof. Lauro Pires Xavier Herbarium (JPB). Material was identified based on the specific literature, and with specimens of the herbaria (EAN and JPB).

Adult leaves were collected from the third to fifth node, and were fixed for 48 h in a solution of FAA (formaldehyde: ethanol:acetic acid), and was

sustained in alcohol (70%). Sections of leaf blades were performed by paradermal cuts on the both epidermal surfaces. Petiole, mesophyll, midrib, and edges were cross-sectioned by freehand. The sectioned samples were discolored by sodium hypochlorite (2%), and subsequently washed in distilled water, and neutralized in acetic acid (1%). Paradermal sections were colored by safranin, and cross-sectioned samples were stained with Astra blue. They were studied and micrographed by a Qwinsystem.

Leaf fragments of t of ~1 cm^2 that have been previously dried were placed on aluminum stubs with double-sided adhesive tape, metalized by gold, and analyzed by scanning electron microscopy (SEM), JEOL JSM-5600, on a 15 KV voltage accelerator. The terminology of wax morphology followed Barthlott[34].

Results

Psychotria carthagenensis showed the leaf blade epidermis, in frontal view, with cells of anticlinal walls straight to slightly curved on both surfaces (Fig. 1ab, Fig. 2a-e), and strongly thickened on the adaxial surface (Fig. 1a, Fig. 2a-e). The leaf is sparsely lepidote on the both surfaces, with glandular-peltate trichomes (Fig. 1c, Fig. 2bc). In cross-section, the epidermis varies from 1-seriate to 2-seriate (Fig. 1cf), with tabular cells whose outer periclinal walls are covered by a smooth, thickened cuticle on the upper face and a thinner cuticle on the lower face (Fig. 1c). Epidermis is hypostomatic, with different types of stomata: anisocytic, anomocytic, and mainly paracytic (Fig. 1b, Fig. 2f), which are positioned just above the level of the cells of the epidermis when opened (Fig. 1cf).

In transverse section, leaf blades showed sharp edges (Fig. 1f) and a dorsiventral mesophyll (Fig. 1c), with the palisade parenchyma 1-seriate and the spongy parenchyma 4-6-seriate. Circular vascular bundles occur within the mesophyll near the leaf edge (Fig. 1f).

The midrib has a plano-convex contour (Fig. 1f), broader and more prominent on the abaxial face; the epidermis is 1-stratified, and the outer periclinal cell walls are coated with a thin cuticle. The collenchyma is angular, with 6–7 layers of cells, which is continued

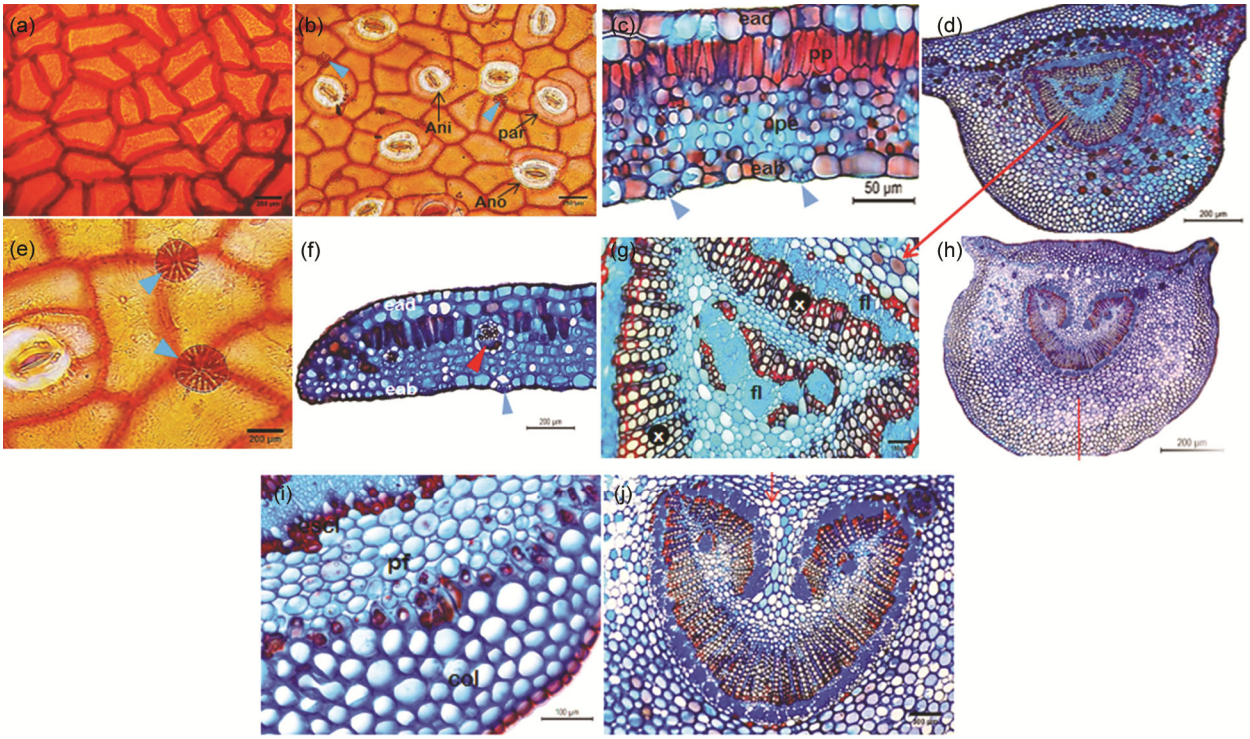

Fig. 1 — *Psychotria carthagenensis* Jacq. (from *Agra et al. 7899*) a-j: A) Epidermis on the upper face with straight and thickened anticlinical cell walls, B) Lower face of epidermis - hypostomatic - with paracytic (Par), anomocytic (Ano), and anisocytic stomata (Ani), C) Detail of the epidermis, showing glandular-peltate trichomes (blue arrows), D) Leaf blade margin, E) Dorsi-ventral mesophyll showing 1-seriate palisade parenchyma (pp), and 4-seriate spongy parenchyma (sp), F) Midrib, G) Detail of the midrib inner vascular system, H) Petiole, I. Detail of the angular collenchyma (Col) and the petiole parenchyma; J. Vascular system with invaginated extremities.

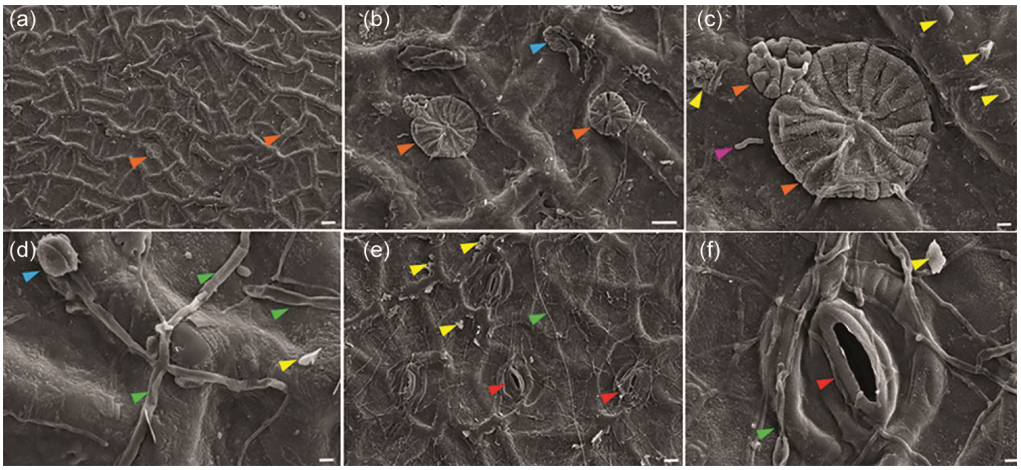

Fig. 2 — *Psychotria carthagenensis* Jacq. viewed by scanning electron microscopy (from *Agra et al. 7899*). a-f: A) Epidermis, with smooth cuticle and glandular-peltate trichomes, B) Detail of the epidermis, with glandular (blue arrow) and glandular-peltate trichomes (orange arrows), 10 µm, C) Glandular-peltate trichomes (orange arrows), epicuticular waxes as platelets (yellow arrows), and coiled rodlets (pink arrow), 2 µm, D) Epicuticular waxes as threads (green arrow), irregular platelets (yellow arrow), and glandular trichome (blue arrow), 2 µm, E) Epicuticular waxes as threads (green arrow) and platelets (yellow arrows) on the abaxial surface, 10 µm, F) Detail of a stoma (red arrow), and epicuticular waxes as threads (green arrow) and platelets (yellow arrow), 2 µm.

by the parenchyma with rounded cells of irregular sizes with thin walls. The vascular system is composed of four collateral bundles, one large and triangular, and three accessory bundles, with one medullary and two facing the adaxial surface (Fig. 1dg). An undeveloped sclerenchymal sheath surrounds the phloem (Fig. 1g).

The petiole has a semicircular outline, winged laterally towards the upper face (Fig. 1h). Its epidermal tissue is 1-stratified and coated by a thin cuticle (Fig. 1i). Underlying the epidermis is the collenchyma (angular type) and the fundamental parenchyma, both with 5-7 cell layers (Fig. 1i). The vascular system is collateral and consists of nine bundles, one large, central, and V-shaped, with strongly invaginated extremities and eight smaller accessory bundles, two of which are medullary (near the invaginated extremities), and six facing the adaxial surface (Fig 1h). Discontinuous sclerenchymal bundles surround the vascular system (Fig. 1j) of the central vein and petiole, as well as the vascular system (Fig. 1h-j) of the midrib and petiole.

The occurrence of a smooth cuticle and more than one type of epicuticular wax (designated syntopism by Barthlott 1998) was covering the epidermal cells, trichomes, and stomata of observed on the both surfaces of *P. carthagenensis*. Those observations were confirmed by SEM, with the deposition of waxes as films, smooth layers, threads, irregular platelets, coiled rodlets, and granules (Fig. 2a-f).

Discussion

Psychotria carthagenensis showed the epidermis on the upper face with anticlinal walls forthright, to arched and thickened – similar to those observed in various species of the genus[35], like *P. velloziana* Benth.[36], *P. viridis* Ruiz & Pav.[37], and *P. schlechtendaliana* (Müll. Arg.) Müll. Arg.[38], constituting a common character in all species of the genus. That configuration was also described for other genera of Rubiaceae, including *Tocoyena*[39], and *Palicourea*[40].

In relation to the leaf indument, studies considering the leaf anatomy of Rubiaceae species have reported the presence of simple trichomes[41]. Those reports differ from our observations of *P. carthagenensis,* which showed only glandular-peltate trichomes on both surfaces of the leaf epidermis.

In terms epidermal appendages, hypostomatic pattern observed in *P. carthagnensis* is seen in several other genera of Rubiaceae, such as *Palicourea*[40], *Tocoyena* [39], *Cordiera*[42], and *Posoqueria*[43], *inter alia*. Paracytic and parallelocytic stomata are inherent characteristics of Rubiaceae species[30].

Dorsiventral mesophyll with 1-seriate palisade parenchyma observed in *P. carthagnensis* differed from that observed for the same species[44] (described as dorsiventral with 2-3-seriate palisade parenchyma). That difference can probably be explained by mesophyll plasticity in response to different environmental factors (such as different levels of luminosity) that can have direct effects on leaf anatomy[44,45].

The midrib contour of *P. carthagnensis* was quite distinct, differentiating it from other species of *Psychotria*[45] and other studied genera of Rubiaceae[29-32]. Angular collenchyma support tissues are common characters in Rubiaceae. The presence of sclerenchyma bundles was also mentioned for *P. carthagenensis*[44], and for genera of Rubiaceae, corroborating Metcalfe & Chalk[30] who considered it to be a common character in Rubiaceae.

The semicircular petiole type with two lateral projections (wings) observed here in *Psychotria carthagenensis*, corroborates with a report for this species and for *P. velloziana,* differing from the circular pattern common to other species of *Psychotria*[27]. Those lateral projections on the petiole of *P. carthagenensis* represent a common character in other species of that genus – so that the petiole contour in the genus *Psychotria* does not demonstrate a consistent pattern. The vascular system is a distinctive character for *P. carthagenensis*, mainly in relation to the number of bundles of the main vein and petiole, however, differing from the pattern cited for the family Rubiaceae (having only two lateral bundles)[30].

It was possible to observe similarities in the smooth cuticle epidermis on both faces of *P. carthagenensis* leaves with the abaxial surfaces of *P. leicocarpa* Cham. & Schltdl. and *P. tenuinervis* Müll. Arg., as well as the adaxial surfaces of *P. nuda* (Cham. & Schltdl.) Wawra and *P. stenocalyx* Müll. Arg.[35]. Some species of the genus, such as *P. velloziana* Benth.[36], showed an epidermis with a smooth cuticle, or with striated ornamentations, recorded to the upper surface of *P. schlechtendaliana* Müll. Arg.[38]. Great variations of cuticle and epicuticular ornamentations were already recorded in *Psychotria*[27], and micro-morphological characters like ornaments are a support for taxonomic analyses[35].

Conclusions

The leaf anatomy characters of *Psychotria carthagenensis*, such as hypostomatic leaves, a dorsiventral mesophyll, a collateral vascular system, and paracytic stomata correspond to the general characteristics ascribed to Rubiaceae. However, leaf blade anatomy and its epidermal attachments (such as anisocytic and anomocytic stomata and glandular-peltate trichomes), the vascular system of the main vein and petiole, as well different types of epicuticular waxes, are diagnostic features among *Psychotria* species, and provide parameters for the leaf based ethnodrugs, and the systematic of the genus.

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Author's Contributions

EAVS was the main author and contributed to the preparation of the article, with practical aspects in the laboratory, and the analysis, interpretation, and description of the data obtained. RFLS contributed to the practical aspects of laboratory work. ALS contributed to the practical aspects of the work, in addition to image editing. MFA contributed to the interpretation and description of the data, and to the critical review, adding intellectual content to the manuscript, in addition contributed to the specimen collections, identification of the species. All authors provided critical feedback and assisted in the preparation of the manuscript.

Competing interests

The authors declare there are no competing interests.

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