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Morphology and anatomy of *Justicia acuminatissima* leaves

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Abstract: *Justicia acuminatissima* (Miq.) Bremek., Acanthaceae, is a subshrub found in northern Brazil, where it is widely used by the population of this region as an anti-inflammatory medicine. Despite this popular use, there is no pharmacognostical data to support the correct identification of this species. We therefore performed a morpho-anatomical, histochemical and phytochemical analysis of the leaves of this species, using well-known methods. The leaves are simple, exstipulate, green on the surfaces, and pubescent, with a lanceolate shape, crenate margin, pinnate venation and decussate phyllotaxy. The parenchyma is palisade and spongy, and its vascular system is bilateral. Glandular and non-glandular trichome and cystoliths were also detected. There are diacytic stomata on both the adaxial and abaxial surfaces of epidermis. Histochemical tests revealed the presence of phenolic compounds, amide and protein. Phytochemical tests showed the presence of coumarins, tannins, catechins, saponins and steroids.

Introduction

The Acanthaceae family comprises about 250 genera and 2500 species widely distributed in tropical regions of the world, with little occurrence in temperate regions (Braz et al., 2002). Brazil has a high diversity of these species, with an estimated occurrence of 40 genera and 542 species. These are mostly distributed in mesophytic forests, with 45% in "Mata Atlantica" and 25% in forests of the "Planalto Central". Another 15% are found in the Amazonian rainforest, and the remainder is distributed in other regions (Silva, 2011; Vilar, 2009).

Acanthaceae species generally grow in the form of plants, subshrubs, shrubs and lianas, and more rarely, trees (Kameyama, 2006). Their leaves are normally opposite, simple and without stipules. These species commonly present cystoliths in epidermal cells of the stem and leaves, and the presence of crystals of almost all shapes and types is an important feature of the family (Kameyama, 2006; Vilar, 2009). Many species are used for ornamental purposes, with mainly economic importance (Metcalfe & Chalk, 1957). In traditional medicine, their use is emphasized for treating fever, pain and stomach disorders (Angonese et al., 1992; Chen et al., 1996). Chemically, Acanthaceae are rich in alkaloids, lignans, flavonoids, terpenoids and biogenic amines. A dichotomist pattern has been observed between the presence of alkaloids and lignans (Angonese et al., 1992).

The *Justicia* genus has the greatest number of species of the Acanthaceae family, comprising about of 600 members found in tropical and temperate areas of the globe. These species occur in the form of perennial plants or subshrubs, erect or scandent. Their leaves are usually entire and petiolate, with cystoliths in the epidermis (Braz et al., 2002; Kameyama, 2006). In the North and Northeast of Brazil, species of this genus, especially *J. pectoralis* and *J. gendarussa*, are widely used by the population for the treatment of inflammatory processes (Oliveira & Andrade, 2000). The genus proved to be rich in amine metabolites, coumarins and lignans (Al-Juaid & Abdel-Mogib, 2004). There is no accurate estimate for the number of the *Justicia* species in the Brazilian flora (Braz et al., 2002), due to inconsistencies in the circumscription

of the genus in the country, which requires taxonomic revisions (Profice, 2010). Therefore, the importance of pharmacobotanical studies with species of this genus is emphasized.

Justicia acuminatissima (Miq.) Bremek. is a native subshrub of the Brazilian Amazon region, used for ornamental and therapeutic purposes (Santos, 2006). In Amazonas (Brazil), where it is popularly known as "saratudo" or "saratudo-de-quintal", its leaves are widely used in the preparation of teas, which are consumed for healing and inflammation relief. However, there is no scientific data providing pharmacognostical identification of this herbal drug, which has morphological similarities with subshrubs and several other plants of the same genus. Therefore, considering that the correct identification of plant material is the first step in producing accurate scientific studies with good quality and reproducibility, we performed morpho-anatomical, histochemical and phytochemical analyses of leaves of *J. acuminatissima* using gold standard methods, seeking to contribute to pharmacognostical characterization of this species.

Materials and Methods

Plant material

The collection of samples was authorized by the Conselho de Gestão do Patrimônio Genético, chaired by the Ministry of Environment (Brazil), under registration number 034/2008. The plant material was collected at the campus of Instituto Nacional de Pesquisas da Amazônia (INPA), in Manaus-AM, Brazil, on June 24, 2008, in the morning, under intense light. A voucher specimen was deposited in at the Herbarium of INPA, under number 224040.

Leaf morphology

To characterize the leaf morphology, three samples of the species were collected and placed in a bottle containing equal parts of alcohol and water. The phyllotaxy and morphologic characters, such as size, shape, texture, color, coating, margin, venation, midrib and petiole were visually analyzed (Hickey, 1973).

Leaf anatomy

The leaf anatomy was analyzed based on transversal and longitudinal sections of 0.7 mm from the lamina, midrib and petiole of three fresh leaves, obtained by a manual microtome. The material was bleached with 20% sodium hypochlorite and then washed and stained with toluidine blue (O'Brien, 1964). To observe the stomata, as well as the characters from the adaxial and abaxial surfaces, the dissociation of leaf epidermis was performed using commercial sodium hypochlorite (Kraus & Arduin, 1997). The slides were mounted in glycerin and stained with safranin. The structures were analyzed and photomicrographed in an optical microscope with a digital camera attached.

Histochemical analysis

Histochemical tests were carried out using: ferric chloride to detect phenolic compounds; Lugol to detect starch; ruthenium red to detect pectin; and xylidine Ponceau to detect total proteins (Ascenção, 2004; Johansen, 1940).

Phytochemical screening

For the phytochemical screening, the leaves were dried in a oven at 40 °C and pulverized using a mechanical grinder. Phytochemical screening was performed to verify the presence of cyanogenic glucosides, coumarins, flavonoids, terpenes and steroids (Matos, 1997), saponins (Schenkel et al., 2007) and alkaloids (Costa, 2002).

Results and discussion

Considering that flowers and fruits are only available in certain seasons of the year, the wide variety of anatomical characters of the leaves has been a useful tool for the taxonomic identification of various species (Dipa & Daniel, 2011). On the other hand, it is obvious that knowledge of the main groups of secondary metabolites in medicinal plants is important as a starting point for studies of their biological activities, or even for the isolation and identification of compounds that may be the active principles of plants, especially those used in traditional medicine. In this work, we studied the micromorphological and anatomical characters of *Justicia acuminatissima* (Miq.) Bremek., Acanthaceae, and investigated the class of secondary metabolites of this herbal drug.

The leaves of *J. acuminatissima* are simple, lanceolate in shape, with an acuminate apex, cuneate base, crenate margin, and pinnate venation, and are green on both sides. They have a membranous consistency and are pubescent. They are usually between 9 in length and 13 cm and width and between 3 and 5 cm in their central region; they are exstipulate, with decussate phyllotaxy. (Figure 1). The petiole is smooth, flat, and concave-convex in transversal section, and between 1.7 and 2.7 cm in length.

The petiole has a uniseriate epidermis composed of cubic cells with thickened outer periclinal walls and anticlinal walls with some sinuosity. The subepidermal region is composed of an angular

Figure 1. Images of *Justicia acuminatissima*. A. *J. acuminatissima* at the collection site; B. Plant illustration, showing morphology details (credits: Lilian Gois); C. Photo showing decussate phyllotaxy.

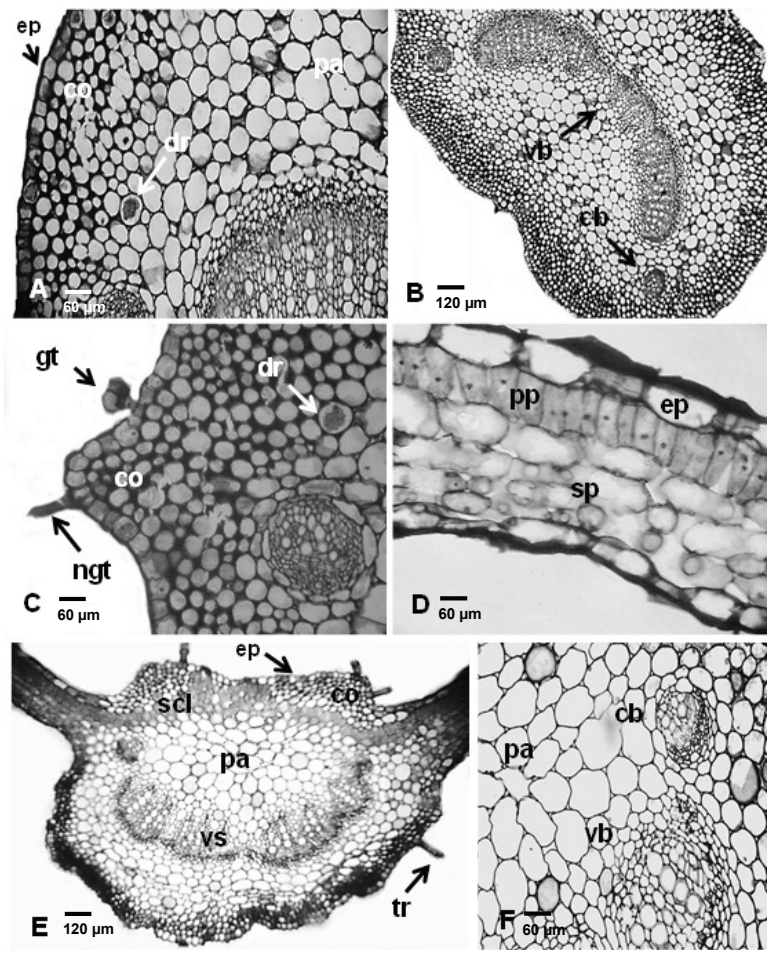

Figure 2. Anatomy of *Justicia acuminatissima* leaves. A. Cross section of petiole showing cubic cells of epidermis (ep), angular collenchyma (co) and parenchyma (pa). Cristals in druse form are also observed (dr); B. Cross section of the vascular system of the petiole showing a semicircular central beam (vb) and two circular complementary bundles (cb); C. Uniseriate epidermis with non-glandular trichome (ngt) and peltate glandular trichome. (gt) and calcium oxalate druse (dr) in the petiole; D. Epidermis (ep), palisade parenchyma (pp) and spongy parenchyma (sp) of the mesophyll; E. Cross section of midrib showing epidermis (ep), angular collenchyma (co), sclerenchyma (scl), parenchyma (pa) and vascular sytem (vs). Presence of trichome (tr) in the epidermis; F. Cross section of the vascular bundles (vb and cb) in the parenchyma (pa) of the midrib.

collenchyma, followed by a parenchyma with many schizogenous spaces (Figure 2A). As in many other Acanthaceae species, the vascular system is composed of a semicircular central beam and two circular complementary bundles (Patil & Patil, 2012) (Figure 2B). Druses were observed in some cells. Overall, calcium oxalate deposits in druse form are common in Acanthaceae species (Vilar, 2009). Glandular and non-glandular trichomes were found in small numbers (Figure 2C). The mesophyll is dorsiventral, composed of one layer of palisade parenchyma, followed by four to five layers of relatively dense spongy parenchyma in which the cells are almost juxtaposed (Figure 2D). The midrib has a uniseriate epidermis composed of cubic cells. The subepidermal tissue consists of angular collenchyma which is interrupted by sclerenchyma. In the adaxial surface of epidermis, the collenchyma is interrupted by sclerenchyma, which is followed by parenchyma, where the bilateral vascular system is dispersed. In the abaxial surface of the epidermis, the collenchyma is continuous. Some trichomes were seen in the epidermis (Figures 2E and 2F).

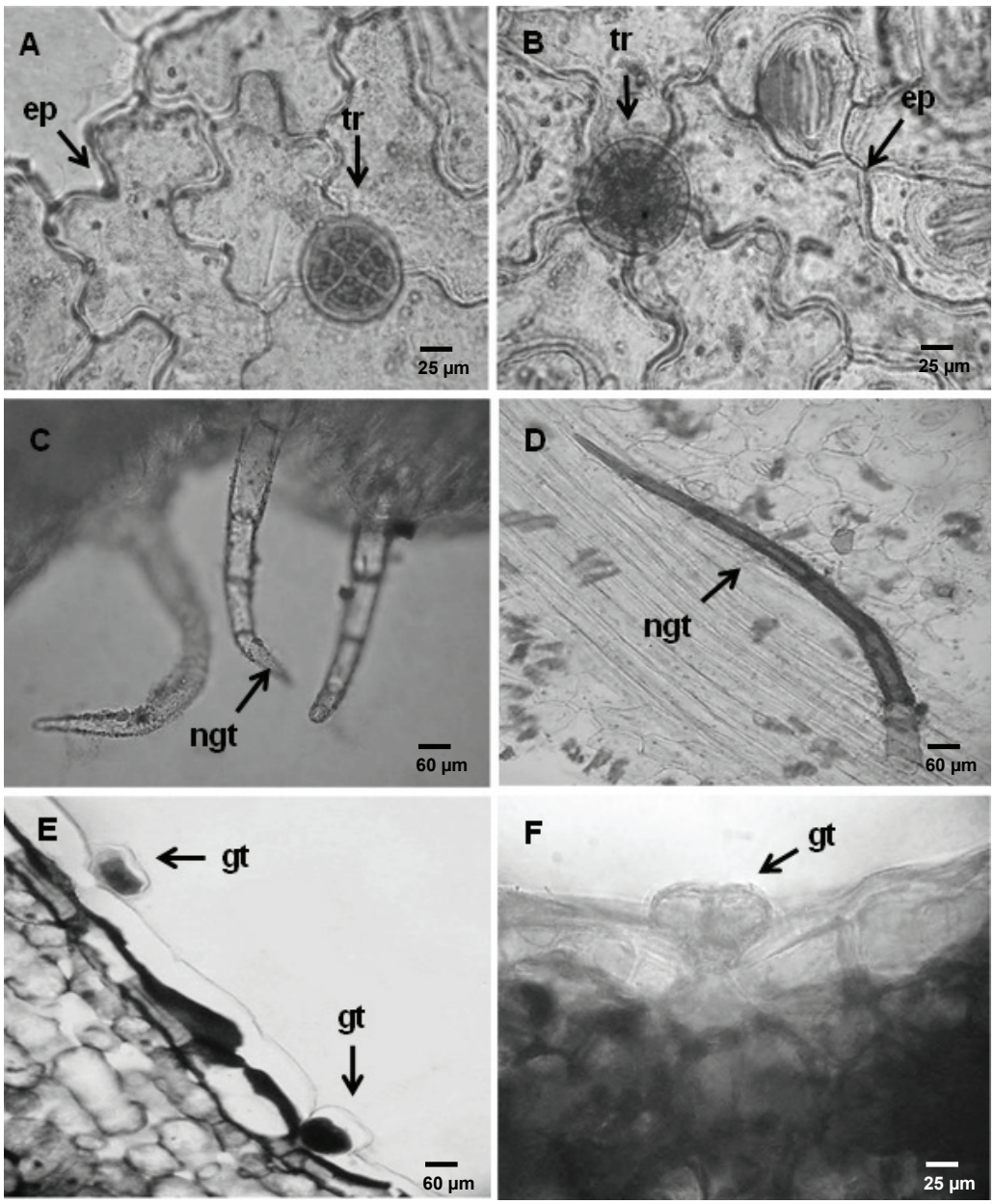

Figure 3. Epidermis of *Justicia acuminatissima*. A. Base of a trichome and sinuous epidermis cells (ep) on the adaxial surface; B. Base of a trichome and sinuous epidermis cells(ep) on the abaxial surface; C. Uniseriate multicellular non-glandular trichome; D. Uniseriate multicellular non-glandular trichome along the veins; E. Sessile glandular unicellular trichomes; F. Sessile glandular bicellular trichome.

On both the abaxial and adaxial surfaces, the epidermal cells were elongated and undulate, with U-shaped sinuses, similar to various other Acanthaceae species (Patil & Patil, 2011a). The bases of the trichomes can be seen on both epidermal surfaces (Figures 3A and 3B). Long multicellular and uniseriate non-glandular trichomes, generally five-celled, were found on both sides of the epidermis (Figure 3C) and along the veins (Figure 3D). Glandular trichomes, with one or two-celled heads, were also found on both sides of the epidermis (Figures 3E and 3F). These were generally sessile, but glandular trichomes with very short stalk were also seen in the petiole. Variations in the form and number of cells of the trichomes can be used to identify these plants, including in the powder form (Dipa & Daniel, 2011).

The epidermis mainly presents diacytic stomata with elliptical guard cells, as in most Acanthaceae species. The subsidiary cells with U-shaped sinuses, like other epidermal cells, but are not elongated and have very different sizes from each other (Figure 4A). Anisocytic stomata were also viewed in small numbers. However, the amphystomatic pattern shown by *J. acuminatissima* is an unusual feature in the *Justicia* genus, and can be used to taxonomically differentiate this species (Patil & Patil, 2011a). Simple and oblong-shaped cystoliths are also found on both sides of the foliar epidermis (Figure 4B).

Species of the Acanthaceae family are known by the presence of various crystal types in their tissues, thus, the presence of cystoliths and druses in *J. acuminatissima* is easily justified (Vilar, 2009; Watson & Dallwitz, 1992). Cystoliths are calcium carbonate deposits formed in specialized cells called lithocysts. They are mostly located in the epidermis and generally consist of a single stalk, but can take different forms, which are easily identified by microscopy. They have been found only in Moraceae, Urticaceae, Acanthaceae and some other families. The occurrence of cystoliths in the vegetative parts of Acanthaceae species have been repeatedly reported by various researchers. In the leaves and sepals of *Justicia*, the lithocysts occurred frequently in the epidermis (Lin et al., 2004). The variations in shapes and sizes have been used to create convenient taxonomic distinctions, either exclusively or in conjunction with other endomorphic or exomorphic features of these plants (Patil & Patil, 2011b).

Histochemical tests showed the presence of proteins in the epidermis and amide in the petiole and vascular system. The parenchyma has high phenolic compound content. Phytochemical screening revealed the presence of coumarins and condensed tannins, which confirms the presence of phenolic compounds observed in the histochemical analysis, as well as steroidal saponins. The presence of coumarins seems to be typical in the *Justicia* genus. In *J. pectoralis*, one of the better scientifically studied species, these compounds are considered the main chemical constituents (Lino et al., 1997; Oliveira & Andrade, 2000).

Saponins present antiprotozoal, antifungal and antiviral activities, as well as cytostatic effects on various cancer cells, lower serum cholesterol, stimulation of cell-mediated immune system and enhancement of antibody production, as demonstrated in experimental animals (Francis et al., 2002). Tannins have biological activities related to their capacity of protein precipitation and astringent propriety, which has led to their use as antidiarrheal, antiseptic and wound sealant, as well as marked antimicrobial, antifungal and antiviral activities (Monteiro et al. 2005). On the other hand, different coumarins showed antioxidant, anticoagulant, anti-inflammatory and analgesic activities, among many other biological effects (Blanco, 2011; Montagner, 2007). The presence of these compounds therefore suggests good pharmacological

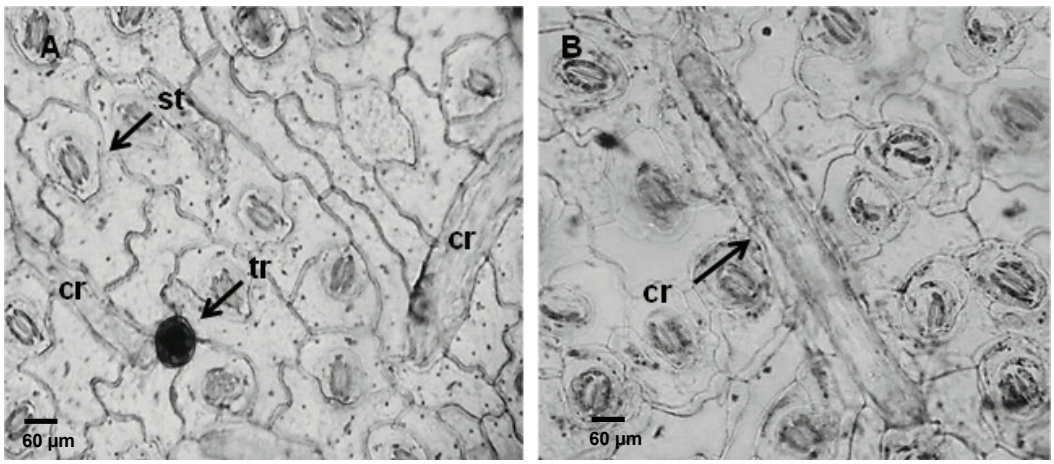

Figure 4. Paradermal section of foliar epidermis of *Justicia acuminatissima*. A. Presence of cystolith/calcium carbonate cristal (cr), diacytic stomata (st) and base of trichome (tr); B. Details of simple and oblong cystolith (cr).

potential for *J. acuminatissima*, which needs to be further investigated.

Overall, *J. acuminatissima* leaves present morphological, anatomical and chemical features common to most species of the *Justicia* genus. Moreover, particular characteristics, such as a crenate margin and amphystomatic pattern, and combined characters, such as long multicellular non-glandular trichomes and sessile glandular trichomes on both sides of the epidermis, and the presence of oblong cystoliths in the epidermis and druses in the mesophyll, enables fast and safe identification of this species by leaf analysis.

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