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Morphology, anatomy and histochemistry of the leaves of *Myracrodruon urundeuva* Allemão (Anacardiaceae)

[Morfología, anatomía e histoquímica de las hojas de *Myracrodruon urundeuva* Allemão (Anacardiaceae).]

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Abstract

The "aroeira" (*Myracrodruon urundeuva* Allemão) is a tropical tree with limited geographic distribution in South America, being found in drier formations such as the *Cerrado* and *Caatinga*. Empirically it is used with antiseptic, antiinflammatory, antiulcer, antidiarrhoeal and others. In this study we used mature leaves and expanded from the third and fourth nodes. Studies venation and morphology, anatomy and histochemistry were performed by the usual laboratory plant anatomy or the usual techniques of plant anatomy. For histochemical study of the fresh cuts various reagents and specific stains were used. The blade is elliptical leaflets with acute apex, oblique base, obtuse angle, entire margin and slightly wavy. Shows the pattern of venation feather-veined, pinnate type based generally asymmetrical and oblique. The indumentum is sericeous with trichomes deciduous. The consistency of the lamina is papyracea. The cuticle of leaflets, is thinner on the lower epidermis than on the upper epidermis, palisade parenchyma with a cell layer and spongy parenchyma with three cell layers with idioblasts containing crystals of $CaCO_3$, tector trichomes simple multicellular with two or more cells are observed on both sides of the leaflets. The histochemical analysis revealed the presence of starch granules, crystals of calcium oxalate, fatty compounds, resins, phenolics and alkaloids compounds. The structural data obtained in this study may assist in ecophysiological characterization of the species and provide evidence for the identification of herbal medicines produced from that plant organ.

Keywords: *Myracrodruon urundeuva*; morphology; leaf anatomy; histochemistry.

Resumen

El "aroeira" (*Myracrodruon urundeuva* Allemão) es una especie de árbol tropical con una distribución geográfica limitada en América del Sur, se encuentra en formaciones más secas, como el Cerrado y Caatinga. Empíricamente se usa en la cicatrización, como anti-inflamatorio y otros. En este estudio hemos utilizado las hojas maduras y ampliadas a partir de los nodos de tercero y cuarto. Para los estudios de venación y la morfología, la anatomía y los procedimientos histoquímicos fue el laboratorio de anatomía vegetal de costumbre. Los estudios de venación y morfología, anatomía e histoquímica fueron realizados por el laboratorio habitual de Anatomía vegetal o por las técnicas usuales de la anatomía vegetal. Los folíolos son elípticos con ápice agudo, base oblicua margen de ángulo obtuso todo ligeramente ondulado. El indumento es seríceo con tricomas de hoja caduca. La consistencia de la lámina es papirácea. Muestra el patrón de venación rectinervia, tipo pinnadas basan por lo general asimétrica y oblicua. La cutícula de los folíolos, es más delgada en la epidermis inferior de la epidermis superior, parénquima en empalizada con una capa de células y el parénquima esponjoso con tres capas de células idioblastos con drusas de oxalato de calcio, tricomas tectores multicelulares simples con dos o más células en la base se observado a ambos lados del folíolo. El análisis histoquímica reveló la presencia de gránulos de almidón, cristales de oxalato de calcio, compuestos grasos, resinas, fenoles y alcaloides. Los datos estructurales obtenidos en este estudio pueden ayudar en la caracterización ecofisiológica de la especie y aportar pruebas para la identificación de los medicamentos herbarios producidos a partir de ese órgano de la planta.

Palabras Clave: *Myracrodruon urundeuva*; morfología; anatomía de hojas; histoquímica

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INTRODUCTION

The family Anacardiaceae Lindl. is represented by approximately 70 genera and 700 species (Souza and Lorenzi, 2005), presenting predominantly pantropical distribution with some species in temperate regions (Cronquist, 1981). This family is characterized by the presence of secretory canals or ducts resiniferous latex and terpenes, also showing tannin compound and calcium oxalate crystals in parenchyma and silica in some cells of the xylem tissue (Engler, 1896, Metcalfe and Chalk, 1950; Cronquist, 1981).

Myracrodruon urundeuva Allemão, popularly known as “aroeira-do-sertão”, is a tropical tree with geographic distribution, is restricted to South America, being found mainly in drier vegetation such as the “Cerrado” and the “Caatinga” (Rizzini, 1971; Lorenzi, 1992; Santin and Leitão-Filho, 1991; Florsheim, 1992; Pacheco et al., 2006). It features dense hard wood, with high concentration of tannins (Medina, 1966; Rizzini, 1971; Santos, 1987; Mainieri and Chimelo, 1989; Carvalho, 1994; Rizzini, 1995; Gonzaga et al. 2003), is included in the group of wood rot-proof timber (Nogueira, 1977). According to Medina (1966); Rizzini (1971), Nogueira (1977), Santos (1987); Mainieri and Chimelo, (1989); Carvalho (1994); Rizzini (1995) and Gonzaga et al. (2003) for its wood has all these qualities, is widely used mainly in rural areas in buildings such as fences and poles.

Together with other plant species with noble characteristics and high economic value, the "aroeira" has been widely exploited, leading to reduced size of natural populations, in many cases, extinction them completely (Brasil, 1992; Santos, 1993; Gonzaga et al., 2003; Freitas et al., 2005; Monteiro et al., 2010). Empirically, the "aroeira" is used to treat various illnesses, his bark is used as an antiseptic, healing, antiinflammatory, antiulcer, antidiarrhoeal, respiratory diseases and urinary tract (Matos, 1999; Gonzaga et al., 2003; Cabral and Carniello, 2004). Scientific studies have proven the anti-inflammatory, healing, antiulcer, anti-histamine, and analgesic antibradicinina, the shells of *M. urundeuva* (Viana et al., 1995; Rodrigues, 1999; Albuquerque et al. 2004). Other studies with out stem bark of *M. urundeuva* isolated tannin compound and chalcones - Urundeuvina A and B (Viana et al., 1995; Rodrigues, 1999; Albuquerque et al., 2004).

Moreover, a recent study Crivalero de Menezes et al. (2010), observed that the aqueous extract of M. urundeuva significantly reduced the oral biofilm formation of Streptococcus mutans in rats, and the accumulation of S. mutans and enamel demineralization.

It is estimated that about 75% pure natural compounds used in the pharmaceutical industry were isolated following recommendations of folk medicine (Yunes et al., 2001). However, research to develop new drugs are time consuming and costly. In order to insure access of the poor to drug compounds, the WHO since 1976 encourages the use of medicinal plants as part of programs for primary health care (Calixto and Yunes, 2001; Cardoso and Verdecia, 1997), due to their efficacy combined with a low operating cost, resulting from the facility for the acquisition of plants. The use of this feature is very useful in the communities where the medical-pharmaceutical assistance is deficient, as is the case of the Brazilian Northeast, the region where mortality rates have remained above the national average (Matos, 1999).

It is known that the morpho-anatomical parameters of the leaves assist in the identification of pharmaceutical inputs and verifying the authenticity of drugs, avoiding possible adulteration, ensuring the proper use of them (Zanetti et al., 2004), also resulting in improved quality production of the species studied.

This study aims to understand the morphology and leaf anatomy, and thus contribute to a basic understanding and characterization of the venation pattern of *M. urundeuva*. Moreover, the results of histochemical tests serve as a basis for identifying classes of active biomolecules which can be used in the manufacture of herbal medicines more accessible mainly to the less favored population.

MATERIALS AND METHODS

Plant material

In this study expanded from the third and fourth nodes and matue leaces were used of *M. urundeuva*. The collection of plant material occurred in New Farm Nova Franca, Santa Maria da Vitoria - BA in June 2007. After collection the leaves were kept at low temperature, and taken to the laboratory of Botany at the Catholic University of Brasilia.

The species was previously identified through literature, comparison with material deposited in the herbarium of the Universidade de Brasília (UnB) and the ecological reserve of the Brazilian Institute of Geography and Statistics (IBGE) and later by experts in taxonomic groups. The herbarium specimens were collected according to the usual procedure and are deposited in the herbarium of the Universidade Católica de Brasilia.

Morphologic studies

To study the venation, the leaves were cleared using techniques (Shobe and Lersten, 1967) with some modifications, with integral mounting of the leaves between two plates of glass, lacquer handicraft (Graciano-Ribeiro et al ., 2004, Paiva et al., 2006). The venation patterns and morphological analysis followed Ash et al. (1999) and Vidal and Vidal (2003).

The organographic study was carried out observations with the naked eye and magnifying glass using LEICA MZ6. For the anatomical study, microscope OLYMPUS Cx31 was used.

Anatomical studies

For the anatomical study transverse and longitudinal sections of median region and midrib of the leaflets were made which were made freehand with the aid of a cutting blade and a base of styrofoam. The sections were cleared in a solution of NaClO 2% according Shobe and Lersten (1967. The sections were washed in distilled water three times to remove the sodium hypochlorite. They were then stained with safranin/astra blue solution (Bukatsch, 1972).

To determine the type of stomatal apparatus were made in sections paradermic abaxial and adaxial surfaces with the aid of a cutting blade and forceps. The same sections were cleared in NaOH solution at 20% and 2% NaCl according Shobe and Lersten (1967), with some modifications. Thereafter, all the histological sections were mounted on semipermanent slides with glycerin gelatin Kaiser (Kaiser, 1880).

Histochemical studies

For the histochemical study were done freehand cuts, the middle region and midrib of leaflets. the sections were subjected to various reactive dyes. the reagents used were: ethanolic sudan iii and sudan iv for detection of long-chain fatty such as lipids and other lipophilic compounds, and suberized walls cutinized (Foster, 1942); ferric chloride (Johansen 1940) and potassium dichromate (Gabe, 1968) for phenolic substances; lugol for the identification of starch grains (Sass, 1951); dilute sulfuric acid to test the nature of the crystals (Johansen, 1940); formalin with ferrous sulfate for evidence tannins (Schneider, 1977). For detection of alkaloids was used dragendorf reagent (Yoder and Mahlberg, 1976), wagner's reagent and dittmar's reagent (Furr and Mahlberg, 1981).

The histochemical reactions were examined under a microscope OLYMPUS Cx31, and photographed with a digital camera attached to the eyepiece Sony DSC-P93. All photomicrographs were taken with digital camera Sony DSC-H1 coupled to said eyepiece microscope and magnifying glass. In all of the equipment ranges were obtained under the same conditions of optical images.

RESULTS

Leaflet and leaf morphology

M. urundeuva and has deciduous leaves are compound, petiolate, featuring a blade elliptic leaflets consistency papyraceous, acute apex, oblique base, obtuse angle, entire margin, with the absence of glands in limbo. The indumentum is sericeous, with multicellular trichomes that cover non-branched across the leaf surface. The average length of the leaf blade is 22.7 x10.4 cm, and the leaflets 6.4 x3.4 cm (Figure 1).

Shows the pattern of venation feather-veined, pinnate type. Venation of the second category is last with irregular marginal vein, the venation of the third category is dichotomized, the venation of the fourth category is regular polygonal reticulate. The veinlets are twice as white, the latter forming laciniosa entire marginal vein. Generally it has four ribs forming laciness (Figure 1).

Leaflet anatomy

M. urundeuva presents a blade to leaflet anfi-hypostomatic. Paradermic sight the epidemics cells present sinusoids anticline walls. In the epidermis directed toward the adaxial face they are gifts stomata varying between anomocitic and tetracitic, surrounding the ribbings, being the anomocitic found in most frequency. In the abaxial face stomata of the anomocitic type are found (Figure 2).

In transversal section is observed thin cuticle thinn both the faces. The epidermis is unistratified, being the adaxial and abaxial face formed by tabular cells, with stomata in the same level of the usual epidemics cells (Figure 2). The limb to leaflet presents heterogeneous, collateral blade, mesophyl, where parenchyma palisade possess an only cellular layer and the apongy three cellular layers. Vascular bundles of the collateral type of small diameter are found in mesophyl. Idioblasts with crystals of oxalate of calcium in form of star, is found occasionally in parenchyma palisade (Figure 2).

Figura 1. Morphology and anatomy leaflets of *Myracrodruon uruneduva*. **a**. Morphology and general appearance of the leaf. **b**. Diaphanized leaflet, explaining the pattern of venation. **c**, **d** and **e.** Details of the venation pattern.

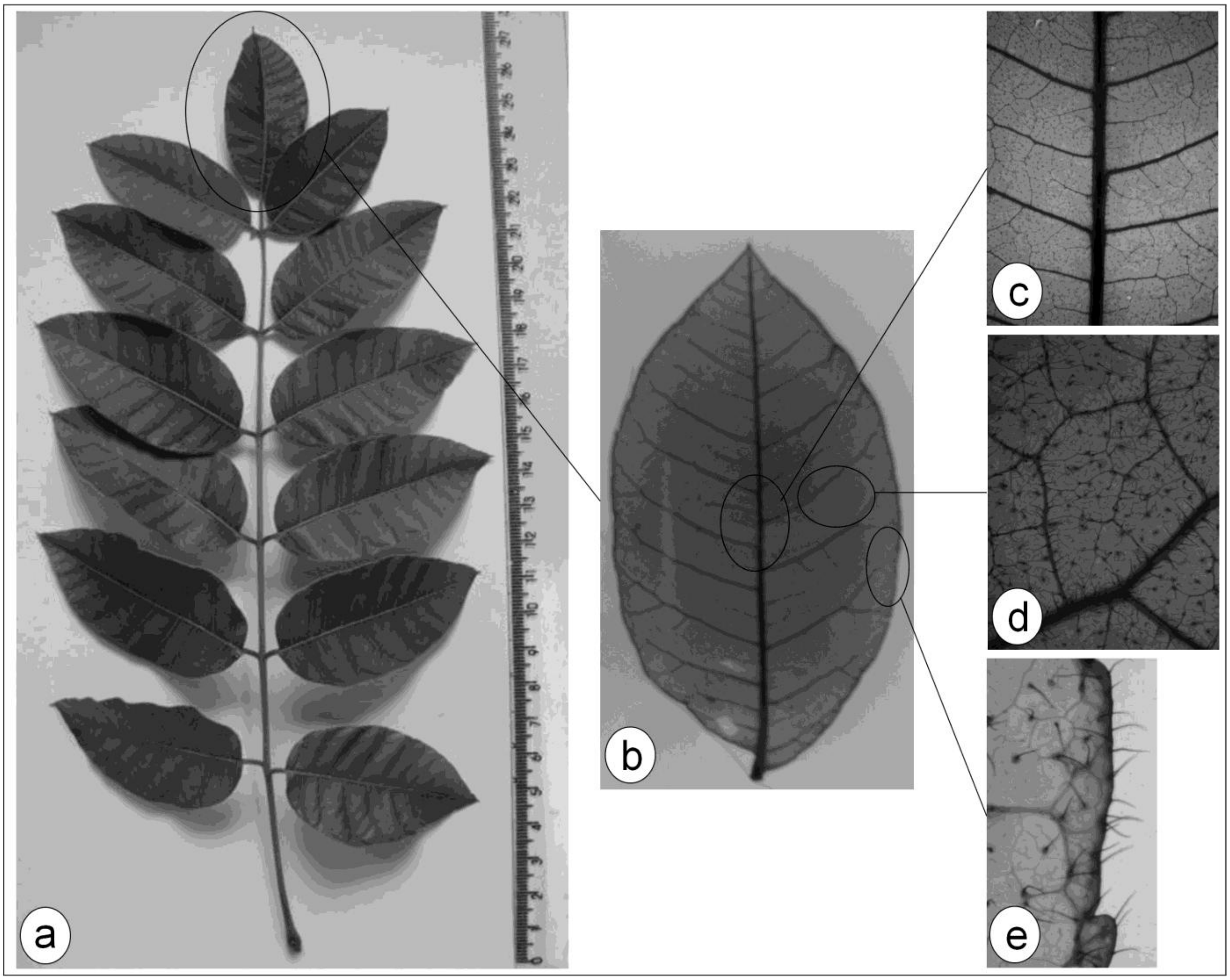
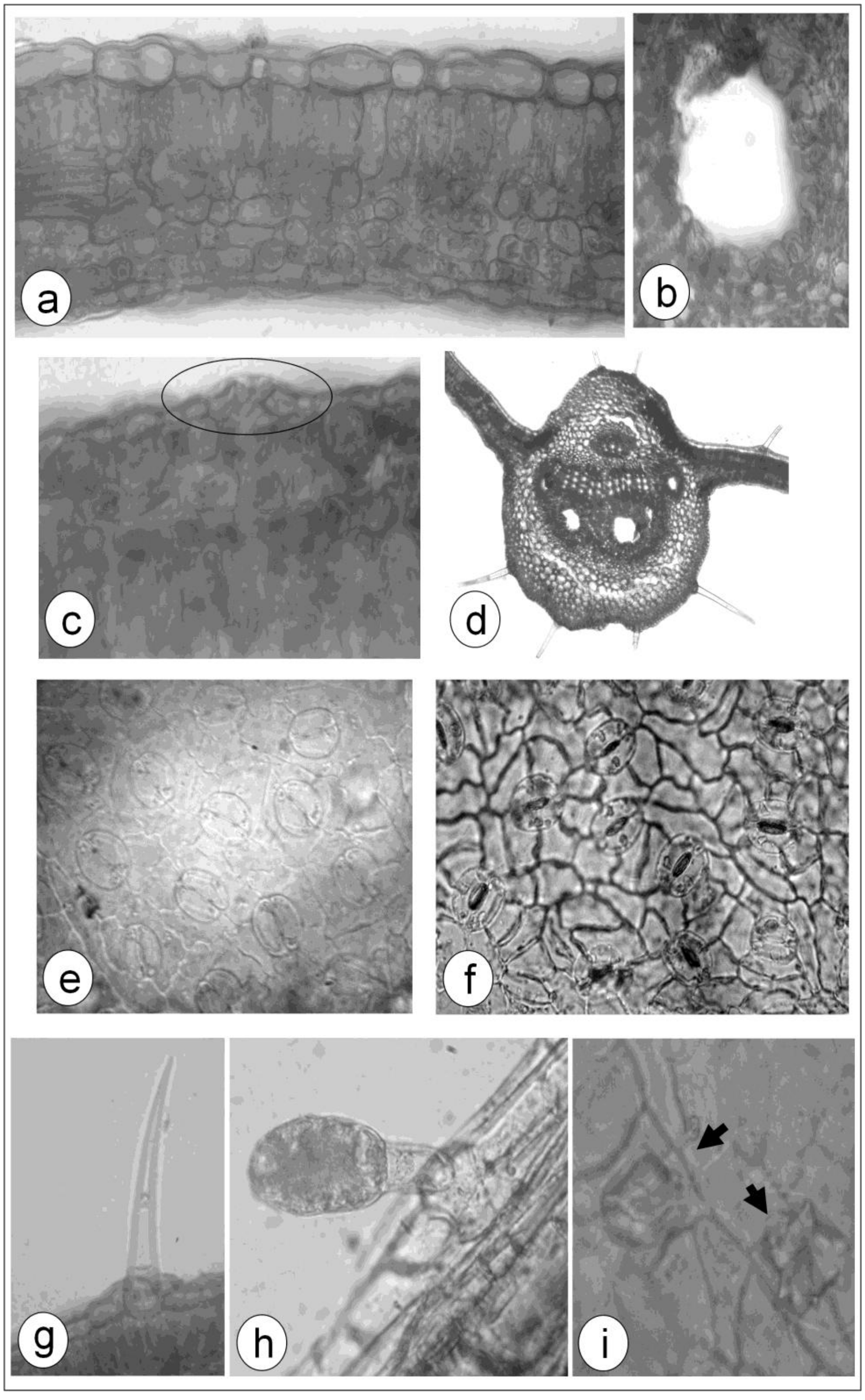


Figura 2. Sections transverse and paradermic of leaflets *Myracrodruon uruneduva*. **a**. Transverse section of middle third of the leaflets. **b**. Central vein. **c.** Leaflet stomata. **d**. Transversal section through leaflet midri. **e**. Transection of stomata on abaxial epidermis. **f**. Transection of stomata on adaxial epidermis. **g**. Non-glandular trichome. **h**. Glandular trichome. **i**. Aggregate crystals of calcium oxalate.



The main ribbing, in transversal section, presents a similar covering system with characteristics to the described ones for the remainder of the leaf. Below of the epidermis stratum of chollenchyma meets. Throughout the ribbing, is presented after lamellar parenchyma basic angular tending to lamellar in the abaxial and angular face in the adaxial face. The vascular system is formed by a vascular beam of the collateral type. Staple cellulosic fibers can be seen, next to the phloem and xylem. Involving the vascular beam an endoderm is observed. Secretory structures of the schizogenous type are found surrounding the vascular beam.

Leaflet Histochemistry

Histochemical analysis showed that there is a greater impregnation of fatty compounds in the cuticle toward the adaxial side (Table 1). Phenolic compounds were found in almost every tissue leaflets in different concentrations (Table 1). They are found in abundance in the palisade and impregnating the periclinal walls of trichomes and the anticlinal and periclinal of walls cell collenchymatic and in smaller amounts in the cells surrounding the secretory structures (Table 1). Tannins were found in all tissues leaflets in large quantities (Table 1).

Table 1. Composite localization analyzed in leaflets of Myracrodruon urundeuva Allemão, by means of specific Histochemistry tests.

Tissue/anatomical structure	**Phenolic composite**		**Alkaloids**	**Fatty compounds**	**Starch**
	Generalities	Tannins			
Cuticle	-	-	-	+++	-
Epidermes	-	+	-	+	-
Trichome	++	++	-	++	-
Fundamental parenchyma	++	++	++	+	++
Palisade parenchyma	+++	+++	+	+	+
Spongy parenchyma	+	+++	+	+	+
Vascular bundles	+	++	++	+	-
Collenchyma	++	++	+	+	+

(+ + +) strong positive reaction for the composition, (++) positive reaction, (+) weakly positive reaction (–) negative reaction.

The alkaloids were present in moderate quantities in the fundamental parenchyma and pervading the bundle-sheath of parenchyma, and in smaller amounts in collenchymatic tissue and palisade and spongy mesophyll (Table 1). Starch granules were found in small amounts mainly in the fundamental parenchyma (Table 1).

DISCUSIÓN

The morpho-anatomical data and histochemical are parameters that can be used in taxonomy and consequently assist in quality control of phytoterapics (Fank-de-Carvalho and Graciano-Ribeiro, 2005; Zanetti et al., 2004).

The morphological data presented to elucidate the species morpho-anatomical studies relevant to the botanical family Anacardiaceae cited by Cronquist (1981), Ribeiro et al. (1999), Martinez-Millian and Cevallos-Ferriz (2005). Among them, anomocytic stomata, trichomes simple, crystals of calcium oxalate and the presence of secretory structures in the midrib as well as studies conducted by Silva and Paiva (2007), which reveal that *Spondias tuberosa* Arruda has venation of the first and second category similar to those presented by *M. urundeuva*.

The presence of stomata on both sides of the blade leaflets may be related to environmental factors such as light intensity and relative humidity (Passos and Mendonca, 2006). According to Smith and colleagues (1997), this positioning of stomata reduces water loss by evapotranspiration. According to Pyykkö (1979) a greater abundance of stomata in abaxial prevent excess rainwater block the stomatal pore.

Transverse sections of leaves in *M. urundeuva* provide consistency to the analysis of the leaf with a thin cuticle, a list of environmental adaptation, since the characteristics leaflets are related to environmental characters. Silva and Paiva (2007), studying *S. tuberosa*, also observed a thin cuticle. Both *S. tuberosa* and *M. urundeuva* are in the same area soils and phytogeographical, thereby warranting the similarity between species, since the environment is a determining factor for the phenotypic expression of the vegetative organs.

Idioblasts with prismatic (druse and/or raphide) in the form of crystals also occur along the vascular system. In this sense, once more the characteristic of the presence of these crystals in the venation of the Anacardiaceae is seen, as alleged by Martinez-Millan and Cevallos-Ferriz (2005) and Silva and Paiva (2007).

Leaflets of lamina mesophyll dorsiventral heterogeneous characteristics are also mesomorphic species has. This fact can be explained as a result of the specimens was collected in the forest transition in western Bahia. In this region rain 700-1800 mm per year concentrated in 83% of the wet season from October to April, with luminosity around 3,000 hours per year (Pinto et al., 2006), features typical of mesophytic environment.

Vascular bundles of small caliber, which are found in *M. urundeuva*, can be interpreted as self-defense of the organism in the environment in which it is, since there are periods of drought, which would favor excessive loss of water in structures of thick bundles calibres. Such data can also be restated because the vascular bundle-being surrounded by parenchymatous sheath cells of light shot, which is nothing more than an endoderm cells that favors the xylem and phloem tissue of the water reserves in times of drought. Another relevant factor is the deciduousness. In general, when the drought is established, there is a drop leaf, allowing the plant, a survival without water loss. The presence of trichomes is another feature that provides the reflectance of light, avoiding high temperature inside leaflets, thus preventing the loss water (Valkama et al., 2003). These trichomes are thick anticlinal cell walls and they act as mirrors, reflectors of sunlight, since the cuticle is deposited on the epidermal appendage, favoring the incidence of light rays.

The histochemistry analysis, showed presence of fatty compounds on epidermal tissue, this compound promotes water retention, and prevents excessive loss of this. The lignification of auxiliar cells of trichomes indicates the same stiffness, which makes their removal via mechanical difficult, favoring the fixing the same.

Phenolic compounds play an essential role in regulating plant growth and interaction with other agents, and promote plant chemical defense against microorganisms, herbivores, pathogens, UV radiation, also possessing allelopathy (Croteau et al. 2000, Oliveira et al., 2003, Taiz and Zeiger, 2004). From the standpoint of pharmacological activity they are antiseptic, anaesthetic, anti-inflammatory, antioxidant, have gonadotropic action, choleretic, bile duct, antitumor, hipocholesterolemic, antipyretic and anti-flu (Diaz et al. 1999; Barbosa, 2004).

The alkaloids are considered the most important active compounds from the standpoint of pharmacology and medicine due to its physiological or psychological activity in humans, insects and other animals. They are often used as poisons, hallucinogens and stimulants (Salisbury and Ross, 1991). They have allelopathic action (Medeiros, 1990), antimicrobial, antifungal, and are toxic to some insects and mollusks (Robbers et al., 1996). Yet such compounds to provide bitter taste plant organs (Kuklinski, 2000, Henriques et al., 2002). According to Martins et al (1995), the alkaloids can be restricted to plant organs in different seasons of the year. According to Martin et al (1999), alkaloids have no definite action in plant organs, but Kuklinski (2000) cites a defensive action against pathogens, because they are toxic. Alkaloids have several pharmacological actions, some of them worth mentioning, as laxative, emetic, sedative for cough, antigout, antitumor (Cunha et al., 2005a) antimalarial, antispasmodic (Cunha et al., 2005b, Martin et al. 1999) stimulant, depressant central nervous system and hallucinogen (Cunha et al. 2005c; Kuklinski, 2000, Martin et al., 1999).

Tannins are compounds found in greater abundance and in all plant tissues. Such compounds provide protection against dehydration plant, rotting (Macedo et al. 2005; Harbone, 1993; Von Teichman and van Wyk, 1994, Rocha et al., 2002) and attack of pathogenic microorganisms (Scalbert, 1991; Trugilho, et al., 2003). Another function related to these compounds is the protection against attack by herbivorous animals, because it blocks the action of digestive enzymes and can reduce fertility in moths (Souza and Marquete 2000). Pharmacologically, the tannins have astringent, healing, antiseptic, antioxidant (Kuklinski, 2000; Cunha and Batista, 2005), vasoconstrictors, haemostatic (Cunha and Batista, 2005) and antiinflammatory (Raphael and Kuttan, 2003; Osadebe and Okoye , 2003). It is believed that there are other functions related to them, although there is evidence that the work (Rocha et al., 2002).

Tawaha et al. (2010) isolated new trimeric proanthocyanidin tannin, this compound was found to have a potent inhibitory effect on COX-2, and exhibited moderate inhibition against COX-1.

Queiroz et al. (2002) found high amounts of phenolic compounds in the wood of *M. urundeuva*, about 20.2% of income earned for the crude methanol were gallic acid. Moreover, these authors tannins found in the wood are mainly the type of proanthocyanidins.

CONCLUSIONES

The structural data obtained in this study may assist in ecophysiological characterization of the species and provide evidence for the identification of herbal medicines produced from that plant organ.

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