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Macro and microscopical identification of two *Acanthospermum* medicinal plants

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***Acanthospermum hispidum* and *Acanthospermum australe* (Asteraceae) are widely distributed in arid regions, in northeastern Brazil, and the first specie is used in folk medicine as anti-asthmatic while the second specie as antiseptic, anti-inflammatory and diuretic. They are often confounded and used indifferently for the same medicinal purposes due to both having similar morphological characteristics. The aim of this work was to carry out a macro and microscopical investigation to characterize the vegetative organs, including the macerate of both species. The plant material was collected, fixed and processed according to usual light microscopy techniques. A short-petiole in *A. hispidum* is a diagnostic trait that distinguish it from *A. australe*. Types of glandular trichomes can distinguish these two species. The different types of xylem vessels found in root, stem and leaf of both species identify the presence of one of them into raw drug. These findings will be useful in establishing pharmacognostic and phytochemical standards for identification, as well as assessment of accuracy to avoid contamination and insure quality control, which definitely is gaining relevance in plant drug research and establishment of plant monograph.**

Key words: *Acanthospermum hispidum*, *Acanthospermum austral*, anti-asthmatic, macerate, traditional medicine, folk medicine, Brazil.

INTRODUCTION

People around the world solve health problems using fresh and dried plant parts, and some of these plants are acquired in the market without accurate botanical identification. This accuracy is a result of an authentication through the description of parts of plants used by people. The knowledge of macro and microscopical traits of root, stem and leaf is fundamental for the standardization of plants used as medicines to contribute to the Brazilian Pharmacopoeia. The anatomical characters are extremely valuable to the taxonomical identification, and many species of Asteraceae family have been investigated in consequence of its wide distribution (Adedeji and

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Jewoola, 2008). Medicinal species of Asteraceae have been highlighted through secretory substances as essential oils which are accumulated inside glandular trichomes, and can be distinguished in a level of specie (Costa et al., 2001; Adedeji and Jewoola, 2008).

Frequently, Brazilian communities use traditionally, many plant species as phytomedicine and identify it merely through the common names, and this can lead to serious health problems. Two species of *Acathospermum* (Asteraceae) are easily mixed up in consequence of the morphological semblance of individuals of *Acanthospermum hispidum* and *Acanthospermum australe* (Loef) O. Kuntze and have the same vulgar names. Both species are known as “espinho-de-cigano”, “carrapicho-de-carneiro” and “carrapicho rasteiro”, and are native and occur in abundance at the same areas of arid regions in northeastern Brazil (Araújo et al., 2008). The common name “espinho-de-cigano” is in consequence of the sticky traits of fruit without technical information strictly attributed to each species. The roots of *A. hispidum* are indicated by local people as phytomedicine for treating dysentery, cough, bronchitis and usually allergic bronchitis with expectorant properties (Novy, 1997; Lorenzi, 2002; Diniz et al., 1997). A syrup thick, viscous liquid consisting primarily of a solution of sugar in water made with root fragments of *A. hispidum* is market-up by more than 20 years (Araújo et al., 2008).

Despite the recognized success of this syrup and easy mistake between these two species, adequate information about them that assist and ensure the quality control does not exist in the literature. Aiming to guarantee the correct taxonomical identification, ensuring the genuine-ness, and contributing to the quality control of plant material used as phytomedicine, descriptions of macro-scopic and microscopic traits are made to distinguish individuals of *A. hispidum* and *A. australe*.

MATERIALS AND METHODS

Individual plant parts of *A. hispidum* DC. were collected at the Laboratório de Fitoterapia da Prefeitura de Olinda, Pernambuco, Brazil, in March, 2006, and individuals parts of *A. australe* (Loef) O. Kuntze were collected on Campus at the Federal University of Pernambuco, Brazil, in April, 2006. The vouchers are in the Herbarium IPA (Dárdano de Andrade Lima) (IPA – 73350 and 73437, respectively). Expanded leaves of both specie, without apparent damage, were fixed in FAA 50 (formaldehyde, acetic acid, ethanol, water, 5:5:45:45, Merck) for 48 h. Semi-permanent slides were prepared according to standard techniques (Johansen, 1940); the transversal sections were obtained with common handmade razor blade, stained with safranin and Astra blue (1% aqueous solution) for 15 min and mounted in glycerin 30% (Johansen, 1940).

To study the cell size variation in xylem fibre and vessel elements, small transversal slices of the roots and stems were macerated in a solution of 10% chromic and 10% nitric acid by conventional Jeffrey’s method described in Kraus and Arduin (1997). All chemicals were obtained commercially (Merck), astra blau and and safranin (Sigma Aldrich). The measurements of cell size and polar length of stomata in transversal and paradermal sections, respectively were made using a digital analysis program image tool (Wilcox et al., 2002) with digital images captured in a compound light microscope (Opton) with a CCD camera (Samsung). The bars in the digital images were determined from images of a micrometer slide under the same conditions as for the plant section images. The descriptions of anatomical structures were based on Metcalfe and Chalk (1950, 1988), and the stomata classification followed Baranova (1987). The anatomical procedures, including the production of semi-permanent slides, digital images capture and posterior analysis were made in the Functional Phytomorphology Laboratory-LAFF at the Universidade Federal Rural de Pernambuco-UFRPE.

RESULTS

Morphological traits

Acanthospermum hispidum

The herb is about 1 m tall; root pivotant up to 20 cm depth with slight sweet odor of about 30 min after the sampling (Figure 1a). The stem and branches are densely pubescent, cylindrical, green when young and become brown in adult plants. Leaves, subsessile, entire, simples, opposite and pubescent are without appendixes; blade is oval and penninerved; the shape is elliptic to oblong and symmetrical, acute to attenuate at the base, acute to acuminate at the apex, margin is entire, slightly wavy, the midrib is prominent beneath, with 7 to 8 pairs of lateral veins. Inflorescence capitule axillar shows some yellow flowers. Flowers are unisexual, fruits are achenium with a triangular and elongated shape, covered by varying bristle.

Acanthospermum austral

The herb is about 20 cm tall; root pivoting to 20 cm depth with strong and sweet odor immediately after the sampling (Figure 1b). The stem is prostrated and densely branched, cylindrical. Leaves are short-petiolated, entire, simple, opposite and pubescent without appendixes; blade is oval, penninerved; the shape is elliptic to oblong and symmetrical, acute to attenuate at the base, acute to acuminate at the apex, margin is serrate, the midrib is prominent beneath, with 7 to 9 pairs of lateral veins; petiole is about 2 to 3 mm long and 1 mm wide. Inflorescence capitulum axillar shows some yellow flowers covered by membranaceous bracts. Flowers are unisexual. Fruit achenium takes a triangular form, elongated, and covered by varying bristle. The morphology description of *A. australe* corroborates Martins et al. (2006). The presence of an extremely short petiole in leaves of *A. australe* differentiated it from *A. hispidum*.

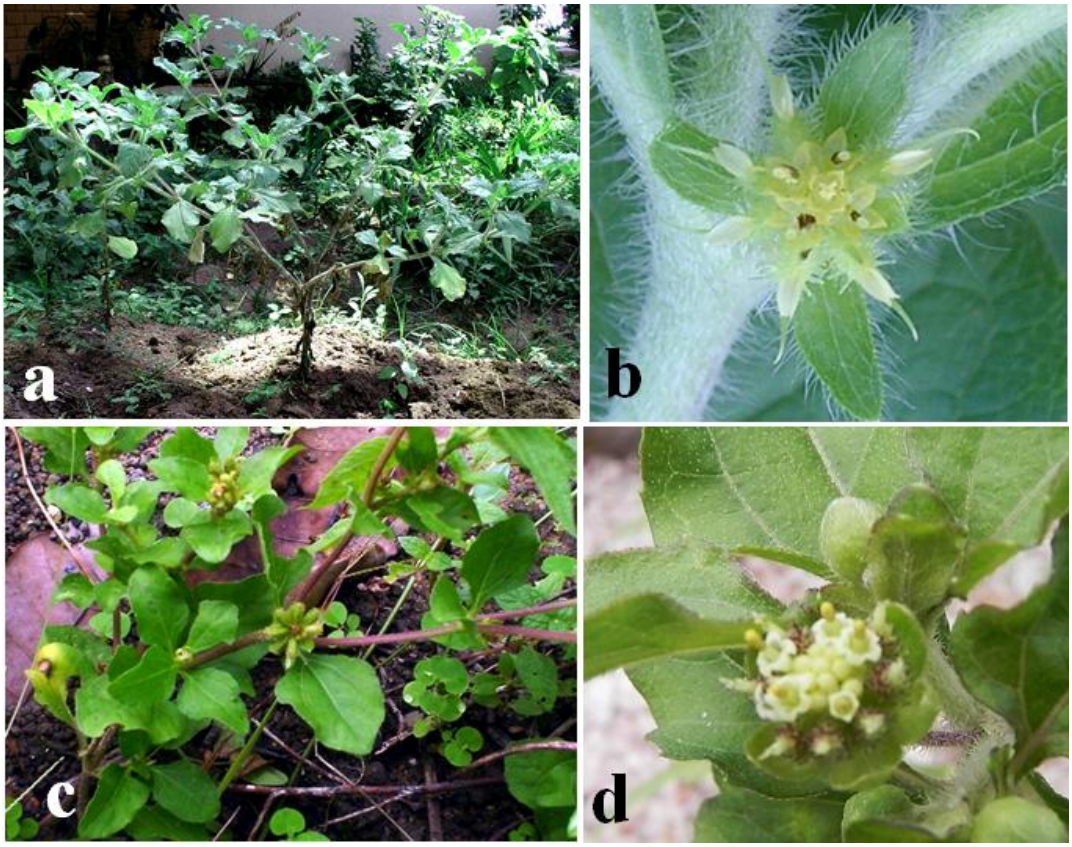

Figure 1. Individuals of *Acanthospermum*. (a and b) *A. hispidum* DC.; (c and d) *A. australe* (Loef) O. Kuntze.

Morphological comparisons

1. Common traits: annual herbs, ramified and pubescent; leaves are entire, simple, opposite, membranous, pubescent without appendixes; blade is oval and penninerved.
2. Non-shared: *A. hispidum* is erectile (50 to 90 cm tall) with subsessile leaves with narrowly decurrent margins, while *A. australe* is prostrate with long-petiole leaves; the basal region of the lamina of *A. hispidum* narrows apparently to an alate petiole. Margin regularly serrates. There are male and female flowers in *A. australe* while female and hermaphrodite flowers in *A. hispidum*. Fruits are achenium elliptic (2.6 to 2.9 mm wide), pappus is absent, elliptical seeds in *A. australe* and triangular (4.7 to 5.6 mm long and 2 to 2.4 mm wide), pappus aristate, spatulated seeds in *A. hispidum*.

Anatomical descriptions

Acanthospermum hispidum

Root: It is cylindrical. In a primary structure, it is covered with an unstratified epidermis, the cortex consists of rounded parenchymatous cells and the medullar area is composed of primary xylem cells (Figure 2a). On a secondary structure, the periderm replaces the epidermis (Figure 2b), the area of the cortex almost exclusively consists of periderm cells, medullar area is composed of secondary xylem cells, and the root is polyarch. On both structures, the endodermis cells showed Casparian strip without “U” thickening. The root vascular cylinder is polyarch stele comprising a uniserial pericycle, more than five strands of both primary phloem and primary xylem, and pith with primary xylem cells.

Stem: It is cylindrical (Figure 2c) and covered by a uniserial epidermis (Figure 2d) with prominent cuticle and tector and capitate glandular hairs, and a pluricellular stalk and one vesicular cell topped. Immediately under the epidermis, there is an angular collenchyma (Fig. 2d). Collateral vascular bundles are inside in a fundamental parenchyma and consist in a circular line (Figure 2c). The vascular bundles have a group of fiber cells over the phloem cells. In the proximity of the vascular bundles, there are small secretory ducts (Figure 2e) close to the

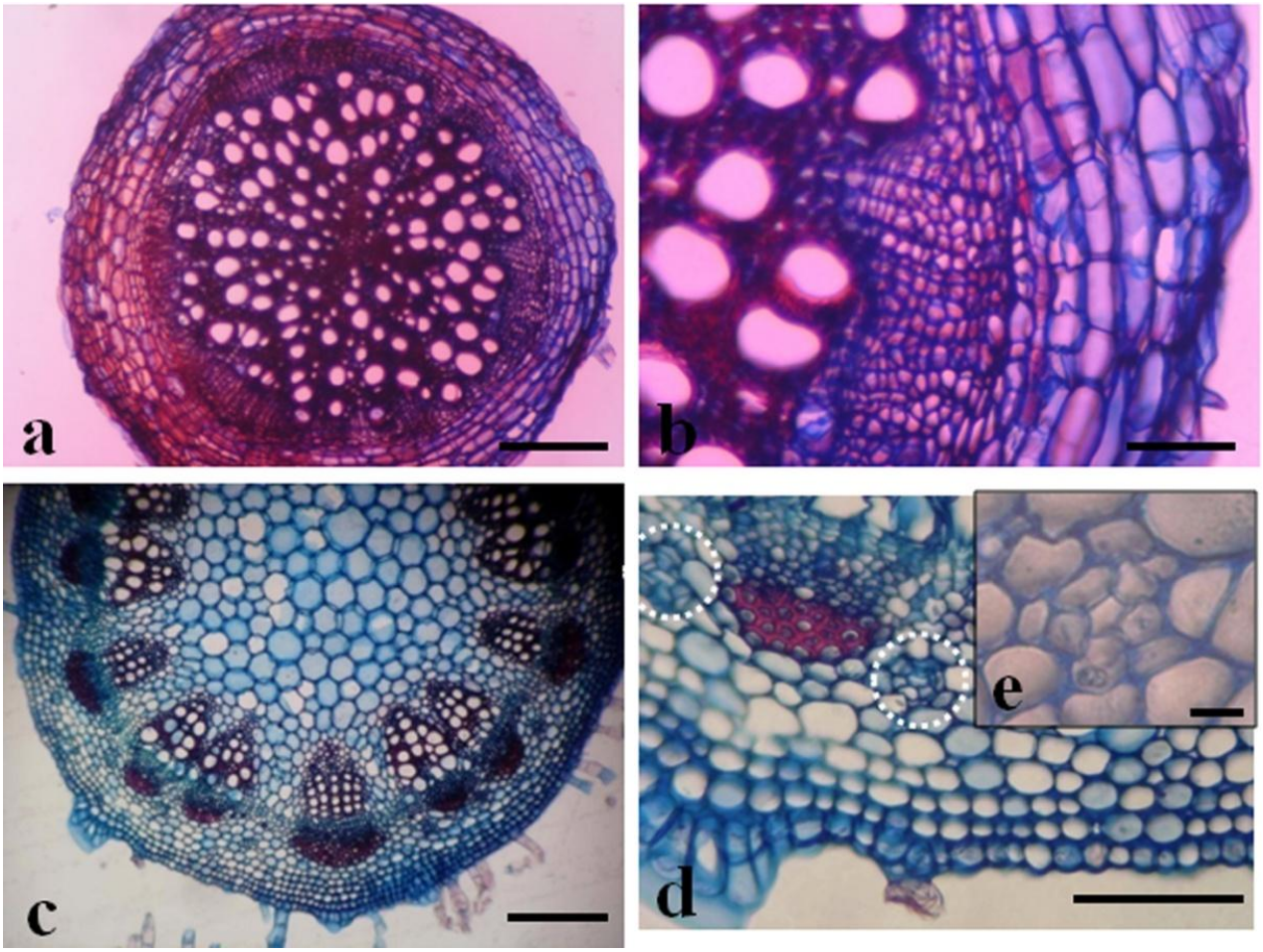

Figure 2. Transverse sections of root and stem of *A. hispidum*: (a) Primary structure in root; (b) secondary structure in root; (c) vascular strands; (d) secretory duct (dotted circle) among vascular bundles; (e) secretory ducts (arrows). Scale bars: (a,b) 100 µm; (c) 200 µm; (d) 100 µm; (e) 50 µm.

phloem. The lumen of these ducts is delimited by a uniserial epithelium, consisting of five diminute and rounded cells (Figure 2e).

Leaf: It shows a dorsiventral mesophyll (Figure 3a), with only one layer of palisade parenchyma. The spongy parenchyma cells are located next to the abaxial epidermis, with 4 to 5 layers of cells densely arranged, with-out intercellular spaces among them. The main nerve (Figure 3b), very protuberant on both surfaces, is strengthened by angular collenchyma. Three collateral vascular bundles are into a fundamental parenchyma. In the proximity of these bundles, there are small secretory ducts close to the phloem or xylem (Figure 3b, arrows). The lumen is delimited by a uniserial epithelium consisting of five minute flattened cells. The leaf shows a unistratified adaxial epidermis; the upper epidermis shows stomata slightly above the level of the epidermis cells (Figure 3a and d, dotted arrow). There are plentiful multicellular trichomes, glandular and capitates (Figure 3a and d, solid arrow) and tector (Figure 3c and d).

Surface view of leaf epidermis: There are anomocytic stomata on both faces (Figure 3c and d), and they are more abundant in the abaxial surface (Figure 3d), and the polar length measuring 23.67 and 27.04 µm in the adaxial and abaxial surfaces, respectively. The epidermal cells of both surfaces show sinuous walls, further accentuated in the abaxial face. Both face show epidermis with multicellular tector trichomes and uniserial trichomes with glandulous capitate cell at the top (Figure 3c and d).

Acanthospermum australe

Root: The pattern of the structure is similar to that observed in *A. hispidum* showing periderm (Figure 4a

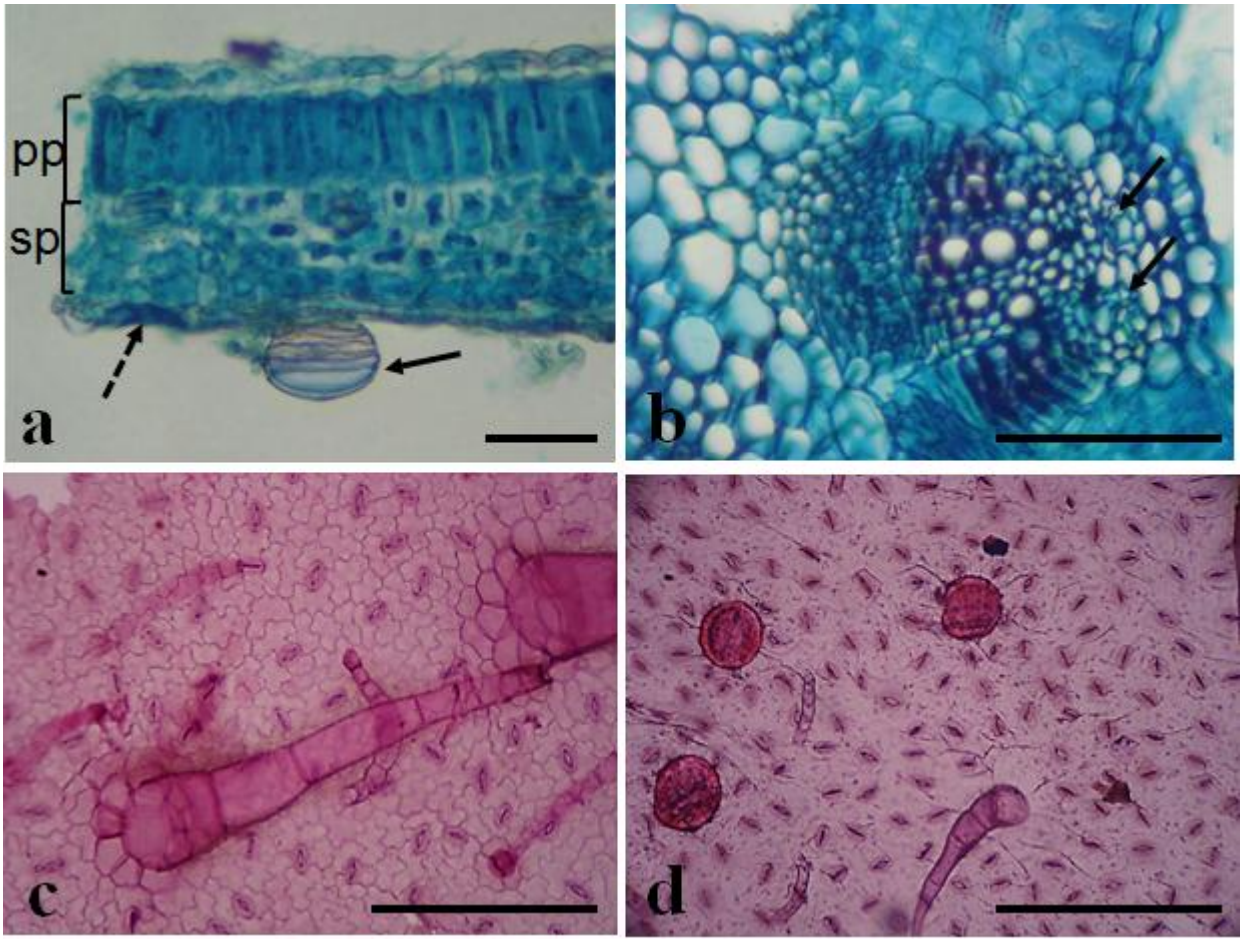

Figure 3. Leaf of *Acanthospermum hispidum*. (a) Transverse section showing dorsiventral mesophyll with only one layer of palisade parenchyma (pp) and 4 to 5 layers of spongy (sp), and stomata slightly prominent (dotted arrow) and glandular and capitates trichome (solid arrow); (b) transverse section showing main vein with small secretory ducts close to the phloem or xylem (arrows); (c and d) paradermal section showing of adaxial and abaxial surface, respectively, showing anomocytic stomata and tector trichomes.
Scale bars: (a to d) 50 µm.

and b) and polyarch organization (Figure 4a). The morphoanatomical characterization of the leaf and stem structure (Figure 4c and d) of *A. australe* corroborate Martins et al. (2006).

Leaf: It shows a dorsiventral mesophyll (Figure 5a), with only one layer of palisade parenchyma. The spongy parenchyma cells are located next to the abaxial epidermis, with 4 to 6 layers of cells densely arranged, without intercellular spaces among them. The main nerve (Figure 5a), very protuberant, on both surfaces, is strengthened by angular collenchyma. The leaf shows a unistratified adaxial epidermis; the upper epidermis shows stomata slightly above the level of the epidermis cells. Figure 5a and d shows the epidermis with plentiful multicellular trichomes, glandular and capitates and tector.

Surface view of leaf epidermis: There are anomocytic stomata on both faces (Figure 5c and d), and they are more abundant in the abaxial surface (Figure 5d), and the polar length measuring 102.97 and 92.82 µm, in the adaxial and abaxial surfaces, respectively. The epidermal cells of both surfaces show sinuous walls, further accentuated in the abaxial face. Both face show epidermis with multicellular tector trichomes and uniserial trichomes with glandulous capitate cell at the top (Figure 5c and d).

Trichomes and stomata in *A. hispidum* and *A. australe*

The stem of both species and the leaf of *A. australe*, on both surfaces, show simple tector trichomes uniserial of

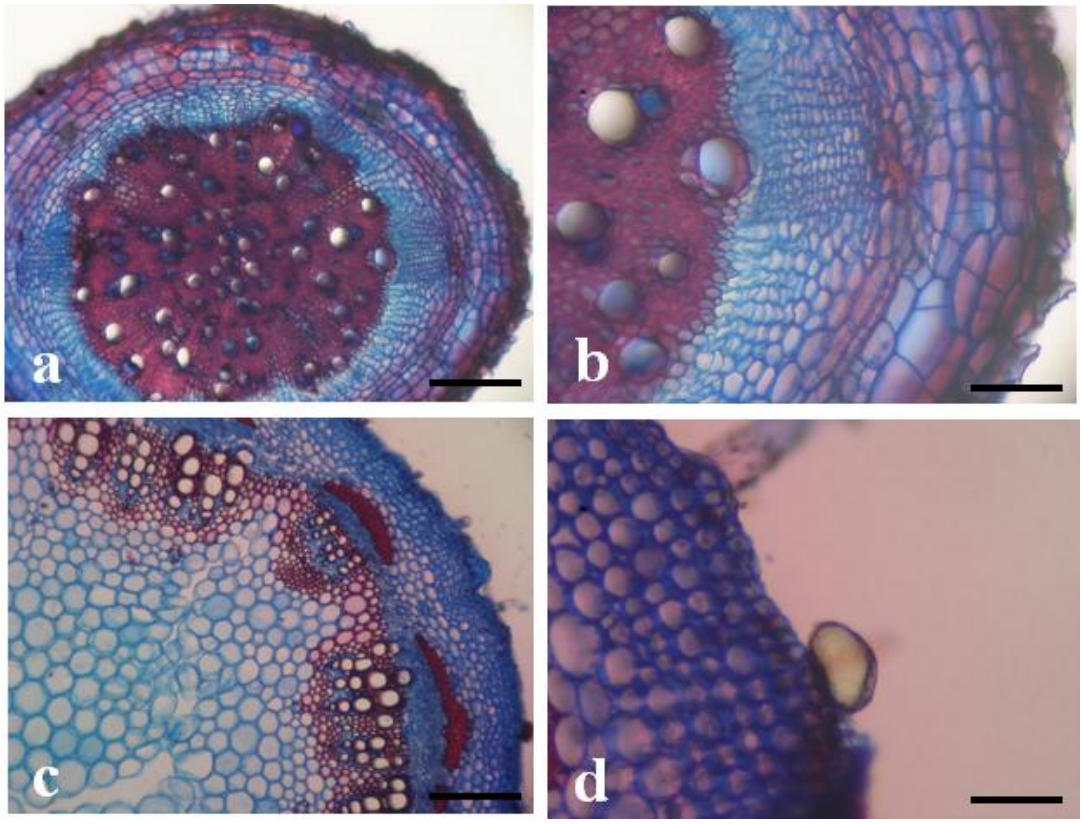

Figure 4. Transverse sections of root and stem of *A. australe*: (a) Secondary structure in root; (b) detail of cortex and vascular tissues in root; (c) transversal view of stem; (d) detail of cortex in stem, showing glandular trichome and angular collenchyma.
Scale bars: (a to d) 100 µm.

varied sizes, with an apical sharp cell (Figure 6a) and different types of glandular trichomes. In stem and leaf of *A. australe*, glandular trichomes are sessile and unicellular with a spherical shape (Figure 6b). In leaves of *A. hispidum*, glandular sessile trichomes are pluricellular with an apical spherical shape (Figure 6c). In the stem of *A. hispidum*, glandular stalked trichomes show 2 to 3 cells in the stalk with one round vesicular cell on the top (Figure 6d). Tector and glandular trichomes, sessile uniserial with a spherical shape does not exist in leaves of *A. hispidum*. *A. hispidum* shows anomocytic stomata on both leaf surfaces (Figure 3d to e), like the *A. australe*. However, these structures have different sizes of polar length, 23.67 and 27.04 µm in *A. hispidum* and 40.57 and 45.00 µm in *A. australe*, in the adaxial and abaxial leaf surfaces, respectively.

Macerate of *A. hispidum* and *A. australe*

Macerate of vegetative organs between these two species showed distinct cellular elements: slender fibers (sf), parenchymatous cells (pc) and xylem vessels (Figure 7). Slender fibers are similar in length and width in root and stem and show different sizes in stem and leaves of both species (Table 1). These cells are shorter (43%) in the stem of *A. australe*, and in the leaf (28%) of *A. hispidum* (Table 1). The width of these cells is similar to the root and stem of both species and comparing the leaf of both species, the range is more than five-fold in *A. australe* (Table 1).

Parenchymatous cells are longer and wider at the stem of *A. australe* (322.80 to 158.11 µm, respectively), shorter at the blade (29.31 µm) and narrow at the root of *A. hispidum* (24.79 µm). The xylem cells (Figure 7) show different traits that permit classifying it into three types. Type 1- cells with both end walls straight (Figure 7a and b), type 2- cells with one straight end wall and one transverse end wall without tails (Figure 7c and d) and type 3- vessels with both tangential end walls diametrically opposite with tails (Figure 7e). These three types of cell were observed in both species varying among root, stem and leaves; type 3 is missing in root and stem of *A. australe* and in leaves of *A. hispidum*, and

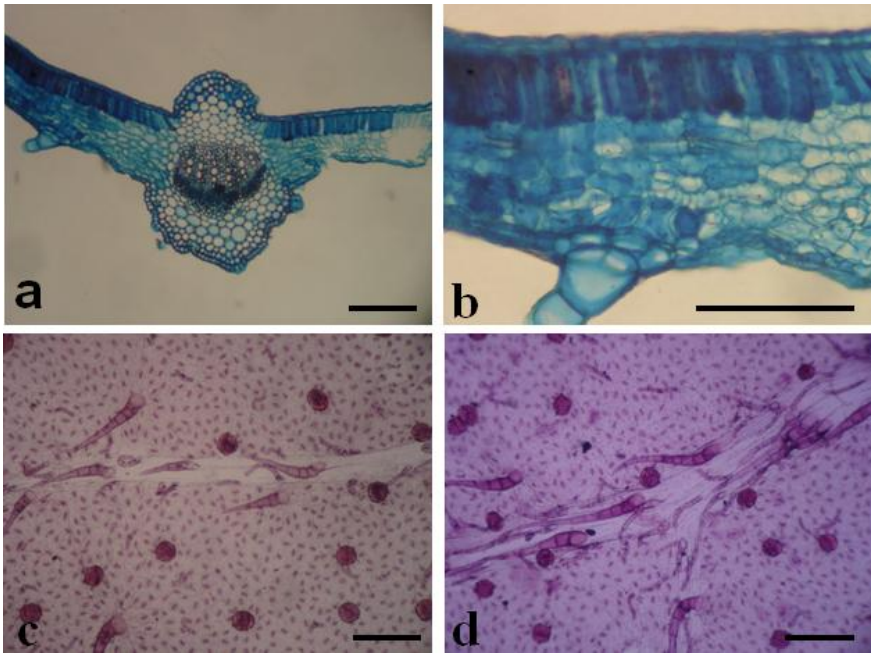

Figure 5. Leaf of *Acanthospermum australe*. (a) Transversal section of leaf, showing the main vein; (b) detail of dorsiventral mesophyll; (c and d) adaxial and abaxial surface of epidermis, respectively, showing anomocytic stomata and tector trichomes. Scale bars: (a to d) 50 µm.

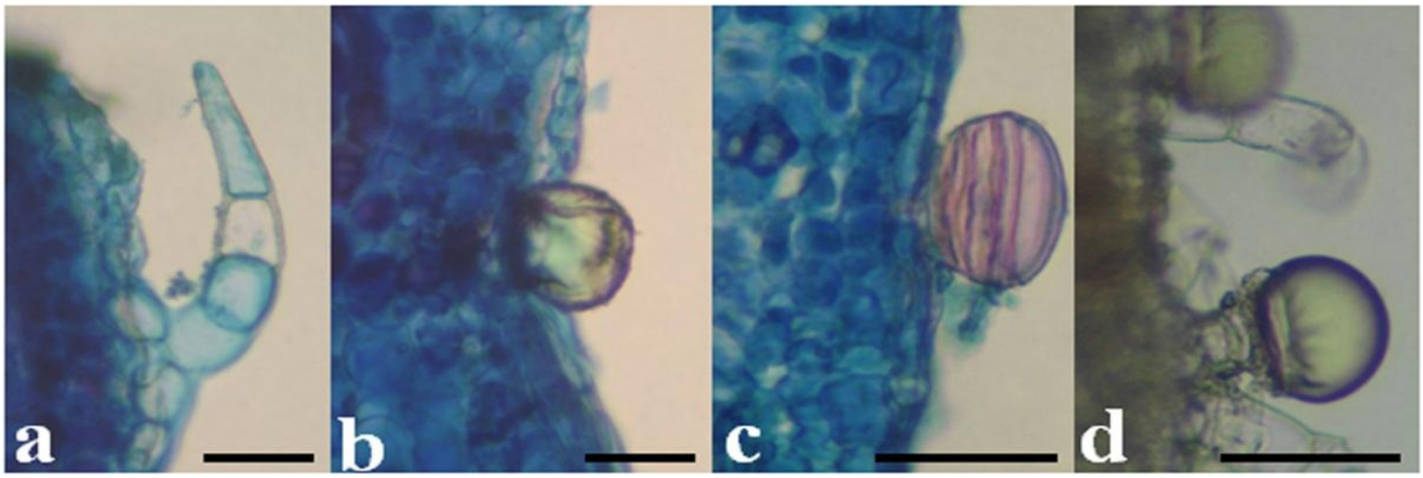

Figure 6. Trichomes of Acanthospermum. (a) Simple, uniseriate trichome with different size and an apical acute cell in the stem of A. hispidum and A. australe and in leaves of A. australe; (b) glandular trichome sessile, unicellular and capitate in the stem and leaf of A. australe; (c) trichome sessile, multicellular, uniseriate, with an apical spherical cell in the leaves of A. hispidum; (d) glandular trichome stalked with 2 to 3 cells in the stalk with one vesicular and rounded cell in the stem of A. hispidum.
Bars: (a to d) 50 µm.

type 2 was not found in leaves of *A. australe* (Table 1). All these cells, independent of type, show scalariform cell wall ornamentation (Figure 7f). The size of these vessels varied among the organs and between the species; type 1 showed two sizes of length in root to *A. hispidum*, with 50% difference between them (Figures 7a-f). Excepting the type 2 in the root of *A. hispidum*, all types of vessels were longest in *A. australe* reaching around 8% in type 1

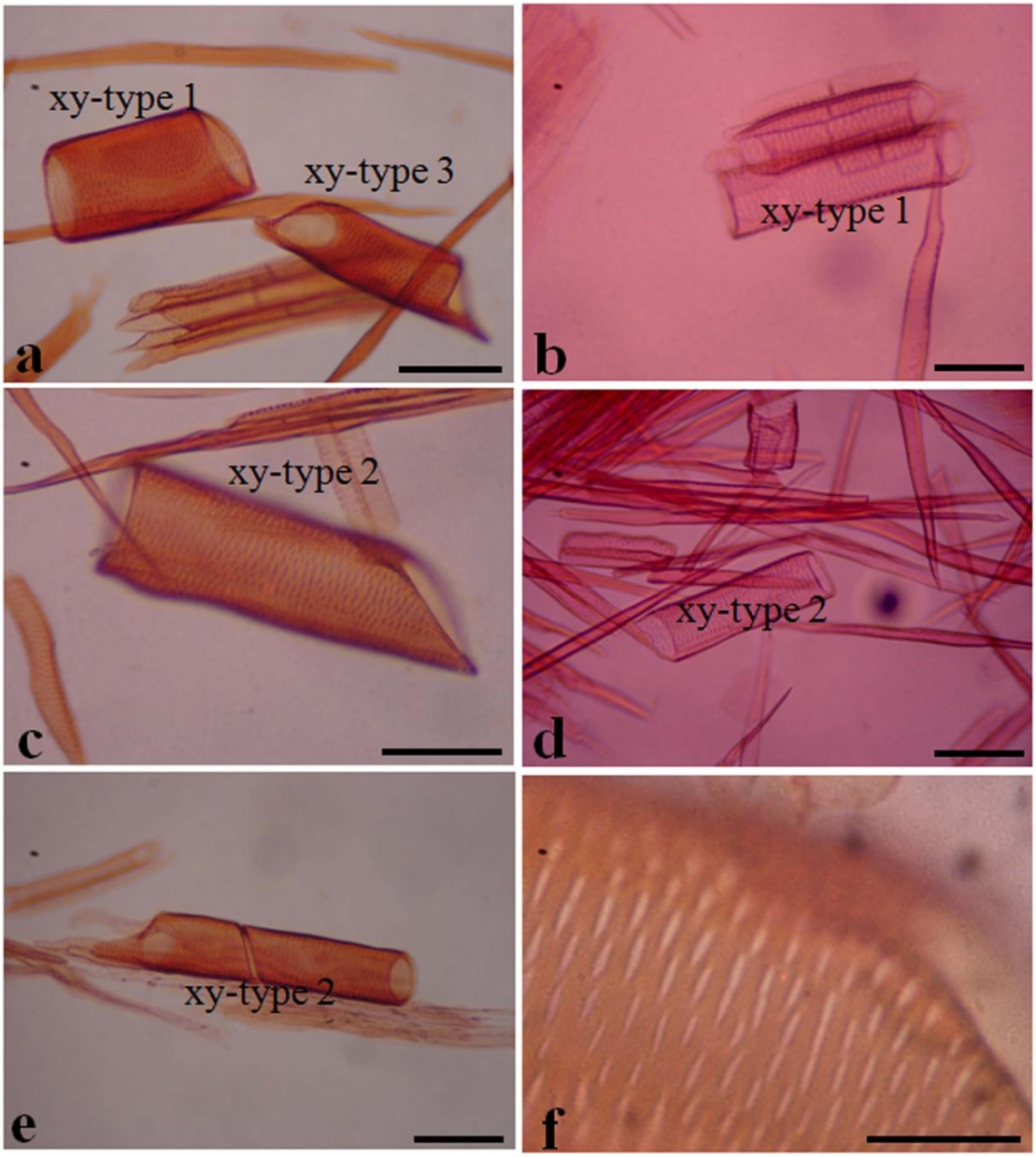

Figure 7. Maceration of *Acanthospermum hispidum* and *A. australe* root showing xylem cell types. (a) Type 1 and 3 in *A. hispidum*; (b) type 1 in *A. australe*; (c) type 2 in *A. hispidum*; (d) type 2 in *A. australe*; (e) type 2 connected in *A. hispidum*.; (f) cell wall showing scalariform ornamentation in *A. hispidum*.
Scale bars: (a to e) 100 µm, (f) 50 µm.

at the leaves of *A. hispidum* (27.16 µm) compared with *A. australe* (311.35 µm).

DISCUSSION

Some morphological traits can help, at first glance, to distinguishing individuals of *A. hispidum* and *A. australe*. Differences in plant height (1 m and 20 cm, respectively) and the length of petiole (subsessile and short, respectively) can identify both species, but despite the fact that these traits can be genetically fixed (Auld and Morrison, 1992), they are insufficient to guarantee the distinction of species.

Table 1. Qualitative traits and dimensions of macerate cells from root, stem and leaves of *A. hispidum* and *A. australe*.

Trait	*Acanthospermum hispidum*	*Acanthospermum australe*
Root		
Slender fibers	Longitudinally elongated cells: 609.70 µm long and 19.57 µm wide	Longitudinally elongated cells: 531.43 µm long and 18.15 µm wide
Parenchymatous cells	Elongated cells: 81.45 µm long and 24.79 µm wide	Cubic cells: 69.70 µm long and 54.41 µm wide
Xylem vessels types	Cells with both straight end walls and two sizes: longer (252.05 µm) and shorter (142.03 µm) length and width-like (110.96 µm and 102.68 µm, respectively)	Cells with both end walls straight: 408.62 µm long and 116.24 µm wide
	Cells with one straight end wall and one transverse end wall without tails: 505.58 µm long and 151.30 µm wide	Cells with one straight end wall and one transverse end wall without tails: 410.02 µm long and 115.74 µm wide
	Cells with tangential end walls at opposing angles with tails: 280.23 µm long and 72.73 µm wide	None
Stem		
Slender fibers	Longitudinally elongated cells: 773.20 µm long and 16.15 µm wide	Longitudinally elongated cells: 437.49 µm long and 20.76 µm wide
Parenchymatous cells	Cubical cells: 92.16 µm long and 74.75 µm wide	Elongated cells: 322.80 µm long and 158.11 µm wide
Xylem vessels types	Vessels with both straight end walls: 191.71 µm long and 30.38 µm wide	Vessels with both straight end walls: 632.81 µm long and 168.11 µm wide
	Vessels with both tangential walls at opposing angles without tails: 373.34 µm long and 45.00 µm wide	Vessels with one straight end wall and one transverse end wall without tails: 643.69 µm long and 72.50 µm wide
	Long vessels with both tangential end wall in opposing angles with tails: 482.55 µm long and 25.50 µm wide	None
Leaf		
Slender fibers	Longitudinally elongated cells: 278.90 µm long and 6.07 µm wide	Consisting of longitudinally elongated cells: 997.87 µm long and 31.08 µm wide
Parenchymatous cells	Isodiametrical elongated cells: 29.31 µm long and 30.70 µm wide	Elongated cells: 75.85 µm long and 42.68 µm wide
Xylem vessels types	Vessels with both transverse end walls: 27.16 µm long and 29.03 µm wide	Vessels with both straight end walls: 311.35 µm long and 60.26 µm wide
	Vessels with one straight end wall and one transverse end wall without tails: 254.76 µm long and 46.14 µm wide	None
	None	Vessels with both tangential end walls with tails: 538.54 µm long and 26.03 µm wide

Data from the literature do not disclose any details about the root structure for *Acanthospermum* species. Martins et al. (2006) was the only reference to the stem and leaf anatomy of *A. australe*. The same is true for data on the macerate of crude drug and the histochemistry of primary metabolic classes for *Acanthospermum* species.

In consequence, the characterization of the plant morphology, the crude drug and the histochemistry of *A. hispidum* is at the first time described. Both species show similar stele root structure, polyarch, common to different families of angiosperm, described for Asteraceae by Machado et al. (2004).

The stem of *A. hispidum* showed glandular stalked trichomes like those found by Mirashi and Bhogaonkar (1974) in types from India. Martins et al. (2006) found trichomes uniseriate, multicellular with a globular form in *A. australe* that was missing in leaves of *A. hispidum*. The types of trichomes seems to be the key factor for distinguishing these species. The presence of trichomes sessile and unicellular with a globular structure in stem and leaves of *A. australe* and trichomes sessile, glandular and multicellular with a globular form, and trichomes 2 to 3 stalked with one-topped round vesicular cell in leaves of *A. hispidum* can be used as diagnostic characters to distinguish both species.

Conclusion

The anatomical and crude drug characterization are the first report for root, stem and leaf of *A. hispidum* DC. and the anatomical description of root and crude drug for root, stem and leaf are the first report for A. *australe* (Loef) O. Kuntze. The association of morphological and anatomical traits of *A. hispidum* and *A. australe* can distinguish them. Individuals erectile with subsessile leaves and narrowly decurrent margins, root with xylem cells with tangential end walls at opposing angles, trichomes sessile, glandular and multicellular with a globular form, and trichomes 2-3 stalked with one-topped round vesicular cell in leaves can identify individuals of *A. hispidum*. It is emphasize that the trichome types can be used to distinguish both species and the identification can be easily obtained through a simple microscopical analysis. The results indicate that investigating the trichome types in other species of this genus can distinguish the species. It will be particularly interesting to the quality control of fragmented plant material used as phytomedicine.

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