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Original article

Morpho-anatomical study of *Ageratum conyzoides*

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ABSTRACT

Ageratum conyzoides L., belonging to the family Asteraceae, is a tropical plant found in some regions of Africa, Asia and South America. This species is popularly known as billy goat weed, "mentrasto" and "catinga-de-bode" and has a large variety of secondary metabolites and biological activities mentioned in the literature. The objective of this work was to contribute the pharmacobotanical standardization of *A. conyzoides*. Cross-sections were obtained, by hand, for microscopic characterization of root, stem, petiole and leaf blade; to the leaf blade were still made paradermal and longitudinal sections, scanning electron microscopy analysis and maceration. The analysis showed that secretory structures ducts are evidenced only in the petiole and the leaf blade. The root has parenchymatous medullar region; stem, petiole and leaf blade exhibit striated cuticle. Non-glandular trichomes are present in stem, petiole and leaf blade, while capitate glandular trichomes are present only in the leaf blade and are restricted to the abaxial face. These anatomical features are useful for diagnosis of the species and provide support to their quality control.

Introduction

Asteraceae is a vast plant family that comprises roughly 1500 genera and 25,000 species in different habitats (Souza and Lorenzi, 2012). This family consists of vegetation quite distinct, which ranges from herbs, subshrubs, and shrubs to trees (Joly, 2002). This vegetation is found into diverse habitats once it has a great environmental adaptation (Venable and Levin, 1983).

Ageratum is one of the genera in the family Asteraceae and consists of about 30 species (Okunade, 2002). Among the species, *Ageratum conyzoides* L., commonly known as "billy goat weed", "mentrasto" and "catinga-de-bode" (Cruz, 1995; Asicumpon, 2005; Matos, 2007), is widely used in traditional medicine in several countries around the world as a purgative, febrifuge, anti-inflammatory, analgesic, anesthetic and in treatment of ulcers (Lorenzi and Matos, 2002; Okunade, 2002; Leitão et al., 2014).

A. conyzoides is a tropical plant very common in the western and eastern regions of the African continent, as well as in some regions of Asia and South America (Bhatt et al., 2012; Iwu, 2014). It is an annual aromatic weed of cultivated fields, however, it is also invasive of pastures, vacant lots and even of forest areas (Sousa et al., 2004; Batish et al., 2006). The occupation of *A. conyzoides* is easily succeeded because of its wide adaptability in the environment, its superior reproductive potential and its allelopathy (Kong et al., 2004).

The species has a wide variety of secondary metabolites, including mono and sesquiterpenes, triterpenes, steroids, flavonoids, coumarins, tannins and alkaloids (González et al., 1991a, 1991b; Kasali et al., 2002; Moreira et al., 2007; Nour et al., 2010; Bosi et al., 2013). Some of these metabolites are biologically active, as the methoxyflavone isolated of the hexane extract of leaves of *A. conyzoides*, which had insecticidal activity (Moreira et al., 2007). Additional properties reported to the plant extracts are antibacterial activities (Adetutu et al., 2012; Odeleye et al., 2014), antifungal (Morais et al., 2014), antiparasitic (Teixeira et al., 2014), anti-inflammatory (Moura et al., 2005), healing (Arulprakash et al., 2012) and cytotoxicity properties (Adetutu et al., 2012).

The essential oil of the leaves or aerial parts of the plant has been widely investigated for its composition and biological activities. The major constituents generally found are the chromenes, precocene I and precocene II, and the sesquiterpenes caryophyllene and germacrene-D (Okunade, 2002). The main activity described in the literature for the essential oil is the insecticide (Lima et al., 2010; Liu and Liu, 2014), but also presents allelopathic (Kong et al., 2004) and antifungal activities (Nogueira et al., 2010; Patil et al., 2010).

Despite the wide range of pertinent studies on the chemical composition and activities of *A. conyzoides*, there are few studies

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on the anatomical description of the plant. Thus, this work aimed to describe the morpho-anatomical characteristics of root, stem, petiole and leaf blade, to contribute to the proper identification of this medicinal plant, besides to providing more information about the *Ageratum* genus.

Materials and methods

Plant material

Several specimens of adult plants of *Ageratum conyzoides* L., Asteraceae, were collected in the city of Camocim de São Félix, Pernambuco, Brazil. A voucher specimen was prepared and deposited in the Herbarium Dárdano de Andrade Lima of the Instituto Agronômico de Pernambuco (IPA), under collection number 89312.

Morpho-anatomical characterization

Several cross-sections were made at the middle region of the root, stem, petiole and leaf blade from the fresh material, by hand, using a common razor blade and petiole marrow from the *Cecropia* sp. as a support material (Oliveira and Akisue, 2009). The cross-sections were subjected to decolorization with sodium hypochlorite solution (50%) (Kraus and Arduin, 1997), followed by washing with distilled water and, lastly, stained according to the technique described by Bukatsch (1972) with safranin and astra blue. Paradermal and longitudinal sections of the leaf blade were also performed, using the aforementioned procedures, but the paradermal sections were stained with methylene blue (Krauter, 1985).

Posteriorly, semipermanent histological slides were prepared containing the sections of botanical material, following common plant anatomy procedures (Johansen, 1940; Sass, 1951). The macroscopic description followed the methodology of Oliveira and Akisue (2009).

Scanning electron microscopy (SEM)

Analyses were performed in samples of fresh leaf blades. The samples were fixed in 2.5% glutaraldehyde (buffered with 0.1 M sodium cacodylate). After that, the material was subjected to post-fixation using 2% osmium tetroxide solution (buffered with 0.1 M sodium cacodylate) and dehydration in ethanol series. Subsequently, the material was submitted to critical point drying (Bal-Tec CPD 030) and mounted onto SEM stubs, using double-sided adhesive tape and sputter-coated with gold (Leica EM SCD 500) (Haddad et al., 1998). Finally, the samples were examined with a scanning electron microscope (Quanta 200 FEG) in the Centro de Tecnologias Estratégicas do Nordeste.

Maceration

The maceration was performed using fresh leaf blades fragments that were disintegrated with the mixture of 10% nitric acid and 10% chromic acid (1:1), according to the method of Jeffrey (Johansen, 1940).

Analysis of histological slides

The analysis of the semipermanent histological slides prepared for anatomical characterization and maceration were conducted on images in software (Toup View Image), obtained by digital camera coupled to a light microscope (Alltion).

Results and discussion

Macroscopic aspects

A. conyzoides (Fig. 1A) has fasciculate root, presenting a coloration yellowish to brown and weakly fixed to the soil (Fig. 1B). The stem is green in the young plant but it may present brown color in older plants. Moreover, the stem is classified as aerial, has cylindrical shape and is covered by trichomes (Fig. 1E). The leaves are simple, opposite, oval shape, acute tip, attenuated base and toothed margin, covered with whitish trichomes. The petiole is straight (Fig. 1C) and has concave-convex contour. The terminal inflorescences bears about fifteen purple flower-head (Fig. 1D).

Microscopic aspects

Root

The root of *A. conyzoides*, in cross-section, has cylindrical contour, presenting suber and some lenticels (Fig. 2A). In the root of *Ageratum fastigiatum* (Gardn.) R.M. King et H. Rob., Del-Vechio-Vieira et al. (2008) evidenced a desquamation process of the peridermis, with consequent elimination of some cortical layers and the epidermis. The cortical parenchyma consists of about five layers of cells, with straight or slightly sinuous walls. It is also observed in this region the presence of inter-cellular air spaces (aerenchyma) (Fig. 2A) and cellular inclusions (Fig. 2B). Aerenchyma was also visualized in the cortical region of the root of *Ageratum houstonianum* Mill., but not in *A. fastigiatum* (Del-Vechio-Vieira et al., 2008; Das and Mukherjee, 2013), which can be a useful feature in the differentiation of these species. Also in relation to the cortical region, in the species *A. houstonianum* were found secretory ducts and in *A. fastigiatum* were found secretory canals (Del-Vechio-Vieira et al., 2008; Das and Mukherjee, 2013). In the species studied in this work were not found secretory structures in the root, which is, therefore, another important diagnostic feature.

The endodermis is uniseriate and has Casparian strips (Fig. 2A and B). The vascular system is formed by xylem, which occupies most of the root (Fig. 2A and C), and by phloem, arranged in few layers, surrounding the xylem (Fig. 2A). In *A. fastigiatum* the secondary phloem is distributed in groups of cells separated by parenchymatic rays (Del-Vechio-Vieira et al., 2008). The pith of *A. conyzoides* is composed of parenchymatic cells with straight walls (Fig. 2C). *A. houstonianum* also has parenchymatic medullar region (Das and Mukherjee, 2013), differing from *A. fastigiatum*, whose central region of root is all occupied by xylem (Del-Vechio-Vieira et al., 2008). Other members of Asteraceae, such as *Bidens pilosa* and *Pluchea sagittalis*, also exhibit parenchymatic medullar region (Colares et al., 2014).

Stem

In cross-section, the stem has cylindrical contour. The epidermis is uniseriate, coated with a thin and striated layer of cuticle (Fig. 3A and B). The presence of striations in the cuticle of stem is also reported in *A. fastigiatum* and in other genera of the family Asteraceae, such as *Baccharis* and *Elephantopus* (Budel and Duarte, 2008; Del-Vechio-Vieira et al., 2008; Empinotti and Duarte, 2008). It is also observed the presence of non-glandular trichomes, which are multicellular and uniseriate (Fig. 3A and C).

According to Metcalfe and Chalk (1950), non-glandular trichomes and glandular trichomes are very common in Asteraceae. In stem, some species have only non-glandular trichomes, as is the case of the species under study, *A. houstonianum* Mill., *Chaptalia nutans* (L.) Pohl, *Mikania lanuginosa* DC and *Vernonia brasiliana* (L.) Druce (Filizola et al., 2003; Duarte et al., 2007; Das and Mukherjee, 2013; Amorin et al., 2014). *A. fastigiatum* (Gardn.) R. M. King et

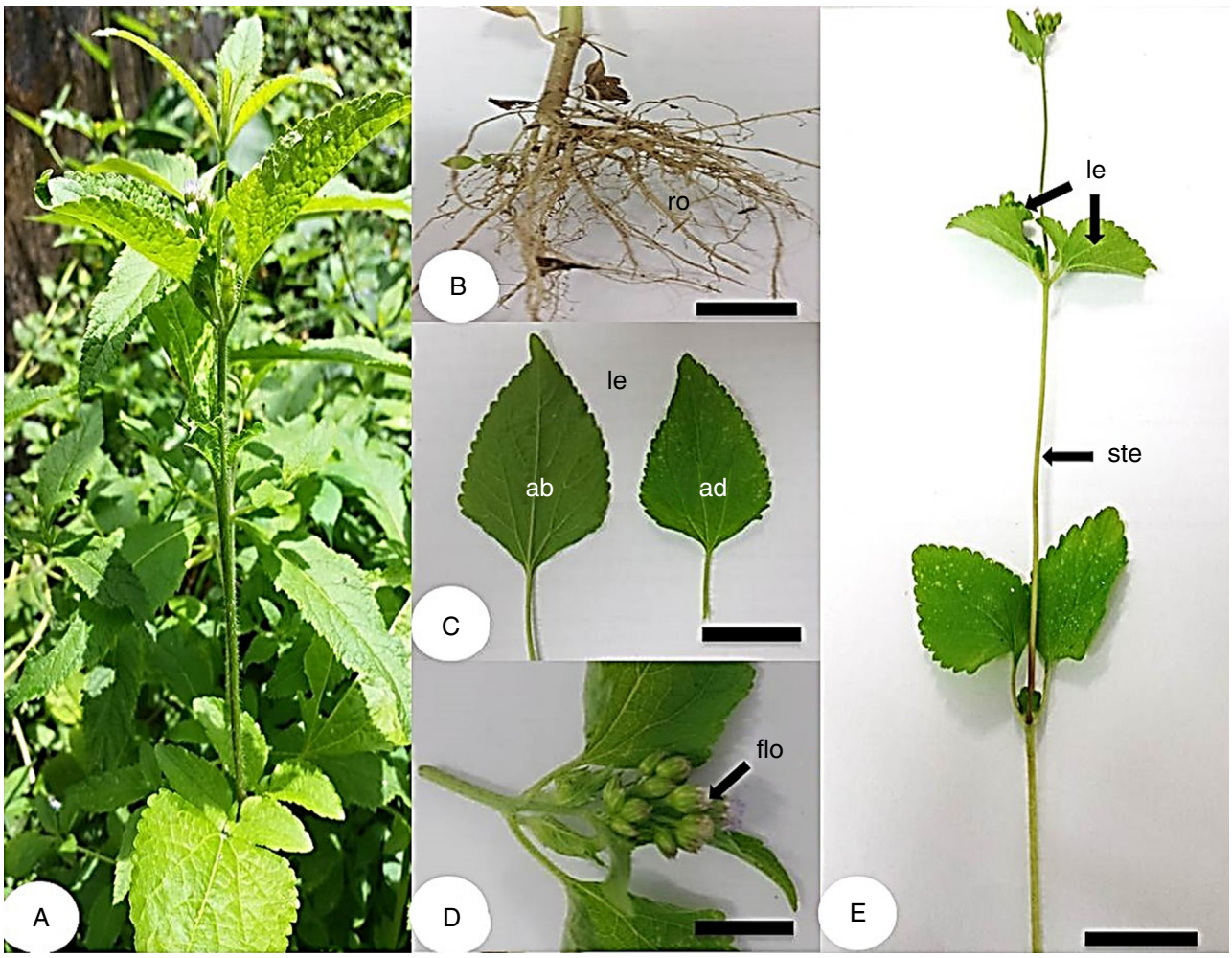

Fig. 1. *Ageratum conyzoides* L., Asteraceae. (A) Plant in its habitat; (B) root (ro) of fasciculate type; (C) Single leaves (le), abaxial (ab) and adaxial (ad) faces; (D) detail of flower-head (flo), which are purple; (E) Stem (ste) and leaves (le) in opposite position. Bars: B, C, and D = 2 cm; E = 5 cm.

H. Rob. has both, glandular and non-glandular trichomes (Del-Vechio-Vieira et al., 2008), as well as *Acanthospermum australe* (Loefl.) Kuntze, *Achyrocline alata* (Kunth) DC., *Ayapana triplinervis* (Vahl) R.M. King & H. Rob., *Baccharis rufescens* Spreng. var. *tenuifolia* (DC.) Baker, *B. uncinella* DC., *Calea uniflora* Less., *Elephantopus mollis* Kunth and *Gymnanthemum amygdalinum* (Delile) Sch.Bip. ex Walp. (Budel et al., 2006; Martins et al., 2006; Mussury et al., 2007; Budel and Duarte, 2008; Empinotti and Duarte, 2008; Duarte and Silva, 2013; Souza et al., 2013; Nery et al., 2014).

The cortical region is formed by two to four layers of angular collenchyma and for about five layers of parenchymatic cells (Fig. 3A and B). There are cellular inclusions in this region (Fig. 3D). The type of collenchyma can be used to distinguish the stems of *Ageratum conyzoides* and *A. fastigiatum*, because in the latter the collenchyma varies between angular and lamellar (Del-Vechio-Vieira et al., 2008). Different from what was observed in the cortical region of the root of *A. conyzoides*, in the stem there is no aerenchyma in this region (Fig. 3A).

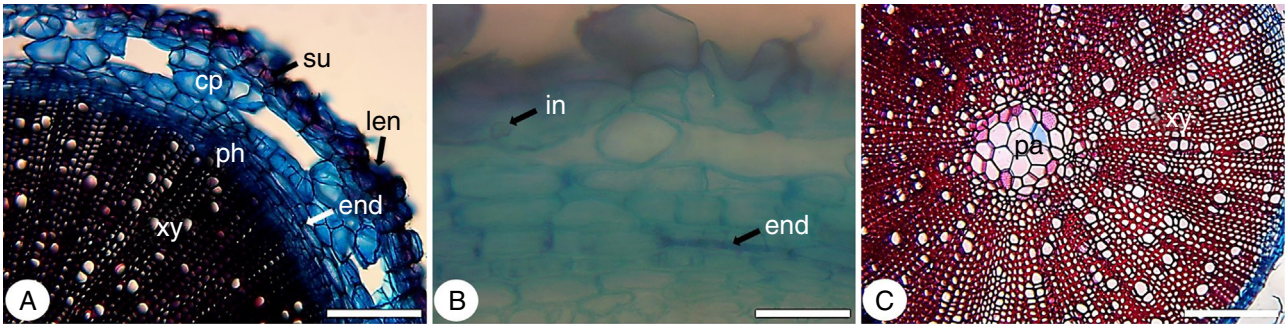

Fig. 2. *Ageratum conyzoides* L. (Asteraceae), cross-sections of the root. (A) Detail of suber (su), lenticel (len), cortical parenchyma (cp) with some air gaps intercellular, endodermis (end), phloem (ph) and xylem (xy); (B) detail of cellular inclusion (in) in cortical region and the endodermis (end); (C) view of the parenchymatic medullar region (pa) and xylem (xy). Bars: A and C = 200 μm; B = 50 μm.

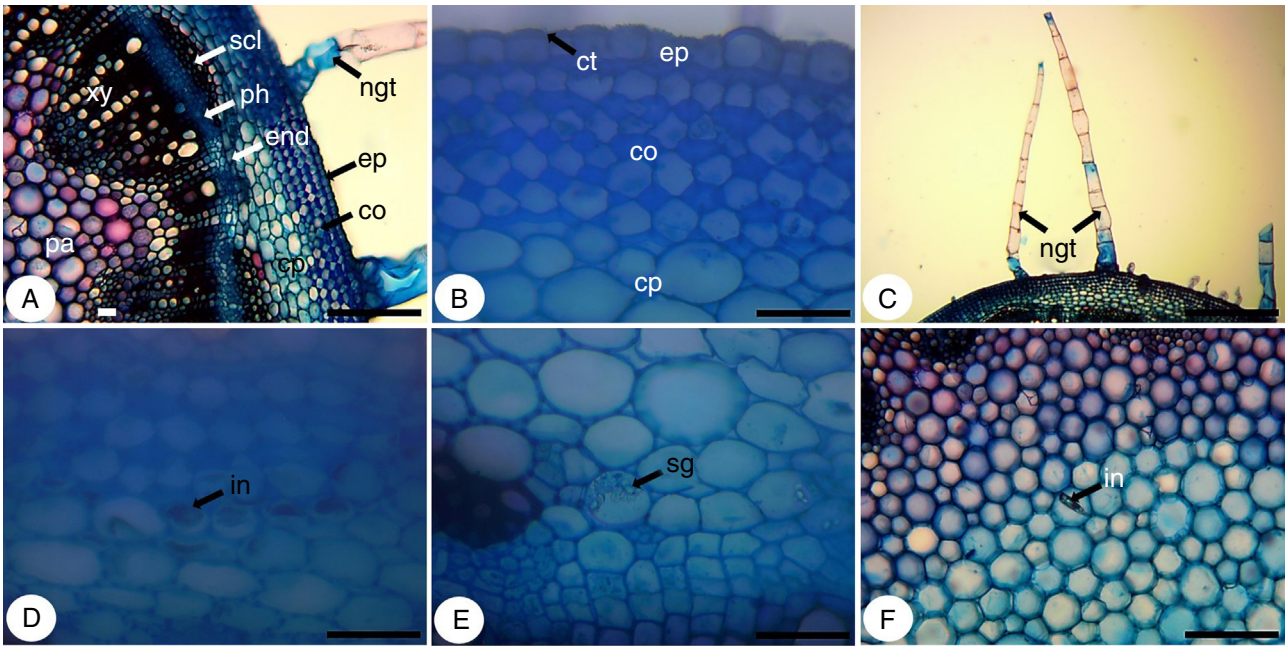

Fig. 3. *Ageratum conyzoides* L. (Asteraceae), cross-sections of the stem. (A) General view, showing epidermis (ep) with non-glandular trichome (ngt), cortical region formed by collenchyma (co) and parenchyma (cp), endodermis (end), sclerenchyma fibers (scl), phloem (ph), xylem (xy) and medullar region composed of parenchyma (pa); (B) epidermis (ep) coated with thin and striated cuticle (ct) and the cortical region formed by angular collenchyma (co) and cortical parenchyma (cp); (C) non-glandular trichomes (ngt); (D) cellular inclusion (in) in cortical region; (E) starch grains (sg) in the endodermis; (F) cellular inclusion (in) in medullar region. Bars: A = 200 μm; B, D, E and F = 50 μm; C = 500 μm.

Starch grains are visualized in the endodermis (Fig. 3E). In the family Asteraceae, the endodermis is well defined and it may present Casparian strips or occur as starch sheath (Metcalfe and Chalk, 1950). In *A. fastigiatum* there are secretory canals near the endodermis, which was not evidenced in *A. conyzoides* and *A. houstonianum* (Del-Vechio-Vieira et al., 2008; Das and Mukherjee, 2013). The vascular system is collateral, consisting of several continuous bundles, distributed in a single ring. Isolated sclerenchyma fibers are located externally to the phloem, forming caps (Fig. 3A). The medullar region consists of parenchyma and inclusions are found in some cells (Fig. 3A and F). The pith cells of *A. fastigiatum* may have lignification (Del-Vechio-Vieira et al., 2008).

Petiole

In cross-section, the petiole has concave-convex shape, with two ribs on adaxial face (Fig. 4A). The same format is common in other species of *Ageratum* (Del-Vechio-Vieira et al., 2008; Das and Mukherjee, 2013). The epidermis is composed of a single layer of cells and covered with a thin and striated cuticle (Fig. 4A and B). The same type of non-glandular trichome found on stem also appears in the petiole (Fig. 4A) and, furthermore, some stomata are inserted in the epidermis (Fig. 4B). The angular collenchyma is almost continuous, encircling the entire parenchymatic internal region of the petiole, composed by one to two layers of cells (Fig. 4A). However, in the adaxial ribs are observed more layers of this tissue (Fig. 4A). In *A. fastigiatum* predominate the angular and lacunar types of collenchyma and it may appear interrupted by lacunary parenchyma, in which are located large spaces of lysigenous origin (Del-Vechio-Vieira et al., 2008).

The vascular system is constituted by three to five defined central bundles arranged in an open arch, with rib traces (Fig. 4A). The bundles are bicollateral (Fig. 4C), while in *A. houstonianum* and *A. fastigiatum* the bundles are collateral (Del-Vechio-Vieira et al., 2008; Das and Mukherjee, 2013). Other species of Asteraceae that have bicollateral vascular bundles in the petiole are *Aspilia africana* (Pers) C.D Adams, *Tithonia diversifolia* (Hemsl) A. Gray and *Vernonia amygdalina* Del. (Mabel et al., 2013). Secretory ducts are found close to the vascular bundles (Fig. 4C). These structures are common in Asteraceae (Castro et al., 1997) and also have been described in petioles of *A. fastigiatum* (Gardn.) R.M. King et H. Rob., *Flourensia campestris* Griseb., *F. oolepis* S. F. Blake and *Siegesbeckia orientalis* L. (Aguilera et al., 2004; Delbón et al., 2007; Del-Vechio-Vieira et al., 2008). In the cells of the parenchyma and in some cells of the collenchyma inclusions can be found, mainly in the regions where these tissues are joined (Fig. 4D).

Leaf blade

The leaf blade of *A. conyzoides*, in surface view, shows epidermal cells with sinuous walls on both sides (Fig. 5A and B). The leaf blade is amphistomatic, with anomocytic and anisocytic stomata, being the firsts more frequent (Fig. 5A and 5B). Metcalfe and Chalk (1950) reported that for Asteraceae the stomata are usually anomocytic. However, data from the literature are quite different with respect to the types of stomata present in the leaf blade of *A. conyzoides*. In some studies were found only stomata anomocytic (Ferreira et al., 2002; Unamba et al., 2008; Folorunso and Awosode, 2013). Rahman et al. (2013) found the same types identified in this work and Adedeji and Jewoola (2008) said that the plant has largely anisocytic and occasionally anomocytic and brachyparacytic.

Non-glandular trichomes, which are the same type described in stem and petiole, are visualized on both faces and capitate glandular trichomes are restricted to abaxial face (Fig. 5C and D). Regarding the types of trichomes, it is also observed divergence in the literature. Ferreira et al. (2002), investigating the plant also collected in Brazil, reported the presence of non-glandular and glandular trichomes, but, different from that found in this study, the authors stated that the glandular trichomes are located on both faces of the leaf blade. In a study of the leaf blade anatomy of *A. conyzoides* cultivated in different substrates, the types of trichomes and its

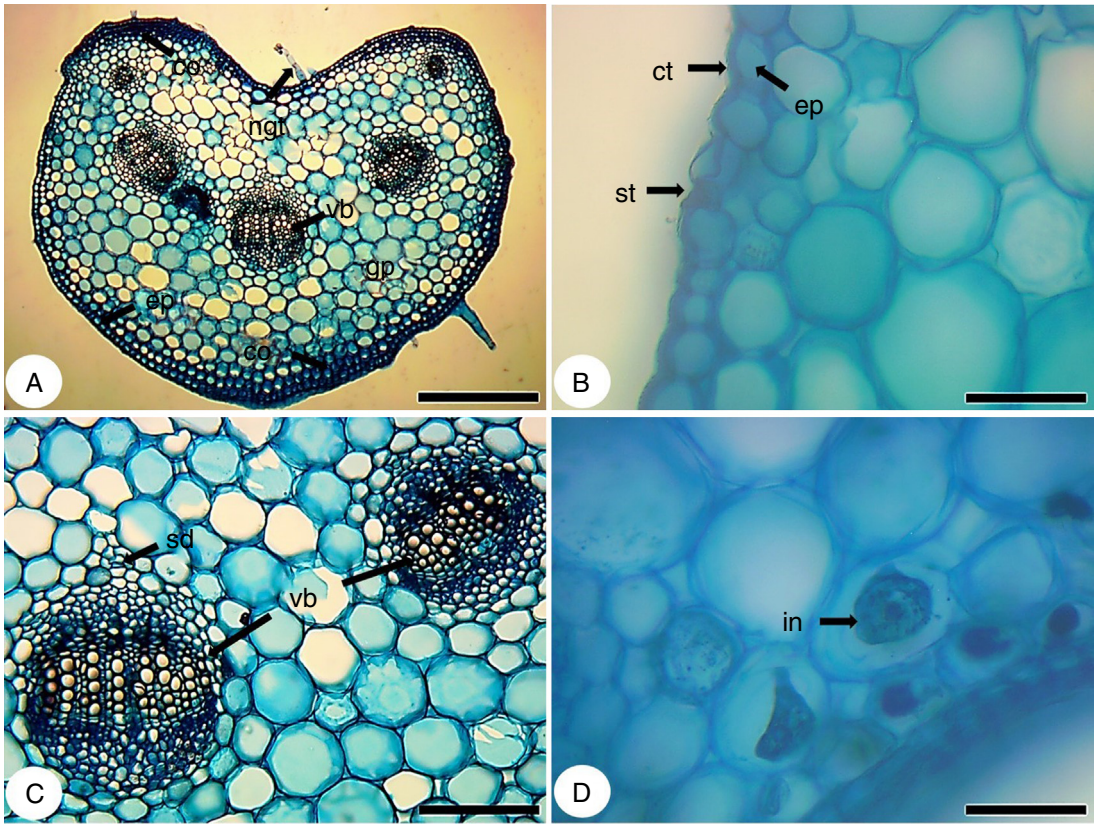

Fig. 4. *Ageratum conyzoides* L. (Asteraceae), cross-sections of the petiole. (A) General aspect, showing epidermis (ep), non-glandular trichomes (ngt), collenchyma (co), ground parenchyma (gp) and vascular bundles (vb); (B) detail of epidermis (ep) coated with thin and striated cuticle (ct) and stomata (st); (C) detail of bicollateral vascular bundles (vb) and secretory duct (sd); (D) cellular inclusion (in) in parenchyma and collenchyma. Bars: A = 500 μm; B and D = 50 μm; C = 200 μm.

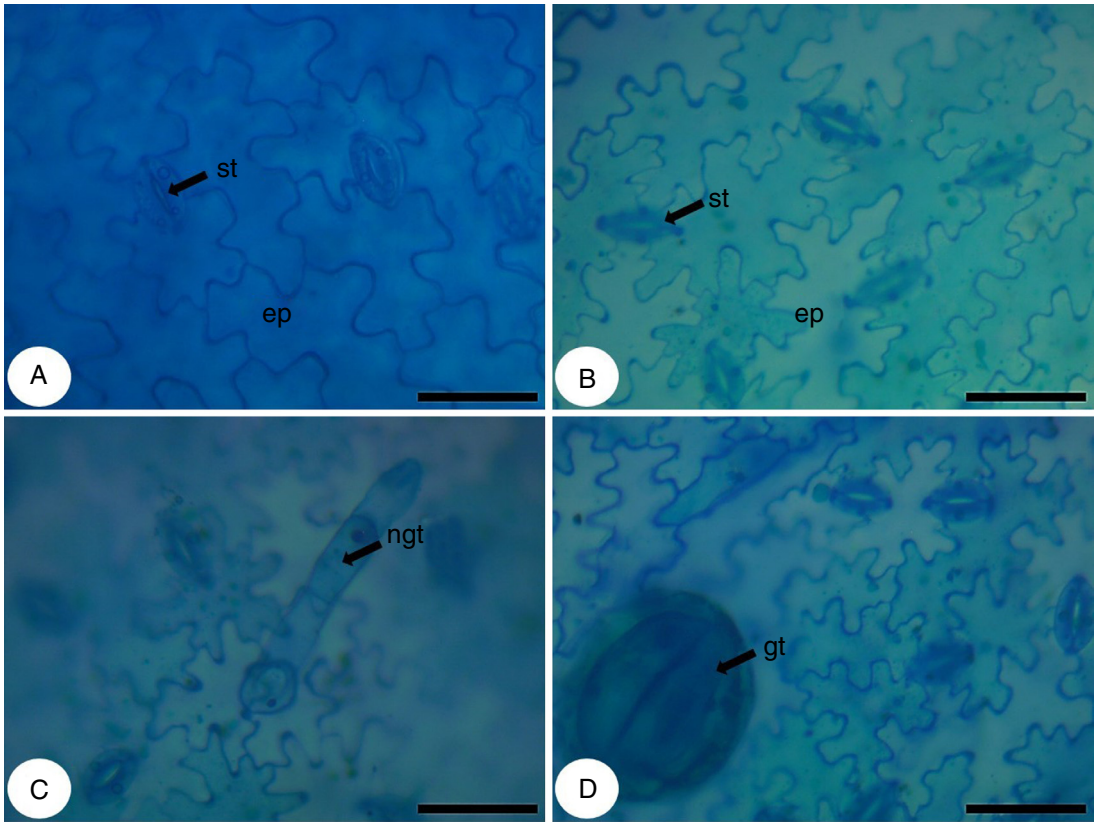

Fig. 5. *Ageratum conyzoides* L. (Asteraceae), frontal view of the leaf blade. (A) Adaxial face showing epidermal cells (ep) and stomata (st); (B) abaxial face showing epidermal cells (ep) and stomata (st); (C) non-glandular trichome (ngt) on the abaxial face; (D) capitate glandular trichome (gt) on the abaxial face. Bars: A–D = 50 μm.

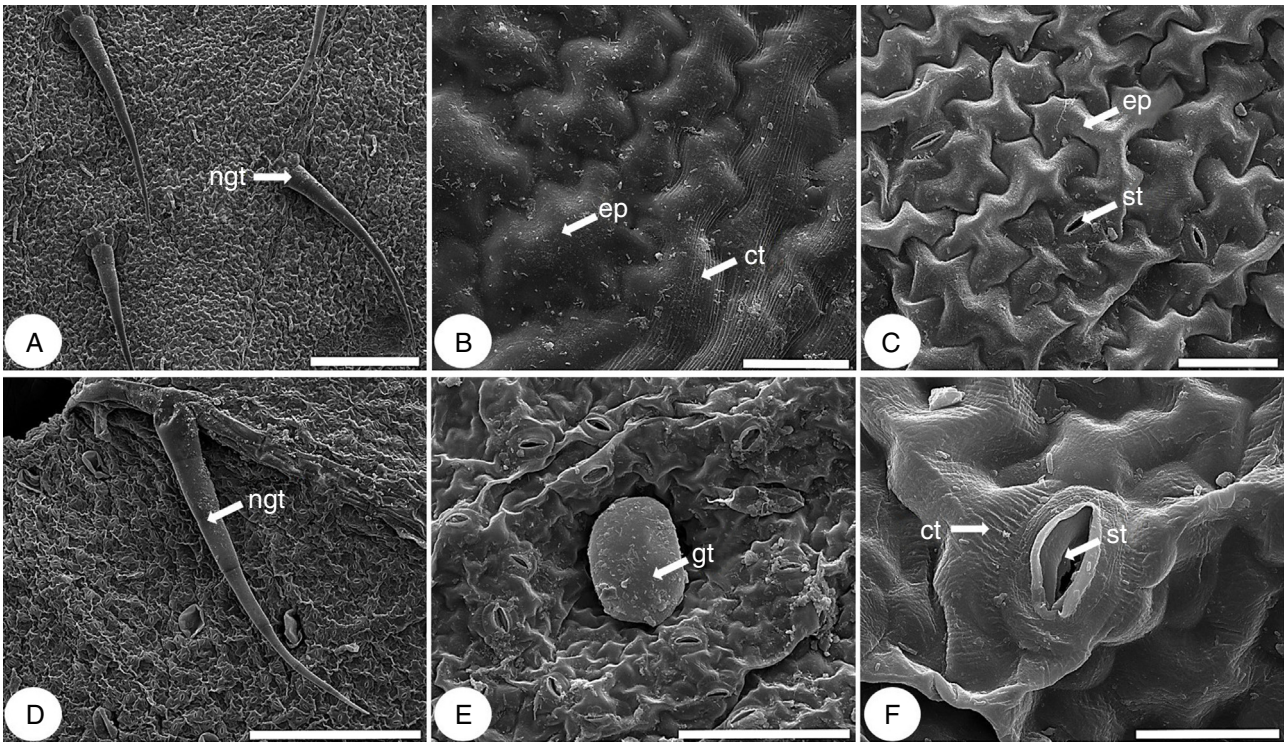

Fig. 6. *Ageratum conyzoides* L. (Asteraceae), SEM of the leaf blade. (A) View of the adaxial face showing non-glandular trichomes (ngt); (B) epidermal cells (ep) and striated cuticle (ct) on the adaxial face; (C) epidermal cells (ep) and stomata (st) on the adaxial face; (D) view of the abaxial face showing non-glandular trichome (ngt); (E) capitate glandular trichome (gt) on the abaxial face; (F) stomata (st) and striated cuticle (ct) on the abaxial face. Bars: A = 500 μm; B and C = 50 μm; D = 400 μm; E = 100 μm; F = 20 μm.

distribution in the leaf blade corresponded to those described in this work (Millani et al., 2010).

The studies conducted on the species collected in Nigeria and Bangladesh described only the presence of non-glandular trichomes (Folorunso and Awosode, 2013; Rahman et al., 2013; Mabel et al., 2014). According to Werker (2000), although the differentiation of trichomes is genetically controlled, their frequency is affected by biotic and abiotic factors.

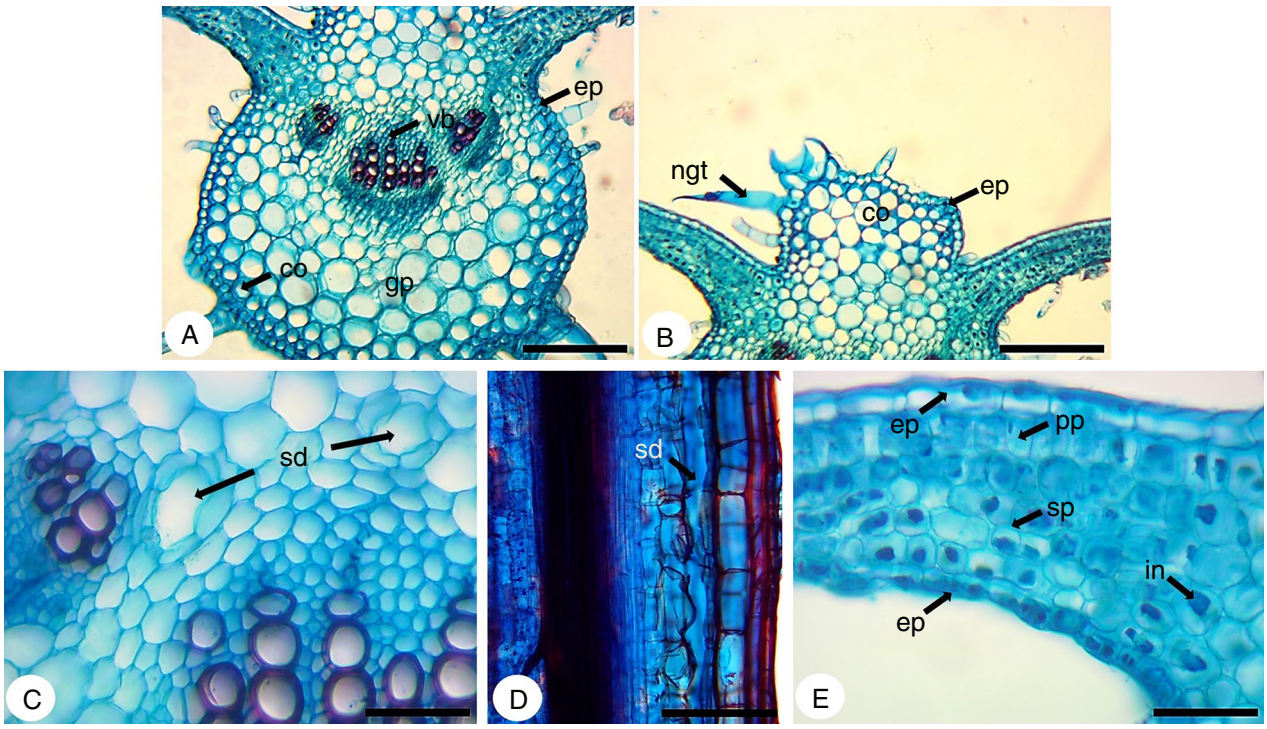

Fig. 7. *Ageratum conyzoides* L. (Asteraceae), cross-sections (A, B, C and E) and longitudinal section (D) of the leaf blade. (A) General aspect of the midrib showing epidermis (ep), collenchyma (co), ground parenchyma (gp) and vascular bundles (vb); (B) detail of epidermis (ep), non-glandular trichome (ngt) and collenchyma (co); (C) secretory ducts (sd) next to the bundles; (D) secretory duct (sd) in longitudinal view; (E) detail of epidermis (ep), palisade parenchyma (pp), spongy parenchyma (sp) and cellular inclusion (in). Bars: A, B and D = 200 μm; C and E = 50 μm.

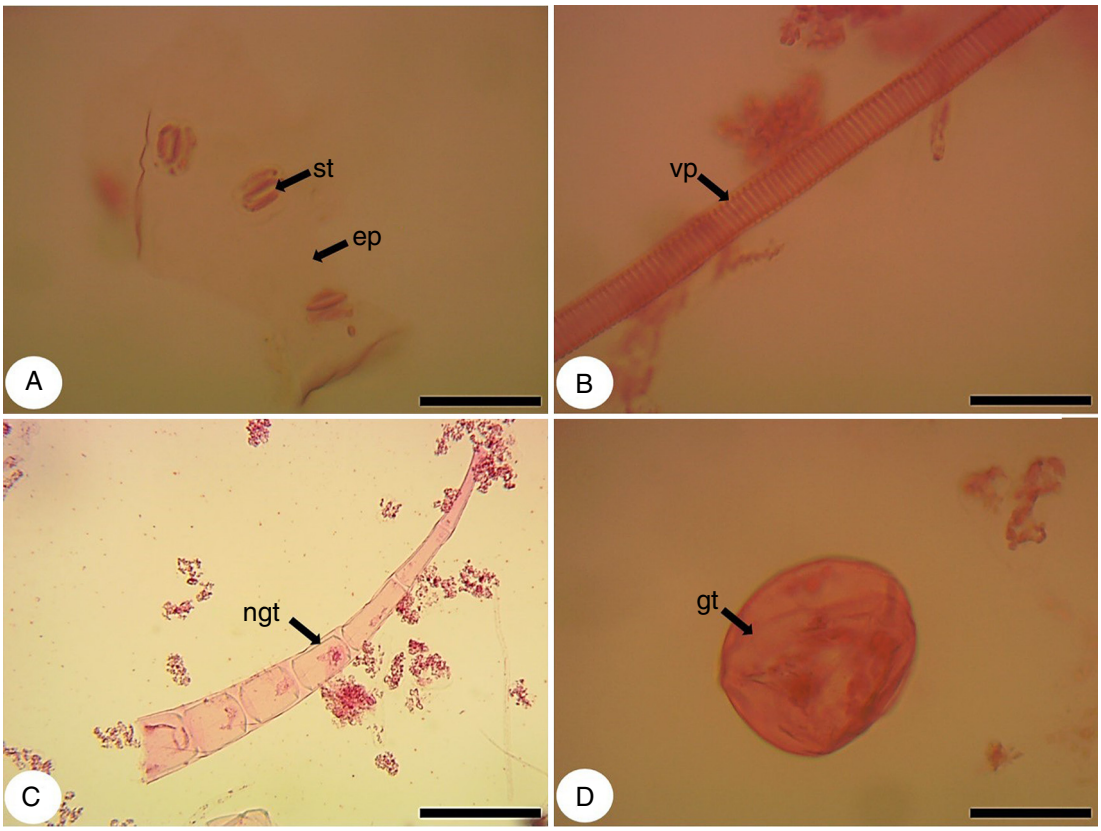

Fig. 8. *Ageratum conyzoides* L. (Asteraceae), maceration of the leaf blade. (A) Epidermis (ep) and stomata (st); (B) vessel element of the helical type (vb); (C) non-glandular trichome (ngt); (D) capitate glandular trichome (gt). Bars: A, B and D = 50 μm; C = 200 μm.

In the leaf blade analysis in SEM it was also noted the presence of the non-glandular trichomes on both faces and the presence of the capitate glandular trichomes only on abaxial face, as it was previously visualized by light microscopy (Fig. 6A, D and E). But only in SEM it was possible observed, with more detail, that the cuticle is striated on both faces (Fig. 6B and F) and that, on the adaxial face, the stomata are situated slightly below of epidermal cells (Fig. 6C), while on the abaxial face they are situated on the same level or slightly above of epidermal cells (Fig. 6E and F). Regarding the presence of striated cuticle on the leaf blade of *A. conyzoides*, two other studies affirmed that the cuticle is non-striated (Adedeji and Jewoola, 2008; Mabel et al., 2014); however, it is possible that this divergence is due to the fact that, in these works, were not performed the analysis in SEM, which has a higher resolution on the observation of microstructural features (Haddad et al., 1998).

In cross-section, *A. conyzoides* shows a midrib with biconvex contour (Fig. 7A). *A. fastigiatum* exhibits midrib ranging from plane-convex to slightly biconvex (Del-Vechio-Vieira et al., 2008). The epidermis is uniseriate (Fig. 7A and B) and it is also observed non-glandular trichomes throughout the midrib (Fig. 7B). Adjacent the epidermis is located the angular collenchyma, composed of one to two layers on the abaxial face of the midrib (Fig. 7A) and up to four layers of cells in its adaxial face (Fig. 7B).

In *A. fastigiatum* the chlorophyll parenchyma invades the region of the midrib until close of the vascular bundle, which does not happen in *A. conyzoides* (Del-Vechio-Vieira et al., 2008). In the central region of the midrib of *A. conyzoides* can be found one to three collateral vascular bundles, arranged in an open arch (Fig. 7A). In the literature, there is description of only one vascular bundle in the midrib of *A. conyzoides* (Millani et al., 2010; Mabel et al., 2014; Ekeke and Mensah, 2015). Secretory ducts are found next to the bundles (Fig. 7C and D), which corroborates the study of Millani et al. (2010). However, Mabel et al. (2014) and Ekeke and Mensah (2015) did not report the presence of secretory structures in the midrib of the plant.

The mesophyll is dorsiventral, with one layer of palisade parenchyma and three to four layers of dense spongy parenchyma (Fig. 7E). In *Ageratum fastigiatum* the chlorophyll parenchyma is plicated (Del-Vechio-Vieira et al., 2008). Cellular inclusions are found throughout the mesophyll (Fig. 7E).

The leaf blade of *A. conyzoides*, after macerated, presents epidermal cells and stomata (Fig. 8A), as well as vessel elements of the helical type (Fig. 8B). The two types of trichomes viewed in cross-section and paradermal sections are also found in the macerated leaf blade (Fig. 8C and D), and they could be intact or fragmented, especially the non-glandular trichome (Fig. 8C). The maceration data is useful when it is not possible to analyze the plant material through histological sections (Farmacopeia Brasileira, 2010).

Conclusion

This work has contributed to expand the information about *Ageratum conyzoides*, utilizing usual techniques of microscopic analysis of plants. It was shown that the root, stem, petiole and leaf blade of *A. conyzoides* present anatomical characteristics that are useful in the identification and differentiation from other species of this genus and are also essential parameters for the quality control of vegetable raw material.

Authors' contributions

RFS contributed in collecting plant sample, confection of a voucher specimen, running the laboratory work, analysis of the data and drafted the paper. BMN and RDS contributed in preparation of the semi-permanent histological slides. LALS contributed to

critical reading of the manuscript. KPR designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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