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Original Article

Morpho-anatomical study of rhizome of *Limonium brasiliense*

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ABSTRACT

Limonium brasiliense (Boiss.) Kuntze, Plumbaginaceae, is an herb popularly known as guaicuru, guaicurá or baicuru. The species inhabits salt marshes from the coastal region of southern Brazil, including Rio de Janeiro, to Uruguay and Argentina. Although widely used in folk medicine in the state of Rio Grande do Sul to treat genitourinary infections and to regulate menstrual periods, *L. brasiliense* has been little studied. The present morpho-anatomical study was undertaken to resolve some doubts in the literature as to the nature of the part of the plant that is used for medicinal purposes, a true rhizome or a root. The morpho-anatomical characteristics were analyzed with the aid of light and scanning electron microscopy. The botanical material was characterized as a rhizome with internodes that are evident in the younger but not the older portions. Microscopic analysis revealed the presence of a multilayered periderm with a cortex, ray parenchyma, and pith, formed by collenchyma tissue with abundant intercellular spaces in the outer portions of the cortex, responsible for the rigidity of the body, and with walls impregnated with phenolic compounds. The vascular bundles are collateral with elliptical to elongated cells, and with few conducting and sclerenchymal elements. Groups of sclereids are dispersed through the cortex and pith. These morpho-anatomical characteristics define the structure as a rhizome.

Introduction

Limonium Mill., the most species-rich in the Plumbaginaceae, includes about 350 species of herbs. The genus is best represented in the Mediterranean regions of Europe and in Asia, and can also be found in coastal regions of North and South America, South Africa, and Australia (Mobot, 2015). In general, species of *Limonium* live as halophytes, including in alpine regions (Chant, 1993). In other countries, such as China, stems and roots of various species of *Limonium* are used in folk medicine, and some of them are similar in morphology and anatomy, making them difficult to identify using traditional methods (Ding et al., 2012).

Limonium brasiliense (Boiss.) Kuntze is an herb popularly known as guaicuru, guaicurá or baicuru (Dias da Silva, 1920) in the state of Rio Grande do Sul, Brazil. It is grown and marketed by small farmers in estuarine regions of the River Plate Basin. According to Simões et al. (1998), this herb is common in coastal salt marshes in southern Brazil, from Paraná to Rio Grande do Sul, and in Uruguay and Argentina; Zappi (2015) gave the range as extending from southern Brazil to the state of Rio de Janeiro.

Although it was described in the first edition of the Brazilian Pharmacopoeia (1929), *L. brasiliense* is not included in the current Pharmacopoeia. It is popularly used to treat uterine and ovarian inflammation, vaginal discharge and dysmenorrhea (Moura et al., 1985), and is useful to regulate menstrual periods (Lifchitz, 1981), as well as having an antimicrobial effect (Rosito, 1975). Murray et al. (2004) isolated five antioxidant compounds from extracts of *L. brasiliense* roots. Their chemical composition includes hydrolyzable and condensed tannins, 4-*O*-methyl gallic acid, sitosterol, and triterpenic saponins, the structures of which have not been determined (Rosito, 1975).

The first botanical description of *L. brasiliense* was contained in the *Flora brasiliensis* (Martius, 1840–1906). Dias da Silva (1920) provided a detailed anatomical description, noting the organoleptic characteristics of fresh plants, *i.e.* a strong unpleasant odor that disappears upon dissection, and a spicy astringent flavor. Martius

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(1840–1906) named it *Statice brasiliensis* Boiss., an herb with a more or less scaly rhizome. In 1920, Dias da Silva, in describing the species, initially referred to the rhizome as cylindrical-irregular, short, thick, more or less covered with scales; but in describing the anatomy of this organ as used medicinally, reported that the "roots" are 1–2 cm in diameter, cylindrical-fusiform and crooked. The characterization as a "root" may have been a conceptual error, because his morphological description makes it clear that this is a rhizome. Reitz (1965) described the species as having thick roots with red scales, and this error in terming the rhizome of *L. brasiliense* a root is also found in the studies of Corrêa (1952), Coimbra (1958), Cruz (1982), Moura et al. (1985), Murray et al. (2004), Fenner et al. (2006) and Blainski et al. (2013).

Given the possibility of exploitation of the species and the inexact categorization of the organ used, this study provided a morpho-anatomical description of the part of the plant used in popular medicine, contributing to the pharmacognostic evaluation of *L. brasiliense*.

Materials and methods

Plant material

This study used rhizomes of *Limonium brasiliense* (Boiss.) Kuntze, Plumbaginaceae, collected in May 2010 and January 2013 on the Ilha dos Marinheiros (31°59′33″ S, 052°10′43″ W) in the city of Rio Grande, Rio Grande do Sul, Brazil. The collection of plant material is registered with IBAMA-SISBIO under number 11995-3 of 2 November 2010, authentication code 46367613, under the responsibility of João Carlos Palazzo de Mello. Access to the botanical material was authorized and licensed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), registered under no. 010252/2015-0. Samples of the reproductive phase are held in the Herbarium of the Universidade Estadual de Maringá (HUEM) under registration numbers 21151 and 27725 for the material collected in 2010 and 2013, respectively. The plant material was identified by Prof. Dr. Lilian Auler Mentz (Universidade Federal do Rio Grande do Sul).

Morpho-anatomical analysis

The macroscopic characterization of the rhizome of *L. brasiliense* was based on the notes of Oliveira et al. (1998). For the anatomical analysis under light microscopy (LM) and scanning electron microscopy (SEM), segments of rhizome with different diameters (from 0.77 to 1.53 cm) were used. The segments were rehydrated by boiling in a solution of 10% glycerin for 15–30 min (2 times) and stored in 70% ethanol (Johansen, 1940), with weekly replacement of the ethanol to eliminate the excess red pigment released by their tissues. Sections for light microscopy were made freehand with steel blades, on the standard planes for plant anatomy. The sections were bleached with sodium hypochlorite (30%), double-stained with Astra blue (1%) and safranin (1%), and mounted on semi-permanent slides with glycerin gel as described by Kraus and Arduin (1997). The same procedure was used to prepare slides with the rehydrated powder from rhizomes.

Histochemical tests were done with cross sections, prepared as above, of samples hydrated in glycerin–water, which were stained with Lugol's iodine solution to reveal the presence of starch grains; iodinated zinc chloride, for lignin; Sudan IV glycerin, for lipophilic substances; ferric chloride, for polyphenols; and 60% chloral hydrate with 25% sulfuric acid, for calcium oxalate crystals (Johansen, 1940; Berlyn and Miksche, 1976; Kraus and Arduin, 1997; Farmacopeia Brasileira, 2010).

For analysis under scanning electron microscopy (SEM), the segments of rhizomes were rehydrated and cut with steel blades into 0.3 mm sections on different anatomical planes, and then fixed in 1% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 (Kraus and Arduin, 1997). After 72 h in glutaraldehyde, the samples were dehydrated in an ascending ethanol series (30, 50, 70, 90, 95%, v/v) for 15 min each, ending in absolute ethanol for 10 min twice, and then critical point-dried with CO_2 (Balzers CPD 30 critical-point dryer) (Horridge and Tamm, 1969). Then the samples were positioned on the different anatomical planes on metal stubs, attached with double-sided carbon tape, and sputter-coated with gold in a Shimadzu IC-50 unit. The photomicrographs were obtained with an Olympus BX50 optical microscope, and the ultrastructural analyses used a Shimadzu SS 550 SEM (at 15 kV).

Results and discussion

The rhizome of *L. brasiliense* is long and thick; the older portions measure 0.77–1.53 cm in diameter (Fig. 1A–C) with a very evident radial appearance when broken (Fig. 1D). The side buds more clearly show internodes with a mean length of 0.38 cm, partially coated with fragments of the petiole base that are reddish when dry (Fig. 1B), similar to the descriptions of Martius (1840–1906) and Dias da Silva (1920). The presence of rhizomes and tubers that function as storage organs and for asexual reproduction is common in herbaceous species of wetlands, as concluded by Braendle and Crawford (1999).

The surface of the rhizome of *L. brasiliense* is dark brown and bears delicate longitudinal striae, and small or deep transverse fissures (Figs. 1C and 2A). The periderm is black and the inner tissues are reddish brown (Fig. 1D), as described by Dias da Silva (1920). To the naked eye, the rhizome in cross section (Fig. 1D) appears grainy in the cortical and in the pith, while the middle portion, filled with vascular bundles, appears streaked. Because of its color and growth partially buried in coastal sandy soil, the rhizome of this species can be confused with a tuberous taproot, as seen in the descriptions of Reitz (1965). No structural differences were observed in the two groups of samples.

The LM and SEM analyses revealed that the periderm of the rhizome of *L. brasiliense* is thick, formed by dozens of cell layers (Fig. 2) which react positively to Sudan IV. In frontal view these cells are polyhedral and relatively elongated (Fig. 2B and C). In cross-section they appear quadrangular to tabular, with slightly thickened walls (Fig. 2D–F), becoming collapsed with the organ surface, but always with a reddish-brown content that does not bleach in the presence of sodium hypochlorite. Both the color and the cell structure of the periderm, described above, are in accord with the observations of Dias da Silva (1920), although the author has designated the structure as roots.

The cortex of the rhizome of *L. brasiliense* is well developed (Fig. 3A), composed of cylindrical cells like collenchyma annular with wide primary pit-fields that in most cases are elongated tangentially into the outer portion of the organ. These cells sometimes have small lobed projections (Figs. 3B and 4A, B), allowing the formation of intercellular spaces that compose an aerenchyma. An aerenchyma is formed in roots and stems of species that inhabit wetlands, or dry locations under adverse conditions, as a result of abiotic stress (Evans, 2003), as where *L. brasiliense* grows in flooded soils and saline salt marshes of Paraná (Zappi, 2015). Macroscopically, this aerenchyma lends a friable appearance to the dried rhizomes.

Near the phloem, the cortical tissue has isodiametric to longitudinally elongated cells, collenchymatous like those of the outer portion of the cortex, and similar to the cells of the interfascicular parenchyma rays and also those which comprise the pith of the

Fig. 1. Dried samples of *Limonium brasiliense*. Long thick rhizome (closed arrows) (A) with thinner lateral branches surrounded by reddish petiole bases (open arrows) (B); details of periderm (C) and internal tissues (D).

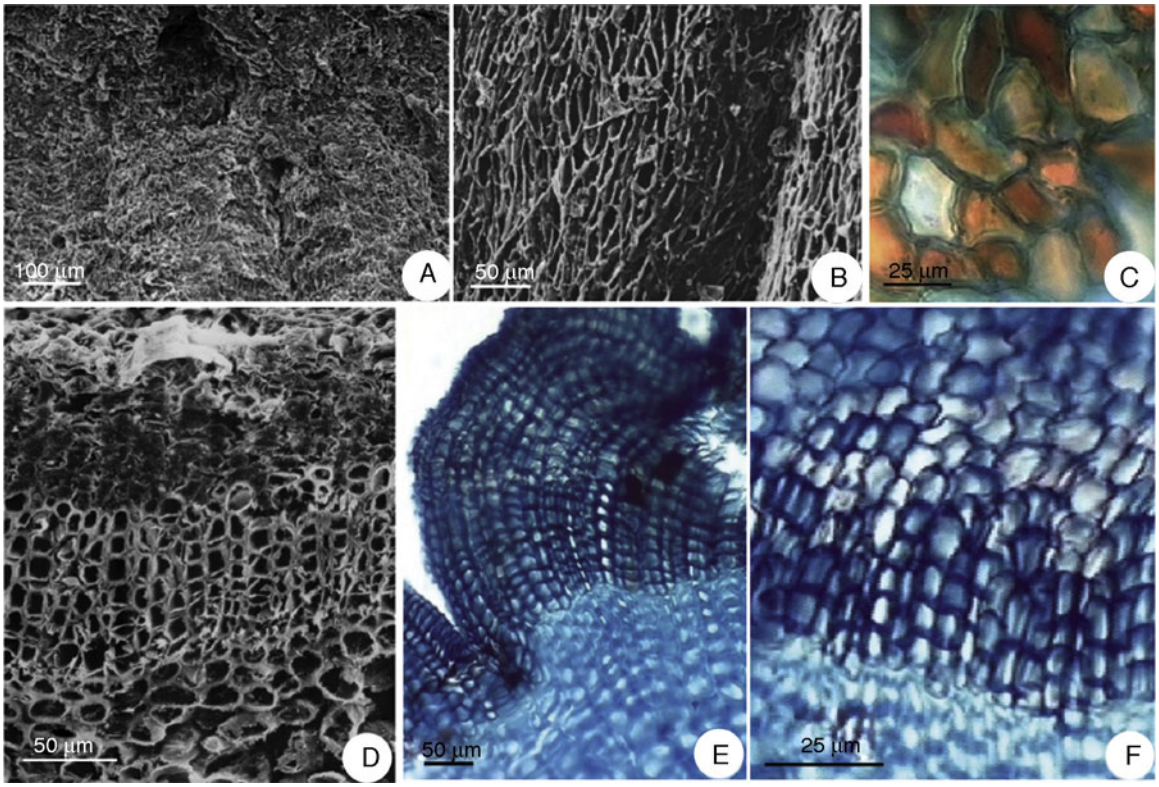

Fig. 2. Periderm of rhizome of *Limonium brasiliense*. General appearance of surface striae (A) and periderm cells (B and C); cross-sections of periderm (D and E) and detail of the cells (F). A, B and D: in SEM; C, E and F: in LM.

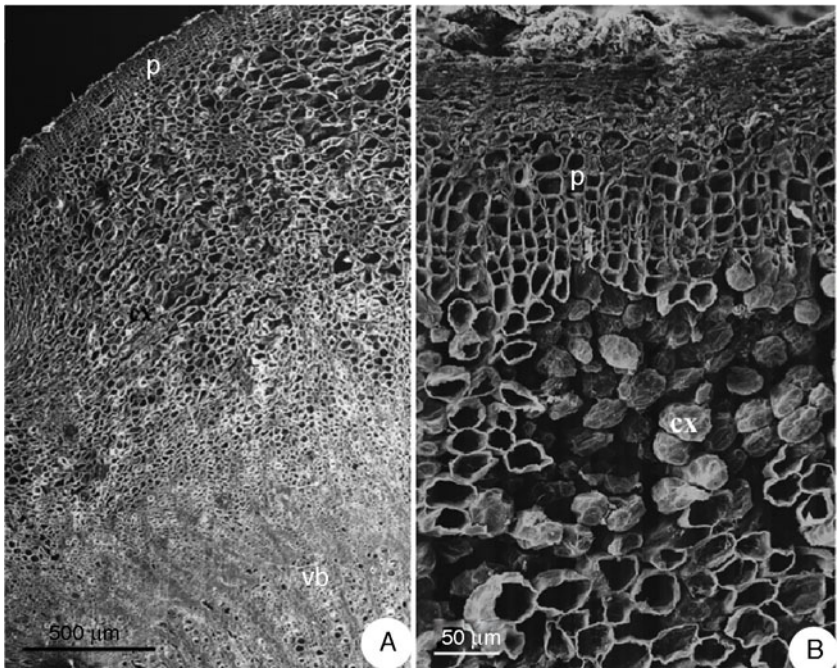

Fig. 3. Rhizome of *Limonium brasiliense*. General appearance (A) and detail of aerenchyma (B) in transversal sections under SEM. cx: cortex, p: periderm, vb: vascular bundles.

stem, but always allowing the formation of relatively large intercellular spaces.

Sclereids with very varied dimensions are scattered through the cortex, individually (rarely, as in Fig. 4A) or in groups of five to a few dozen elements, positioned parallel to the major axis of the rhizome. These cells have a small to voluminous lumen, rounded, beveled or anomalous ends, but always with branched pits (Fig. 5A–C), similar to those described by Metcalfe and Chalk (1950) in the cortex of the underground stems of *Limonium bellidifolium* (Gouan) Dumort and *Limonium binervosum* (G.E.Sm.) C.E. Salmon. The multiple layers of the secondary wall of this sclerenchymatic cell type become even more apparent in SEM (Fig. 5D). The presence of groups of sclereids was also reported by Grigore et al. (2014) in the cortex of the rhizome of *Limonium furfuraceum Kuntze*, although in large groups. Similar groups of sclereids are also present in the pith of the rhizome of *L. brasiliense*.

The vascular bundles of the rhizome of *L. brasiliense* are collateral with an elliptical-elongated shape, short to very long, depending on the specimen (Fig. 6); but always narrow and radially arranged, as described by Dias da Silva (1920), composed of a small number of conducting elements in relation to the parenchyma cells. Solitary, randomly positioned vascular bundles were observed in the cortex and in the pith (Fig. 6A), sometimes transverse to the major axis of the rhizome.

The vascular cambium was detected only within the vascular bundle (fascicular type), with a delicate and inconspicuous appearance (Fig. 7A), similar to the rhizome of *Senecio juergensii* Mattf., a species of Asteraceae analyzed by Bagatini (2008), which, like *L. brasiliense*, occurs in flooded areas. No sclerified cells were found in the phloem (Fig. 7), while in the xylem (Fig. 8), the vessel elements, solitary or in small groups, may be accompanied only by parenchyma cells or by short fiber-sclereids with a large lumen

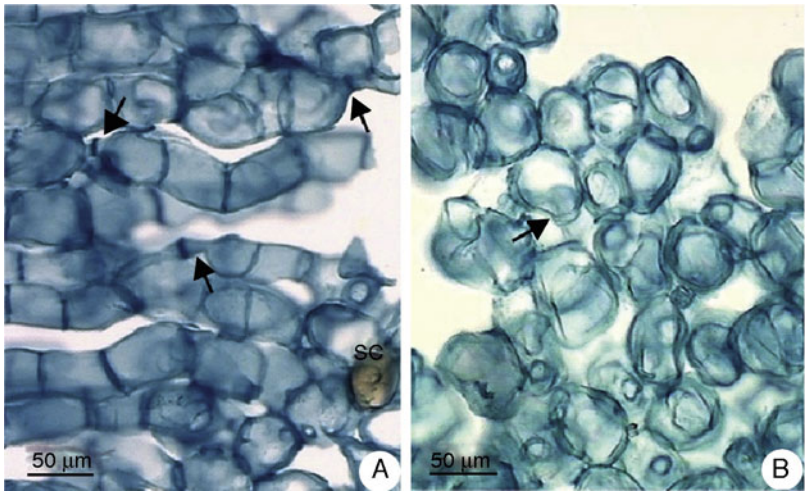

Fig. 4. More superficial strata of cortex of rhizome of *Limonium brasiliense*. Detail of tangential section (A) and radial section (B) under LM. Arrows indicate small lobed projections. sc: sclereid.

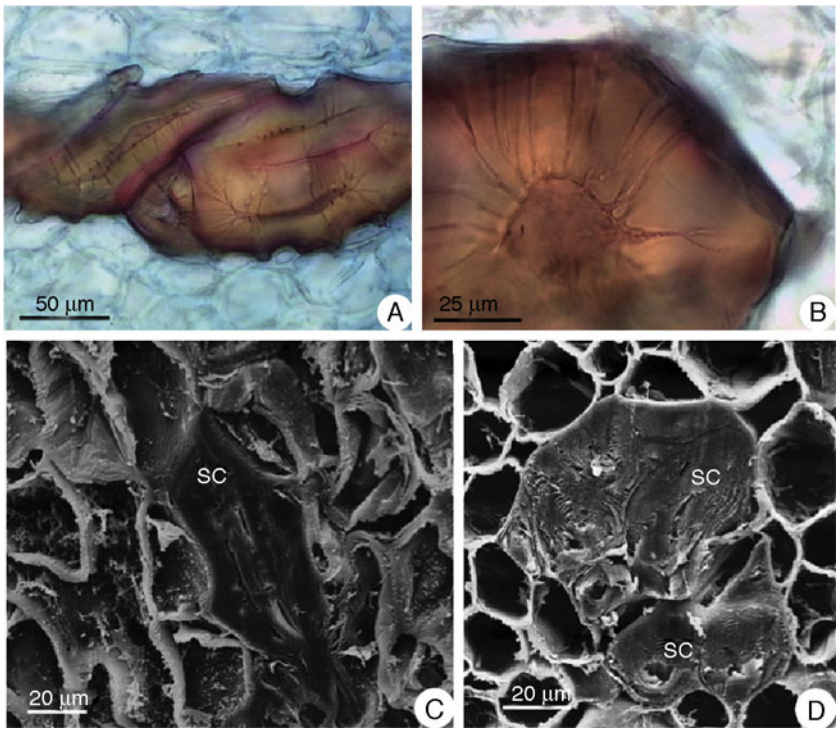

Fig. 5. Group of sclereids in rhizome of *Limonium brasiliense* in radial section (A and C) and detail of secondary wall in cross section (B) with multiple layers (D). A and B under SEM, C and D under LM. sc: sclereid.

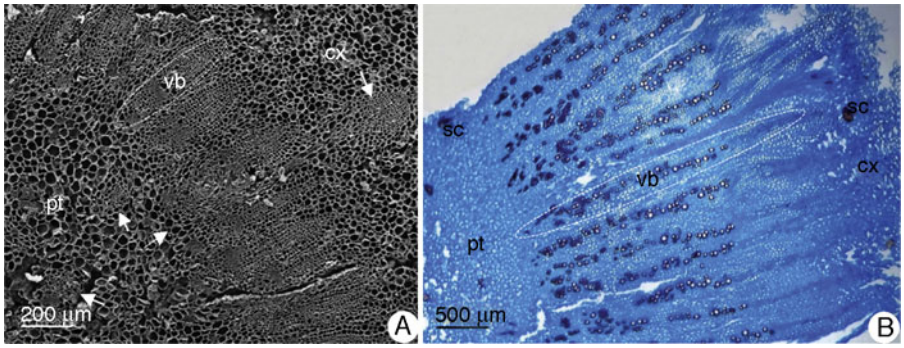

Fig. 6. General view of vascular bundles (highlighted) from different rhizome samples of *Limonium brasiliense* in cross section. Shorter, under SEM (A) or longer, under LM (B). cx: cortex, sc: sclereids, vb: vascular bundle. Arrows indicate anomalous vascular bundles in cortex and pith.

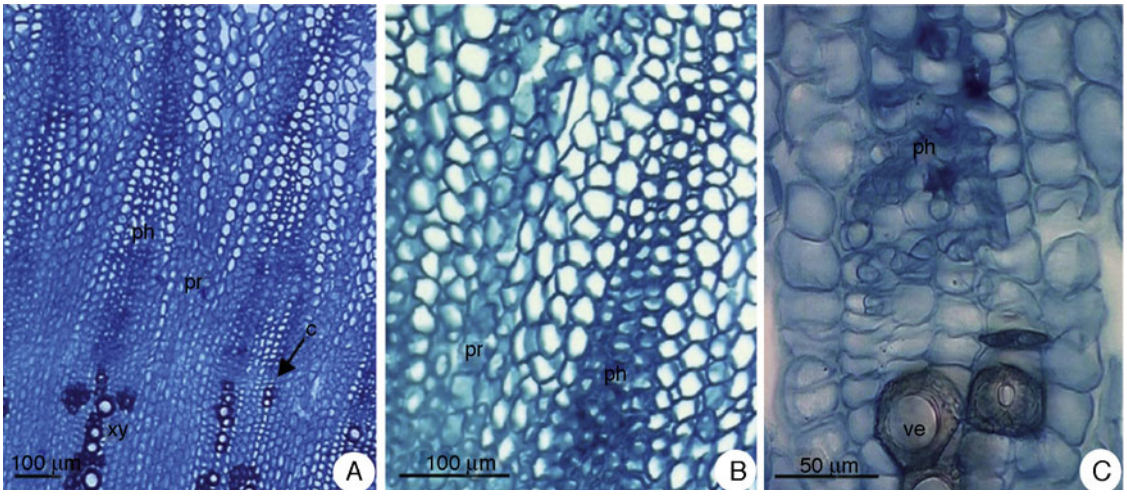

Fig. 7. Detail of some vascular bundles of rhizome of *Limonium brasiliense* under LM, indicating general aspect of delta phloem organization (A), details of parenchymatic ray (B) and phloematic cells (C). c: vascular cambium, ph: floem, pr: parenchymatic ray, ve: vessel element, xy: xylem.

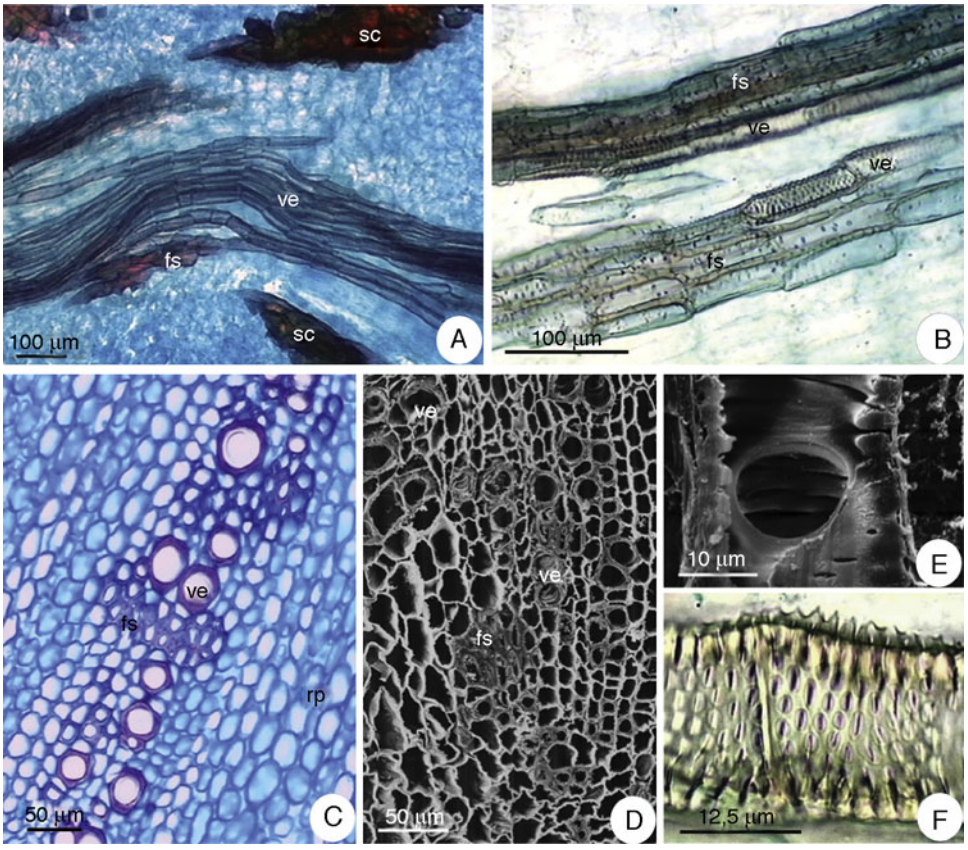

Fig. 8. Xylem of rhizome of *Limonium brasiliense*. Overall appearance of unaligned vessel elements (A); details of fiber-sclereids accompanying vessel elements (B), both in radial section; detail of xylem elements in cross section (C and D), and vessel elements in tangential section (E and F). fs: fiber-sclereids, rp: radial parenchyma, sc: esclereids, ve: vessel element. A, B, C and F under LM; D and E under SEM.

and beveled ends (Fig. 8B and C). These characteristics are very different from the stem of a species of *Plumbago* L., Plumbaginaceae, native to South Africa (Galal et al., 2013), which has thick strands of sclerenchyma surrounding the large vascular bundles; and from the species of *Limonium* analyzed by Colombo and Trapani (1992) and the rhizome of *L. furfuraceum* (Grigore et al., 2014), although the secondary xylem of this last species appears to be richer in sclerenchyma than those of the others.

When examined in more detail, the phloem elements are arranged in the form of delta (Fig. 7A and B), with only a few conductor elements (Fig. 7C). The xylem vessel elements (Fig. 8A) are short and unaligned with the major axis of the organ, having secondary walls with scalariform thickening with bordered pits and a simple perforation plate (Fig. 8B–F).

The pith of the rhizome of *L. brasiliense* is bulky, comprised of collenchymatous cells similar to those of the cortex, allowing the formation of large intercellular spaces (Fig. 9A). Groups of sclereids are visible (Fig. 9A), similarly to the three species of *Plumbago* analyzed by Galal et al. (2013), *P. auriculata* Lam., *P. indica* L., and *P. zeylanica* L.; as well as anomalous vascular bundles, as described above (Figs. 6A and 9B).

The rehydrated powder of the rhizome of *L. brasiliense* (Fig. 10) contains easily identified fragments with collenchymatous cells characteristic of the cortex, parenchyma rays, and pith, as well as groups of sclereids and vessel elements with their typical wall ornamentation and simple perforated plate.

All parenchyma cells of the rhizome in this species of *Limonium* show a strong reaction to ferric chloride, indicating that its

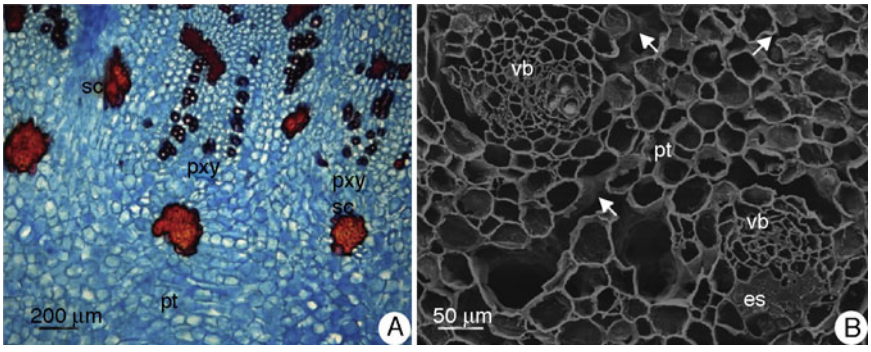

Fig. 9. Pith internal tissues (protoxylem and pith) of the rhizome of *Limonium brasiliense* under LM (A) and detail of isolated vascular bundles presents in the pith SEM (B). pt: pith, pxy: protoxylem, sc: sclereids, vb: vascular bundle. Arrows indicate small lobed projections in medullar parenchyma.

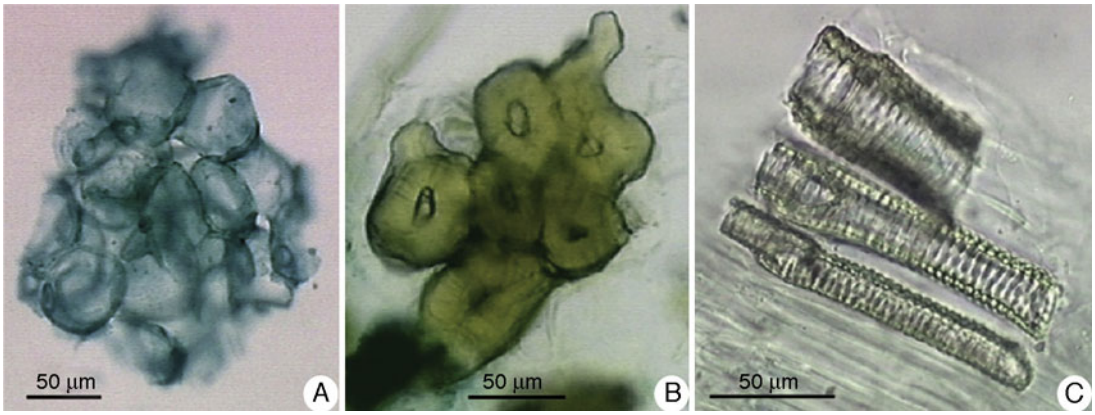

Fig. 10. Fragments observed in rehydrated powder of rhizome of *Limonium brasiliense* under LM. Cortex cells (A), sclereids (B) and vessel element (C).

walls are impregnated with polyphenols, similarly to the observations of Colombo and Trapani (1992) on three species of *Limonium*, *L. albidum* (Guss.) Pignatti, *L. intermedium* (Guss.) Brullo, and *L. lopadusanum* Brullo, native to the Pelagic Islands (Italy). Lin and Chou (2000) confirmed the presence of flavonoids and 20 phenolic compounds in the leaves and stem of *L. sinense* (Girard) Kuntze, and Grigore et al. (2014) observed tannins impregnating the sclerified cell walls of the rhizome of *L. furfuraceum*. No starch grains or other ergastic substances were detected in the parenchyma cells of the rhizome of *L. brasiliense*.

Conclusion

The analyses confirmed that the organ of *L. brasiliense* used in popular medicine is a rhizome, although the internodes, characteristic of this type of stem, are evident only in the younger portions. The main pharmacognostic features observed in powder from this species is the collenchymatous cortex tissue with small lobed projections, whose cell walls are impregnated with phenolic compounds; the elliptical-elongated vascular bundles with few conducting elements and sclerenchyma; and groups of sclereids with very thick walls and branched pits.

Authors' contributions

TMAU assisted in the laboratory work, analysis, discussion, and writing and formatting the article. AB collected and dried the plant material, prepared the voucher specimen, and assisted with writing. NCG and FG (undergraduate students) conducted the laboratory work, prepared the plant material for microscopic analysis, and assisted with writing. KAKC contributed to the scanning electron microscope analysis. EVSLM assisted in the project design and reviewed the manuscript. JCPM was responsible for conceiving the project and assisted with the writing, review and supervision of the study. MAMG supervised the laboratory work, performed the microscopic analyses, and supervised the writing.

Conflicts of interest

The authors declare no conflicts of interest.

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