Pharmacogn J. 2017; 9(3): 361-366
A multifaceted peer reviewed journal in the field of Pharmacognosy
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Original Article

Microscopic Characterization of *Croton cordiifolius* Baill. (Euphorbiaceae)

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ABSTRACT

Background: *Croton cordiifolius* Baill. is popularly known as "quebra-faca" in the Brazilian Northeast and is used to treat general inflammation, pain, and gastrointestinal disturbances. **Objective:** This paper aims to study the anatomical characteristics of *C. cordiifolius*. **Materials and Methods:** Cross-sections and paradermic sections of root, stem, petiole and leaf blade were obtained by hand and submitted to double staining with astra blue and safranin process for the anatomical study. Maceration was performed according to the method of Jeffrey. **Results:** The secondary root has peridermis, parenchymatic cortex, collateral vascular system surrounded by sclerenchyma. The secondary stem has peridermis, cortical region with lignified cells, isolated groups of fibers, laticifers, prismatic crystals, druses and starch. The vascular system of the stem is bicollateral. The petiole and the leaf blade have stellate, simple and glandular trichomes. The leaf blade is hypostomatic, with paracytic stomata. The mesophyll is isobilateral and the midrib shows a concave-convex contour. Laticifers occur in stem, petiole and leaf blade. Prismatic crystals and druses are found in all organs analysed. Through the maceration it was possible to identify the type of vessel elements. **Conclusion:** The anatomical features are useful for differentiation of other species of *Croton* and provide support to their quality control.

Key words: *Croton cordiifolius*, Euphorbiaceae, Pharmacobotany, Plant anatomy, Quebra-faca.

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History
- Submission Date: 22-12-16;
- Review completed: 05-01-17;
- Accepted Date: 02-02-17.

DOI : 10.5530/pj.2017.3.61

Article Available online
http://www.phcogj.com/v9/i3

INTRODUCTION

Caatinga ecosystem, covering almost one million km² in Brazilian Northeast, is characterized by xerophytic vegetation affected by long and irregular drought, high temperatures and elevated ultraviolet radiation. It is an exclusively Brazilian biome and its plants are widely used in folk medicine of local communities, whose socio-demographic conditions favor this reality.[1-2]

Euphorbiaceae family includes about 300 genera and 7600 species, including trees, shrubs, herbs and creepers.[3] This family is, quantitatively, the second most representative of Brazilian Caatinga, with about 68 species of *Croton* genus.[4] This genus, which presents a high pattern of species diversity and is the second largest in Euphorbiaceae family, consists of about 1200 species, of which 350 are present in Brazil.[4-5] It is considered of difficult taxonomic classification due to the high number of species, problems with the nomenclature, polymorphism and specific delimitation of its representatives.[6]

Traditional uses of *Croton* spp. have often been confirmed by pharmacological tests. In Brazil, among Euphorbiaceae Family members, this genus has the highest number of ethnopharmacological uses, followed by *Euphorbia* and *Jatropha*.[7] Among the proven pharmacological activities can be highlighted antioxidant,[2,8] anti-inflammatory and anti-nociceptive,[9] leishmanicidal,[10] antidiarrheal,[11] antiulcer,[12] anti-atherogenic and anti-ischemic,[13] cytotoxic, antimicrobial, antiviral, antihyperlipidemic, antidiabetic and weight loss actions.[14-17]

Due to the chemical compounds already described for many *Croton* species, such as mono and sesquiterpenes, diterpenoids, flavonoids, tannins and alkaloids, it can be characterized as a promising source of bioactive molecules and as a remarkable potential for research.[18] The growing interest in studying the various species of the *Croton* genus has resulted in the isolation and identification of more than 100 compounds currently known in Brazil.[19]

Croton cordiifolius, popularly known as "quebra-faca" in the Brazilian Northeast, is used to treat general inflammation, pain, and gastrointestinal disturbances.[20-21] Nogueira and collaborators[22] described the antinociceptive activity and chemical composition of its leaf's essential oil.

Although the *Croton* genus is widely studied, including some species found in the semi-arid northeast, no studies characterizing the botany of *C. cordiifolius* have been published. Since the correct species identification is a relevant step for researches reproducibility and the standardization of morphoanatomic markers is one of the parameters required for quality control of herbal raw material or drugs, this paper aims to study the anatomical characteristics of the root, stem, petiole and leaf blade of *C. cordiifolius*.

Cite this Article: Alves IABS, Sá RD, Cadena MB, Ximenes RM, Randau KP. Microscopic Characterization of *Croton cordiifolius* Baill. (Euphorbiaceae). Pharmacogn J. 2017;9(3):361-6.

MATERIALS AND METHODS

Plant material

Adult specimens of *Croton cordiifolius* were collected in the city of Salgueiro, Pernambuco, Brazil. The voucher specimen was deposited in the Herbarium Dárdano de Andrade Lima, of the Instituto Agronômico de Pernambuco (IPA), under registration number 89211.

Anatomical characterization

Several individuals of the same species were used to conduct this study. Various cross-sections at the middle region of the root, stem, petiole and leaf blade fixed in FAA 50% were obtained by freehand,[23] using a common razor blade. For leaf blade were also performed paradermal sections. All sections were clarified in sodium hypochlorite solution (50%)[24] and were stained according to the technique described by Bukatsch,[25] with safranin and astra blue. Then, semipermanent histological slides were prepared containing the sections botanical material, following common plant anatomy procedures.[23,26] The maceration was performed according to the method of Jeffrey,[23] using fragments of root, stem and leaf that were disintegrated with the mixture of 10% nitric acid and 10% chromic acid (1:1). The analysis of the semipermanent histological slides prepared were conducted with a light microscope (Alltion) equipped with a digital camera.[27]

RESULTS

Anatomical characterization

The secondary root, in cross-section, shows cylindrical contour, presenting peridermis (Figure 1A). The cortical region is composed of about seven layers of parenchymatic cells (Figure 1A), some of them containing starch (Figure 1B). The vascular system is formed by xylem occupying the central region of the root, and by phloem surrounding the xylem (Figure 1A). A continuous band of sclerenchyma delimits the vascular system (Figure 1A). Prismatic crystals and druses are displayed in the phloem, sclerenchyma and in the cells of the cortical region located near the sclerenchyma (Figure 1B).

The secondary stem, in cross-section, exhibits cylindrical contour and peridermis (Figure 1C). The cortical region is formed by parenchyma, where are found lignified cells (Figure 1C), isolated groups of fibers (Figure 1C), laticifers (Figure 1C), prismatic crystals (Figure 1D), druses (Figure 1D) and starch (Figure 1C,D). The prismatic crystals and druses are usually visualized near the sclerenchyma surrounding the vascular system and in the sclerenchyma itself (Figure 1D), as well as in the phloem and in the medullar region (Figure 1F). The prismatic crystals are more frequent than the druses. The phloem is situated externally to the xylem, forming a continuous ring, as well as internally the xylem, arranged in the form of isolated nuclei in the medullar region (Figure 1E). In the internal phloem are present groups of isolated fibers (Figure 1E). In the pith are found parenchyma (Figure 1E,F), lignified cells, stone cells, prismatic crystals, druses and starch (Figure 1F).

In cross-section, the petiole also presents cylindrical contour (Figure 1G). The epidermis is uniseriate, coated with a thin cuticle layer (Figure 1G). There are non-glandular trichomes and glandular trichomes. The non-glandular trichomes can be of two types: stellate trichome with multicellular stalk (Figure 1H) and simple trichome (Figure 1I). The glandular trichome has a unicellular head (Figure 1J). In subepidermal position appears about four layers of collenchyma (Figure 1G). The central region of the petiole is filled by parenchyma, in which are inserted several col-

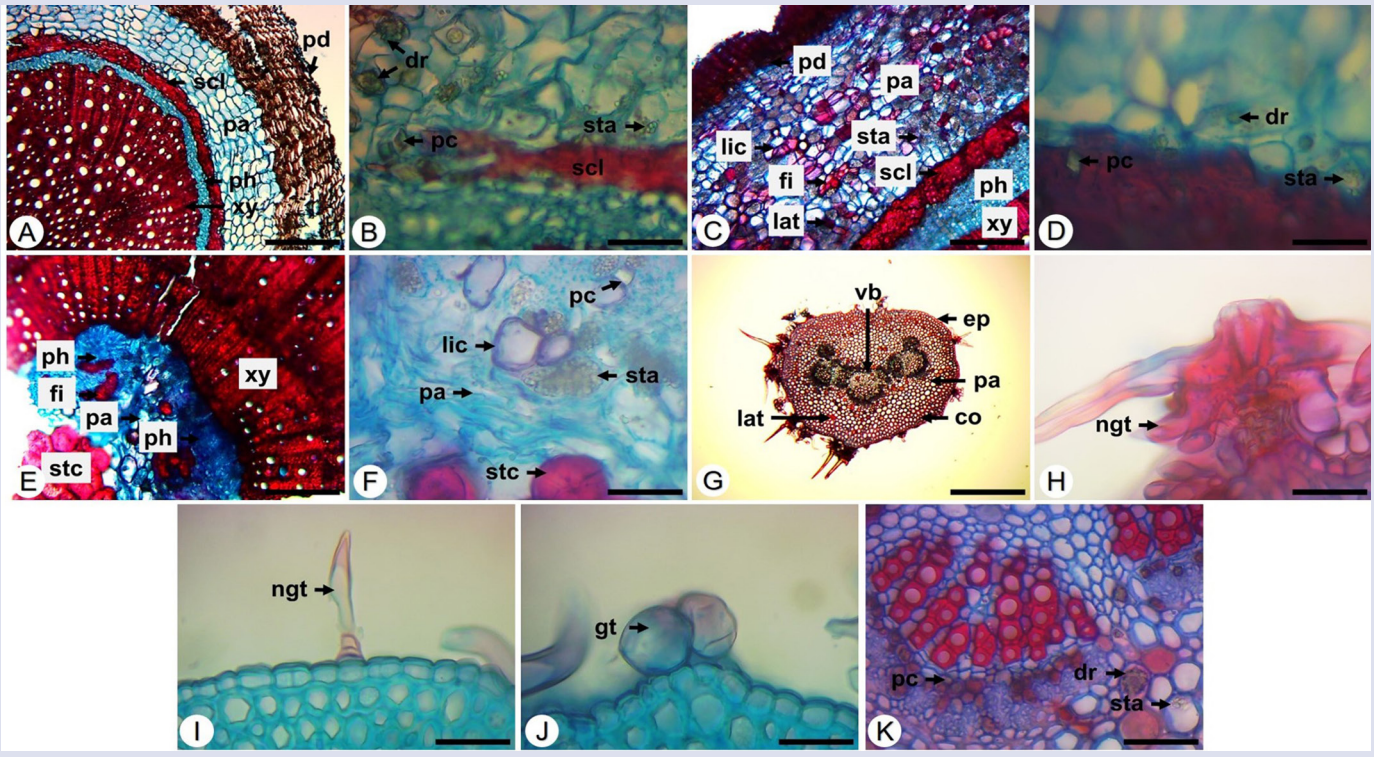

Figure 1: Cross-sections of the root, stem and petiole of *Croton cordiifolius* Baill.

Root: **A.** general view. **B.** crystals and starch. Stem: **C.** cortical region. **D.** crystals and starch. **E,F.** medullar region and vascular system. Petiole: **G.** general view. **H.** stellate trichome. **I.** simple trichome. **J.** glandular trichome. **K.** crystals and starch. co: collenchyma, dr: druse, ep: epidermis, fi: fiber, gt: glandular trichome, lat: laticifer, lic: lignified cell, ngt: non-glandular trichome, pa: parenchyma, pc: prismatic crystal, pd: peridermis, ph: phloem, scl: sclerenchyma, sta: starch, stc: stone cell, vb: vascular bundle, xy: xylem. Bars: **A,C,E:** 200 µm. **B,D,F,H,I,J,K:** 50 µm. **G:** 500 µm.

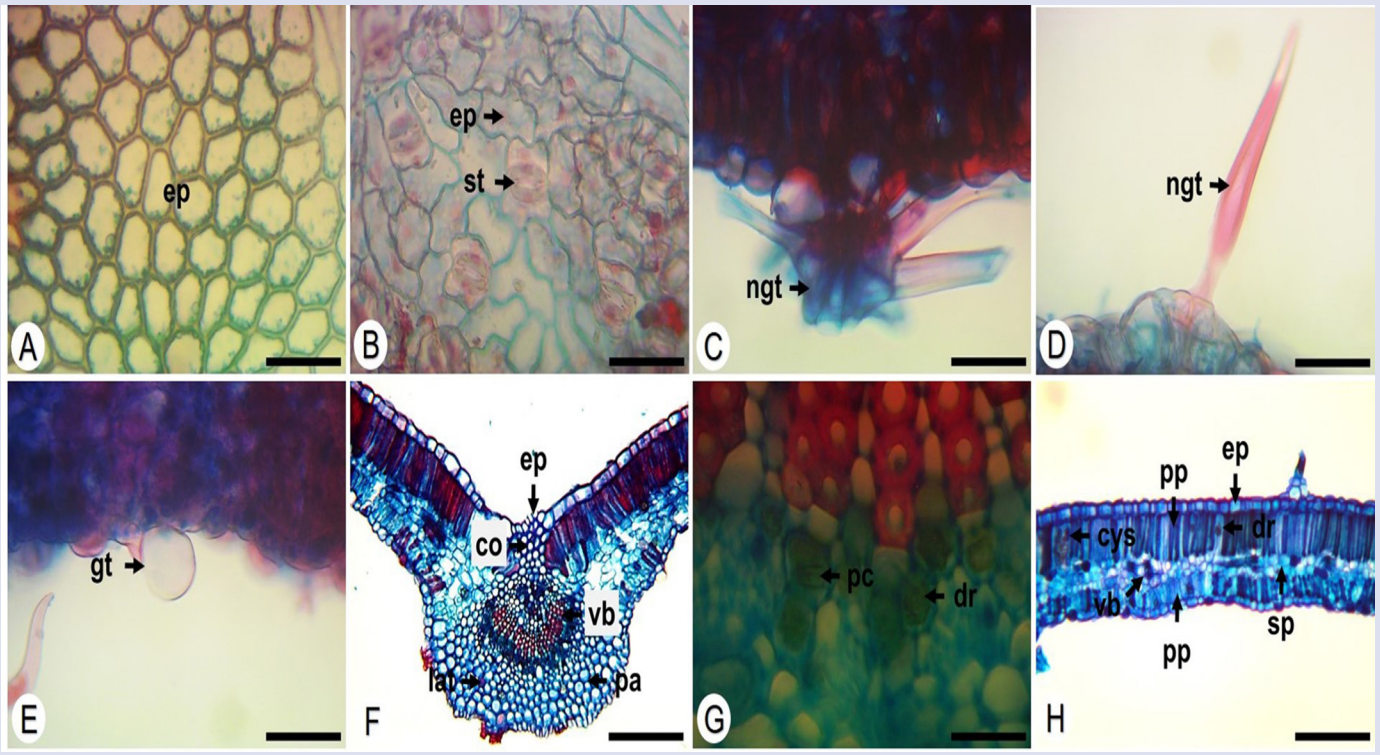

Figure 2: Cross-sections and paradermic sections of the leaf blade of *Croton cordiifolius* Baill.

A. adaxial face. **B.** abaxial face. **C.** non-glandular stellate trichome. **D.** non-glandular simple trichome. **E.** glandular trichome. **F.** midrib. **G.** crystals in the midrib. **H.** mesophyll. co: collenchyma, cys: cystolith, dr: druse, ep: epidermis, gt: glandular trichome, lat: laticifer, ngt: non-glandular trichome, pa: parenchyma, pc: prismatic crystal, pp: palisade parenchyma, sp: spongy parenchyma, st: stomata, vb: vascular bundle. Bars: **A,B,C,D,E.** 50 µm. **F,H**. 200 µm. **G.** 20 µm.

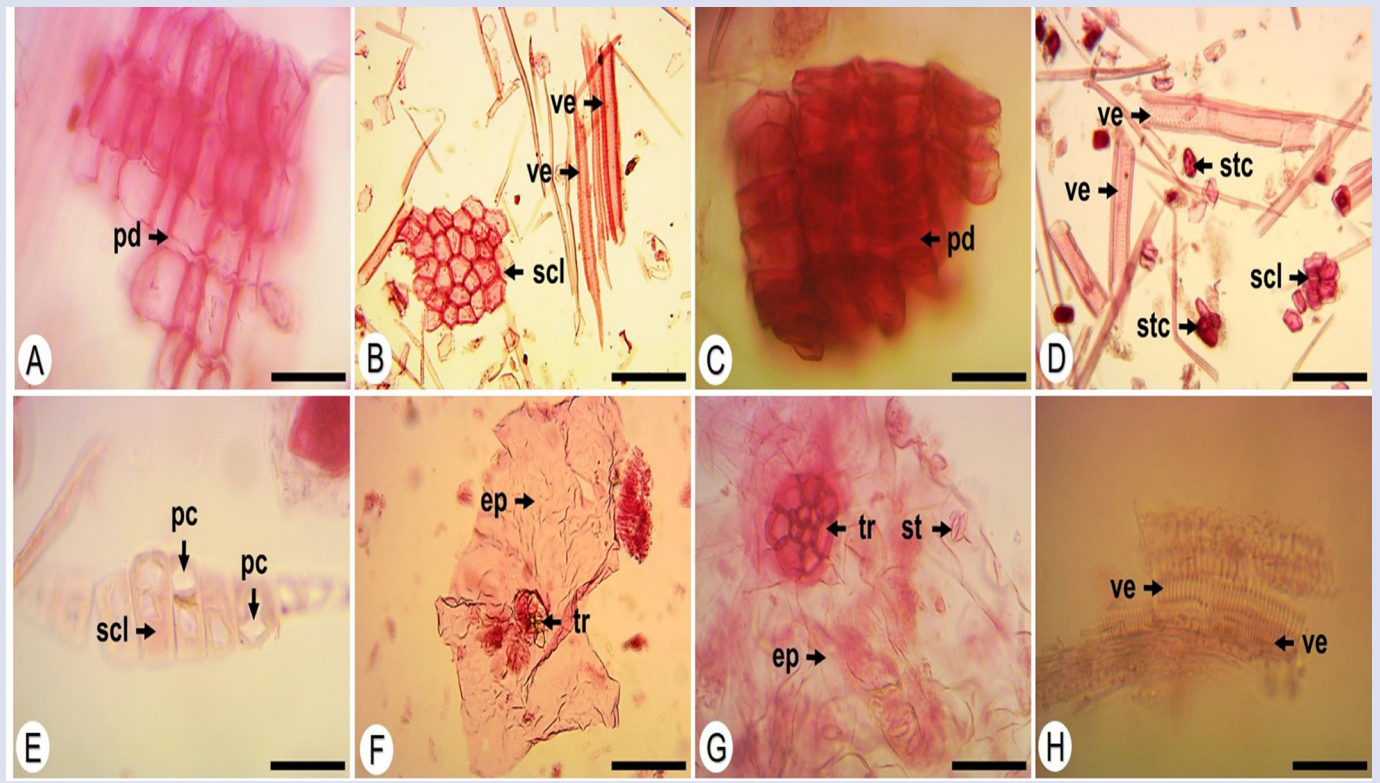

Figure 3: Maceration of the root, stem and leaf of *Croton cordiifolius* Baill.

A. peridermis of the root. **B.** parenchyma and vessel elements of the root. **C.** peridermis of the stem. **D.** parenchyma, stone cells and vessel elements of the stem. **E.** prismatic crystals in the sclerenchyma of the stem. **F.** adaxial face of the leaf blade. **G.** abaxial face of the leaf blade. **H.** vessel elements of the leaf. ep: epidermis, pc: prismatic crystal, pd: peridermis, scl: sclerenchyma, st: stomata, stc: stone cell, tr: trichome, ve: vessel element. Bars: **A,C,E,G,H.** 50 µm. **B,D,F.** 200 µm.

lateral vascular bundles (Figure 1G). Next to the bundles are visualized laticifers (Figure 1G), prismatic crystals, druses and starch (Figure 1K). Different what occurs in the secondary stem, in the petiole the druses are more frequent than the prismatic crystals.

The leaf blade, in frontal view, has cells with straight or slightly sinuous walls on the adaxial face (Figure 2A) and cells of sinuous walls on the abaxial face (Figure 2B). The leaf blade is hypostomatic, with paracytic stomata (Figure 2B).

In cross-section, the leaf blade presents the same types of trichomes described for the petiole (Figure 2C,D,E). The midrib shows a concave-convex contour (Figure 2F). The epidermis is composed of a single layer of cells and is covered by a thin cuticle (Figure 2F). The collenchyma is formed by about three to four layers, located in the adaxial face (Figure 2F). The vascular system is composed of a larger collateral vascular bundle towards to the abaxial face and smaller vascular collateral bundles towards the adaxial face (Figure 2F). Laticifers are presents in the parenchyma (Figure 2F). In the phloem are found prismatic crystals and druses (Figure 2G), being the druses more abundant, as occurs in the petiole. The druses can also be found in the parenchyma of the midrib, as well as in the mesophyll (Figure 2H). The mesophyll is isobilateral, with a layer of palisade parenchyma facing each face of the epidermis and around two to three layers of spongy parenchyma between the palisade tissue (Figure 2H).

Through the maceration it was possible to verify some of the characters observed in the cross-sections and paradermic sections of the analyzed organs. In maceration of the root are observed fragments of peridermis (Figure 3A), sclerenchyma and vessel elements with alternating pits (Figure 3B). In the maceration of the stem are also observed fragments of peridermis (Figure 3C), sclerenchyma and vessel elements with alternating pits (Figure 3D), besides stone cells (Figure 3D) and sclerenchymatic tissue with prismatic crystals (Figure 3E). In the maceration of the leaf are found fragments of the adaxial face (Figure 3F) and abaxial face (Figure 3G), where in the latter the stomata are visualized. The stellate trichome or their stalk are identified in the fragments of epidermis (Figure 3F,G). The other trichomes are difficult to see in maceration. In the maceration of the leaf it is also seen that the vessel elements are of the helical type (Figure 3H).

DISCUSSION/ CONCLUSION

Anatomical characterization

The laticifers are distributed in several genera of Euphorbiaceae[28]. They can be found in all vegetative organs, as in the case of species of *Euphorbia*.[29] According to Rudall[30] in *Croton* the laticifers are non-articulated and their abundance depends on the environment. Randau *et al*[31] did not mention the presence of laticifers in the roots of *Croton rhamnifolius* and *C. rhamnifolioides*, as it is also not identified in this study. Also as a common feature with both species of *Croton* analyzed by Randau *et al*,[31] in *C. cordiifolius* is described a parenchymatic cortex in the root. However, in *C. rhamnifolius* were verified gelatinous fibers in the phloem and in *C. rhamnifolioides* was seen pericycle. None of these characteristics is present in *C. cordiifolius*.

The pericycle was also visualized in the stem of *C. rhamnifolioides*.[31] It is possible to distinguish the stem of the species of *Croton* by the type of tissue that forms the cortical region and the structures found in these tissues. In *C. rhamnifolius* the cortical region is composed by collenchyma and parenchyma, while in *C. rhamnifolioides*, *C. draco* and *C. cordiifolius* only parenchyma is found.[31-32] However, in the cortical parenchyma of *C. rhamnifolioides* were observed fibers, laticifers and starch,[31] while in the cortical parenchyma of *C. draco* were found crystals,[32] besides the other mentioned elements for *C. rhamnifolioides*. In this study is also verified the presence of all these elements previously mentioned for the cortical region of the other species of *Croton*, however, it is also noted the presence of lignified cells.

Hayden and Hayden[33] noted internal phloem in *C. glandulosus*, but did not mention the occurrence of fibers associated with the internal phloem, as occur in *C. cordiifolius*. In *C. draco* was reported the presence of fibers in the external phloem.[32] Farías *et al*[32] also observed crystals in the sclerenchyma. According to Franceschi and Nakata,[34] the morphology of the crystals produced can be of a single type throughout the plant, or several types being each specific for a particular organ, or several types within the same organ. It was verified that this last case occurs in *C. cordiifolius*.

Tadavi and Bhadane[35] investigated the anatomy of rachis, petiole and petiolule in 43 species and 20 genera of Euphorbiaceae and observed that the variation in the distribution of sclerenchyma, collenchyma and vascular patterns, besides the shapes observed in the transverse section, can be used for differentiation of the species. Sá-Haiad *et al*[36] analyzed ten species of *Croton*. In three species, the outline of the petiole was circular; in six species the outline was approximately circular, with a depression in the adaxial face; and in one species the outline was concave-convex. *Croton rhamnifolius* and *C. rhamnifolioides* exhibit a plane-convex contour,[31] differing from the contour displayed by *C. cordiifolius*. In addition, the species of *Croton* can also be differentiated by the types of trichomes that are present in the petiole. In *C. rhamnifolius* and *C. rhamnifolioides* were verified dendritic and glandular trichomes,[31] in *C. cajucara* was identified the multiradiate trichome,[37] while in *C. cordiifolius* is seen stellate, simple and glandular trichomes.

Lucena and Sales[38] have characterized the types of trichomes presents in leaf blade of 14 species of *Croton* as stellate, fasciculate, multiradiate, dendritic, lepidote, simple and glandular. The authors have stated that the type of trichome is an important feature for the taxonomy of the genus. According to Haiad *et al*,[36] are common characters in leaf blades of *Croton* species: amphistomatic leaves, paracytic stomata, dorsiventral mesophyll and biconvex midrib, with collateral vascular bundles. Nevertheless, in *C. cordiifolius* the leaves are hypostomatic, the mesophyll is isobilateral and the midrib has a concave-convex contour. But, some of these characteristics of *C. cordiifolius* have already been described in other species of *Croton*, such as hypostomatic leaves in *C. lanjouwensis*[39] and isobilateral mesophyll in *C. rhamnifolioides*.[31] *Croton rhamnifolius* presents midrib with a plan-convex contour.[31]

No studies were found in the literature on the maceration of the organs of species of *Croton*. However, the studies of Luchi[40] on the root of *C. urucurana*, Luchi[41] on the stem of *C. urucurana* and Hayden and Hayden[33] about the stem of *C. glandulosus* described that the vessel elements have alternating pits.

The characters described in this study are important for the differentiation of *C. cordiifolius* from the other species of the genus *Croton*. Some characters, such as the non-glandular trichomes in the leaf blade, and the crystals, as the druses, demonstrate the adaptation of the species in xeric environments

ACKNOWLEDGEMENT

The authors thanks to CAPES and CNPQ for financial support in the form of fellowship awards.

CONFLICT OF INTEREST

None

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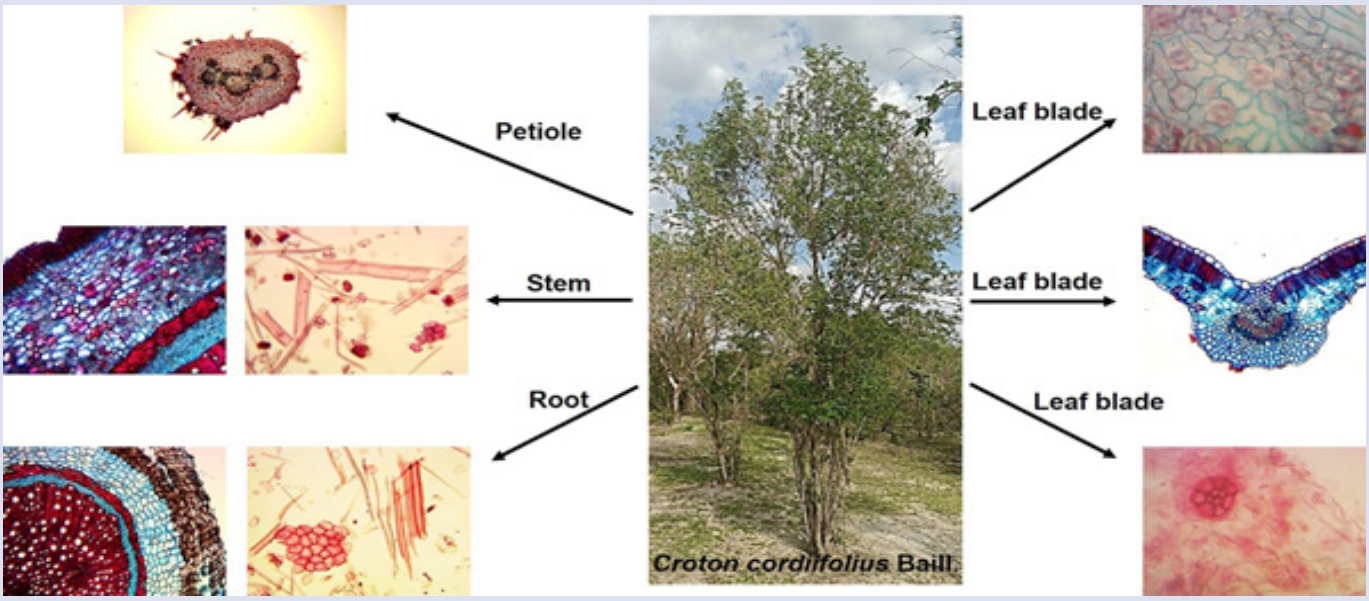
PICTORIAL ABSTRACT



SUMMARY

- The secondary root has peridermis, parenchymatic cortex, collateral vascular system surrounded by sclerenchyma and the secondary stem has peridermis, cortical region with lignified cells, isolated groups of fibers, laticifers, prismatic crystals, druses and starch.
- The petiole and the leaf blade have stellate, simple and glandular trichomes.
- The leaf blade is hypostomatic, with paracytic stomata; the mesophyll is isobilateral and the midrib shows a concave-convex contour.
- Laticifers occur in stem, petiole and leaf blade, while prismatic crystals and druses are found in all organs analysed.
- Through the maceration it was possible to identify the type of vessel elements.

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Cite this Article: Alves IABS, Sá RD, Cadena MB, Ximenes RM, Randau KP. Microscopic Characterization of *Croton cordiifolius* Baill. (Euphorbiaceae). Pharmacogn J. 2017;9(3):361-6.