

Anatomy of leaf and stem of *Erythrina velutina*

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Abstract: *Erythrina velutina* Willd., Fabaceae, known as “mulungu”, is a tree of tropical regions, as northeastern Brazil. Its bark is used in folk medicine as tranquilizer, sedative and insomnia. This study aimed to characterize the stem and leaf anatomy and to provide subsidies to quality control of the plant drug due to its wide use in folk medicine as well as its differentiation from other species with the same popular name. Samples were collected at Cuité, in Paraíba State, Brazil, fixed in FAA50, semipermanent slides were made, following usual procedures in plant anatomy. The stem shows a cylindrical contour, covered by a uniseriate epidermis covered by a thickened cuticle. It shows claviform glandular and branched trichomes with uniseriate stalk. Secretory cavities are into the phloem. The leaf epidermis has branched and glandular trichomes and anisocytic and paracytic stomata, on both sides, with predominance of branched trichomes and stomata on abaxial surface. Secretory cavities in stem and leaf, types of trichomes and stomata, its location and distribution constitute diagnostic characters for this specie. The structural characterization of the stem and leaf allows its distinction from other ones of this genus, ensuring safety for commercial pharmacological uses, allowing certification of the authenticity of raw material.

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Introduction

Comprising more than 650 genera and 18000 species, the Fabaceae (Papilionoideae) family is widely distributed throughout the world, especially in tropical and subtropical regions. According to Joly (1998) is one of families with the greatest representative numbers in the dicotyledons. Among the genres with native species, more common in the northeast region are *Phaseolus*, *Crotalaria*, *Erythrina*, *Andira*, *Sophora*, *Indigofera* and *Mucuna*, among others (Judd et al., 1999).

The *Erythrina* genus comprises about 120 tropical species in warm-temperate regions and is divided into five subgenres and 26 sections. In Brazil, there are 12 known species, of which eight are located in the northeast. *Erythrina velutina* Willd., Fabaceae, presents the botanic synonym *Coralloidendron velutinum* Willd., *Erythrina aculeatissima* Desf., *Erythrina splendida* Diels and *Chirocalyx velutinus* Walp. It is popularly known as suinã, mulungu, canivete and cork tree (Lorenzi & Matos, 2008). It can be found in a variety of biomes in the national territory. Popular medicine indicates the use of

several *Erythrina* genus species as a tranquilizer, sedative, insomnia control and aid in the treatment of inflammatory processes.

Phytochemical studies demonstrated that the plants belonging to the *Erythrina* genus are sources of tetracyclic alkaloids of the *Erythrina* flavonoids type, especially isoflavones, pterocarpanos, flavanones and isoflavanonas (Cabral, 2009), coumarins and saponins (Da Cunha et al., 1996; Rabelo et al., 2001; Virtuoso, 2005; Corrêa et al., 2008; Sousa et al., 2008).

Due to phytochemical employment, this study aim to characterize the anatomy of the leaf and stem of *Erythrina velutina*, in order to provide quality control subsidies for the plant drug, as well as its differentiation from other species with the same name popular denomination.

Material and Methods

Plant Material

The plant material was collected on the Campus

of the Federal University of Campina Grande, City of Cuité, located in the Paraíba State-Brazil, in the Curimatua region during the flowering period. The voucher specimen was deposited in the Herbarium UFP Geraldo Mariz, under registration number 63.317.

Methodology

The samples were fixed in FAA 50, and subsequently semipermanent histological slides were prepared, containing crosssections and paradermal sections of the material previously prepared, following normal plant anatomy procedures (Sass, 1951; Johansen, 1940).

The cross sections of the leaf blade and petiole median regions were obtained, by free hand, using a common razor blade and, as support material, petiole marrow from the embaúba (*Cecropia* sp.). Portions of the leaf-blade were cleared in sodium hypochlorite solution at 30% and stained with safranin and astra (Johansen, 1940) for analysis of epidermal cells in front view.

The stomata and trichome classification followed Metcalfe & Chalk (1950, 1983). The analyses were carried out in digital images captured by optical microscope (*Olympus*) coupled with a digital camera (*Sony*); the density of stomata was determined through the use of an image analysis program, *Image Tool* (Wilcox et al., 2002).

Microchemical tests were performed on fresh material: Sudam III for lipophilic substances (Sass, 1951) ferric chloride for phenolic compounds (Johansen, 1940) and Dragendorff reagent for alkaloids (Furr & Mahlberg, 1981).

Results

Macroscopic features

Erythrina velutina Willd., Fabaceae, is a tree up to 15 m high, aculeate or thorny, and deciduous. The stem bark is smooth to slightly rough. The leaf is ternate with an obtuse apex and symmetrical base. The inflorescence is fascicle type, presenting orangish flowers. The fruit is a legume, with an acute apex and base, with 1-3 seeds. The seeds are reniform, presenting a dark-red to orange-red color (Figure 1).

Microscopic features

Anatomical description of the stem

In cross-sectional view, the young stem presents a cylindrical contour (Figure 2a) with a layered epidermis covered by thickened cuticle (Figure 2b). Claviform glandular trichomes (Figure 2c) and branched tector trichome. (Figure 2d) with uniseriate stalk were

observed.

The angular collenchyma (Figure 2b) is observed immediately under the epidermis with 4-5 layers of cells. A band of collenchyma fibers is found, with 1-2 cells thick, about 4-6 layers of cortical parenchyma with many intercellular lacunas. Styloid and prismatic crystals (Figure 2b) are seen inside of subepidermal cells and in cortical parenchyma. Inside the central cylinder, collateral vascular bundles with fiber caps on the phloem and medullar parenchyma are found. The styloid crystals are also present in large quantities into the parenchyma cells within the vascular bundles and the medullary region.

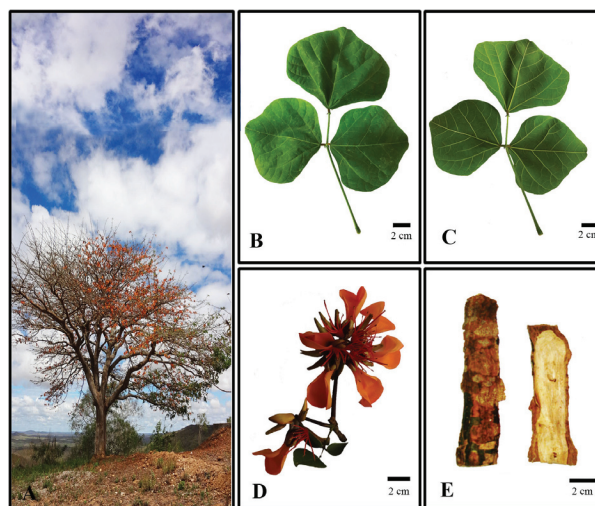


Figure 1. *Erythrina velutina* Willd. (Fabaceae). a. Individual in the field; b. Adaxial surface of imparipinnate leaf; c. Abaxial surface of leaf; d. Flowery branch; e. Stem aspect.

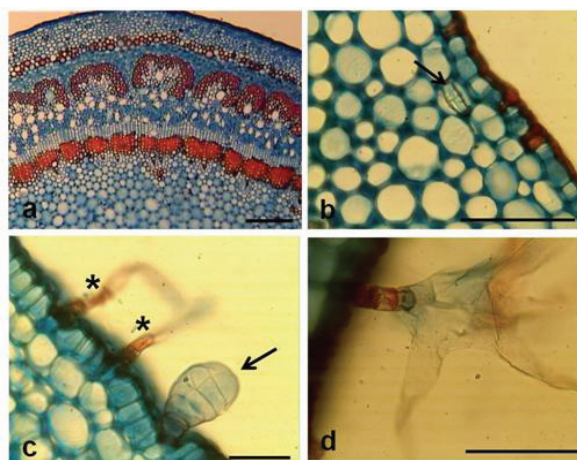


Figure 2. Stem of *Erythrina velutina* Willd. (Fabaceae) (a-d). a. Cross-sectional view of the primary structure; b. Thickened epidermis covered by cuticle and crystal (styloid, arrow) in the subepidermal parenchyma, followed by angular collenchymas; c. Damaged ramified trichomes (*) and glandular claviforme (arrow); d. Ramified trichome in detail. Bars: a=200 μ m; b, d=50 μ m; c=100 μ m.

The secondary growth is evidenced by the presence of phellogen in the cortex (Figure 3a) and vascular cambium in the central cylinder. Phellogen formation does not continuously occurs, and can be found in some areas, just under the epidermis (Figure 3a). Groups of fibers, forming a band over the phloem, were found in the stem cortex (Figure 3b).

The vascular bundles are collateral, with varying sized secretory cavities in the phloem (Figure 3c). In the region of the phloem, the secretory structures have various sizes (Figure 3d). The identification of the substance inside of these secretory cavities was not possible, which have an internal coating by periclinal flat or convex cells, arranged in a single layer (Figure 3d).

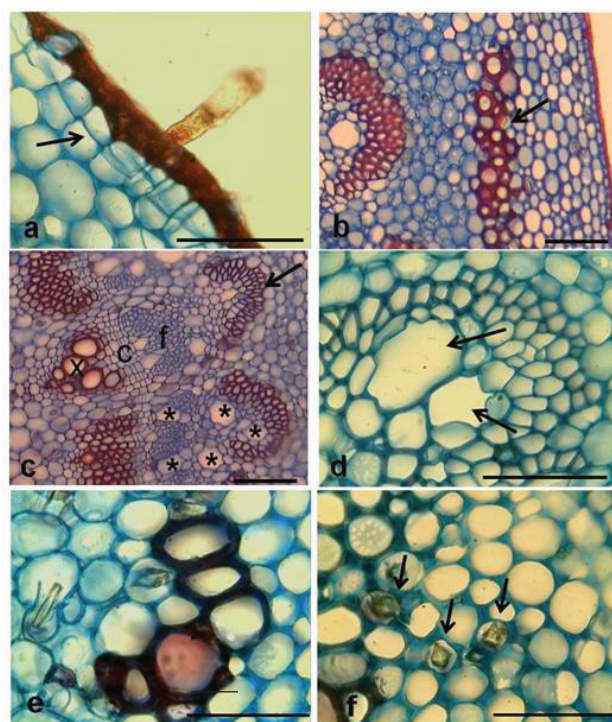


Figure 3. Stem of *Erythrina velutina* Willd., (Fabaceae): a. Cross-sectional view showing initiation of periderm subepidermal; b. Groups of fibers (band) over the phloem; c. Vascular bundles with fiber cap (arrow) over the phloem (f), cambium (c) followed by xylem (x) and secretory cavities inside the phloem (*); d. Secretory cavities (arrows) in the phloem; e. Groups of fibers; f. Prismatic crystals (arrows) in the outer cortical parenchyma. Bars: a, d, e, f=50 μ m; b, c=100 μ m.

Anatomical Description of the petiole

The petiole presents a uniseriate epidermis, branched trichomes, covered by a thickened cuticle, followed by three to four layers of angular collenchyma. The external cortical parenchyma cells exhibit inter cellular spaces of various dimensions (Figure 4a, 4b). Prismatic and styloid crystals were observed inside the collenchyma,

cortical parenchyma, and phloem and xylem cells (Figure 4c, 4d, 4e). Sclerenchyma fiber caps surrounding the crystalliferous sheath cells in the vascular bundles (Figure 4f). There are, approximately, 12 collateral vascular bundles, with 4-5 cells of thickness circumference with lignified cell walls.

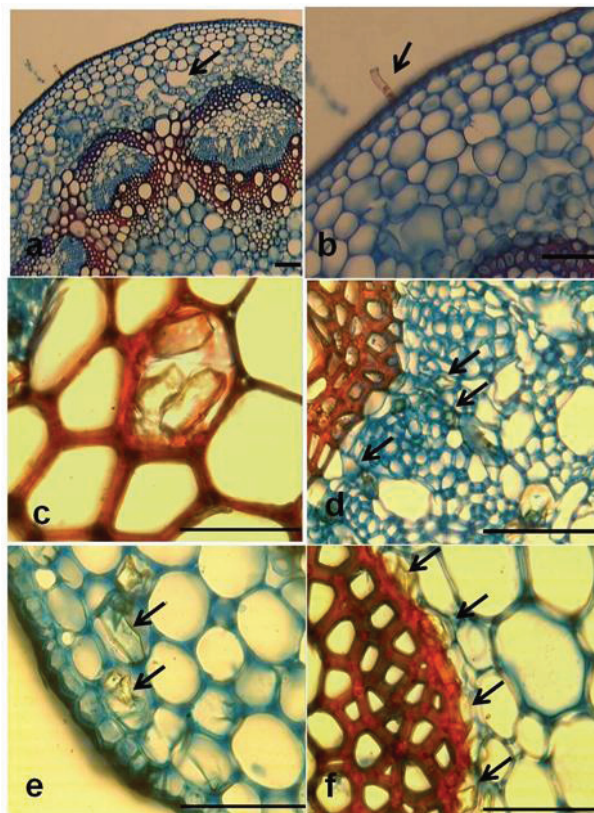


Figure 4. Petiole of *Erythrina velutina* Willd. (Fabaceae): a. Cross-sectional view of the petiole showing vascular bundle collateral and spaces in the cortical parenchyma (arrow); b. Trichoma incomplete (arrow); c. Styloid crystals in xylem cell; d. Prismatic crystals in the phloem (arrow); e. Styloid and prismatic crystals into the collenchyma (arrow); f. Fiber caps surrounded by a sheath of crystal cells (arrows). Bars: c, d, e, f=50 μ m; b=100 μ m; a=200 μ m.

Anatomical Description of the leaf-blade

The leaf-blade, in front view, epidermis with predominance of branched and glandular trichomes and paracytic and anisocytic stomata on both surfaces (Figure 5a-d). In the adaxial surface, epidermal cells on the veins have prismatic crystals on their interior. The anticlinal walls of epidermal cells are sinuous on both surfaces (Figure 5a-d). The stomata density on the abaxial surface was 264.60 ± 16.83 and on the adaxial surface was 46.60 ± 8.82 (Figure 5e-f).

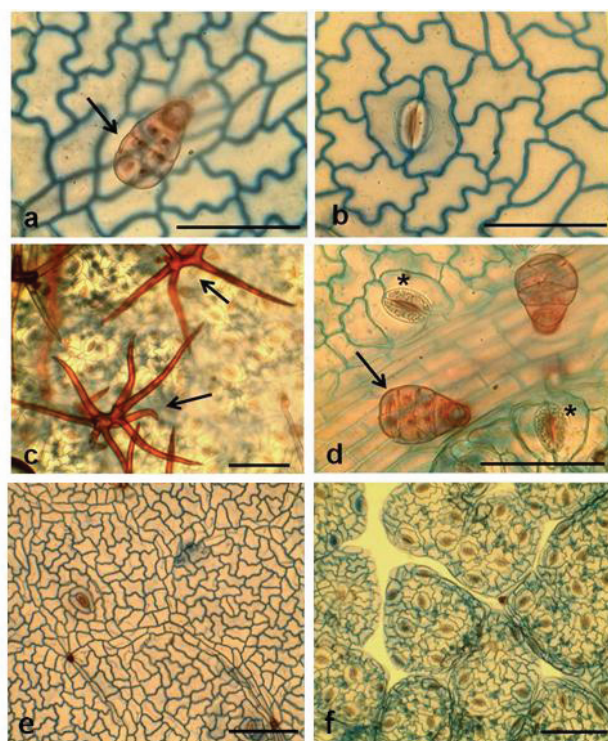


Figure 5. Leaf of *Erythrina velutina* Willd. (Fabaceae): a. Front view of adaxial surface showing capitate glandular trichomes; b. Paracytic stomata on adaxial surface; c. Branched trichomes on the abaxial surface; d. Paracytic stomata and glandular and capitate trichomes on the ribs on the abaxial surface; e. Adaxial leaf epidermis surface; f. Abaxial leaf epidermis surface. Bars: a, b, d, g, h=50 μ m; c, f, i, j, l=100 μ m; e=200 μ m.

In cross-sectional view, the leaf-blade presented branched trichomes with uniseriate stems (Figure 6b). The epidermis surface, on the abaxial surface, presents large furrows in the leaf vein region (Figure 6c). The mesophyll is of the dorsiventral type, with three layers of palisade parenchyma and a number of varied layers of spongy parenchyma, with large inter cellular spaces. The mesophyll presents two layers of colourless parenchyma cells, between palisade and spongy (Figure 6a). Prismatic crystals are present inside the uniseriate layer of cells that involve the fibre cap around the vascular bundles on the mesophyll interior. The vascular bundle is collateral closed, with angular collenchyma over and under the veins. The vascular bundles, in the lower caliber leaf vein region of the show sheath extensions in the direction of the adaxial surface and fiber cap on the xylem and the phloem (Figure 6c). Secretory cavities are present in the vascular bundles, always associated with the phloem, both in the mesophyll as in the petiole. These cavities are always delimited by a uniseriate epithelium of small flattened periclinal cells (Figure 6d).

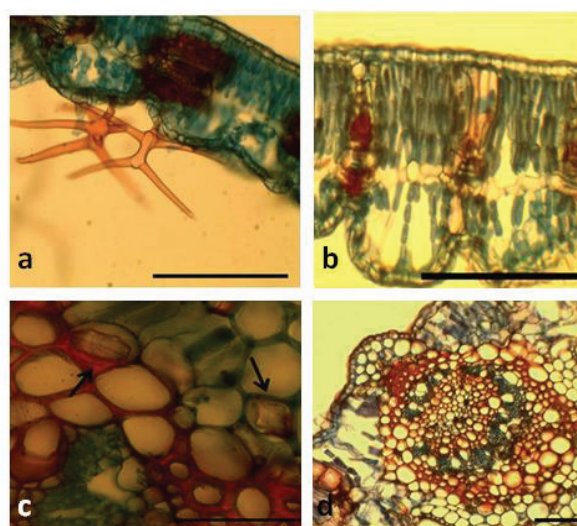


Figure 6. Leaf of *Erythrina velutina* Willd. (Fabaceae): a. Dorsiventral mesophyll and branched trichomes on the abaxial surface; b. Abaxial leaf epidermis with large grooves; c. Prismatic crystals into the bundle sheath (arrows); d. Closed collateral vascular bundle. Bars: a, b, d, g, h=50 μ m; c, f, i, j, l=100 μ m; e=200 μ m.

Histochemical tests indicated the presence of phytoconstituents of lipid droplets, alkaloids and phenolic substances in stem and in leaf of *E. velutina*. In the Stem (Figure 7a-d) and in the leaf (Figure 7e-j), phenolic compounds were found in secretory cavities inside the phloem (Figure 7a) and in branched trichomes (Figure 7c and 7i). Alkaloids is present in parenchyma cells and secretory cavities (Figure 7b), in glandular and tector trichomes (Figure 7d) and in capitate glandular trichome (Figure 7g). The lipid droplets were marked with Sudam III in cuticle (Figure 7e-f) and branched trichomes (Figure 7j). The color absence was performed by a control (Figure 7h).

Discussion

Several representatives of the genus *Erythrina* show pharmaceutical importance, such as *E. cristagalli*, *E. falcata*, *E. speciosa* and *E. verna*, and are frequently used in popular medicine. Due to the similarities among the species, with the same pharmaceutical application, with external leaf (blade and petiole) and stem morphology of *Erythrina velutina* Willd., Fabaceae, showing similar structures to those described for the genus in the literature (Almeida, 2010; Almeida, 2011; Carvalho, 2008; Farmacopéia Brasileira, 2010; Gratieri-Sossela, 2005), the morphological and anatomical description of this species allows distinguish it among other species of this genus. Glandular and tector trichomes, paracytic stomata, dorsiventral mesophyll, and crystals were referenced to the Fabaceae family by Metcalfe & Chalk (1950) and were

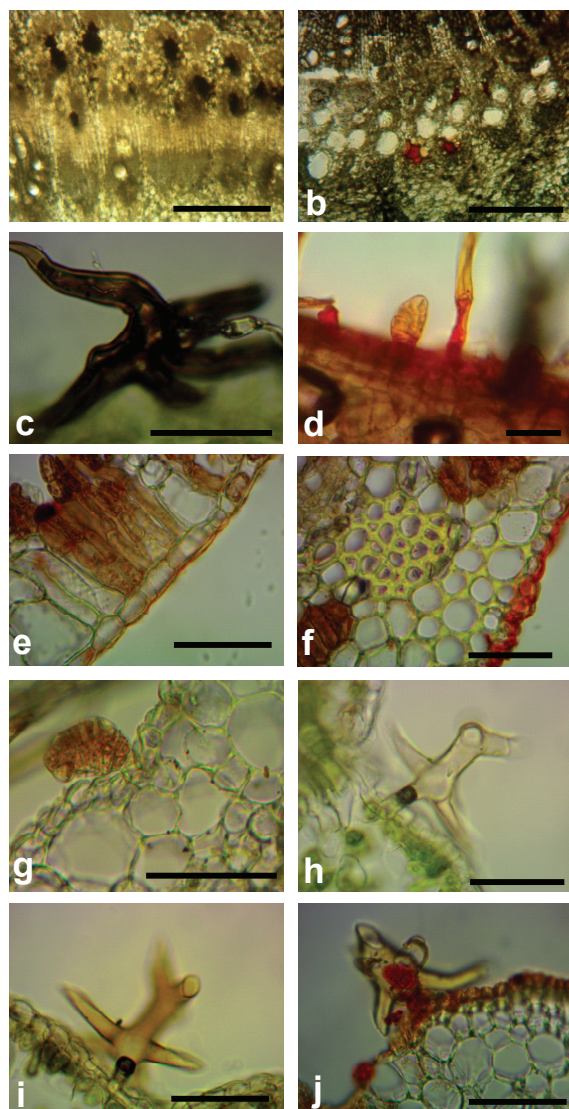


Figure 7. Stem and leaf of *Erythrina velutina* Willd. (Fabaceae): structure and histochemistry: (stem a-d; leaf e-j). a. ferric chloride in secretory cavities inside the phloem, b. Dragendorff reagent presence the alkaloids in parenchyma cells and secretory cavities, c. ferric chloride in branched trichomes, d. Dragendorff reagent in glandular and tector trichomes, e-f. Sudam III in cuticle, g. Dragendorff reagent in capitulate glandular trichome, h. control, i. ferric chloride in branched trichomes, j. Sudam III in branched trichomes. Bars: a, b, e, f=200 μ m; c, d, g, h, i, j=50 μ m.

confirmed in this study for *Erythrina velutina*. Branched trichomes with uniseriate peduncle are characteristic for the *Erythrina* genus, according to Metcalfe & Chalk (1950), however, *E. cristagalli* present no epidermis adorned with trichomes (Gratieri-Sossela, 2005). Similar to *E. velutina*, *E. falcata* and *E. speciosa* it also exhibits this same type of trichome (Almeida, 2010; Almeida, 2011). In addition, *E. velutina* has uniseriate pluricellular and bifurcated tector trichomes. Glandular, multicellular and peltate trichomes were referenced for *Centrolobium tomentosum*, another

genus from to the same family (Erbano & Duarte, 2012).

Considering the types of stomata found in *E. velutina* (paracytic and anisocytic), the same were found in *C. tomentosum*, *E. speciosa* and *E. falcata* (Almeida, 2010; Almeida, 2011; Urbano & Duarte, 2012). In the latter species, in addition to paracytic stomata, anomocytic stomata were found. In *Holocalyx balansae*, a species also from the Fabaceae family; anisocytic stomata were found (Ló & Duarte, 2011), as well as in *E. velutina*.

A greater quantity of stomata was observed on the abaxial surface of the epidermis, similar to that recorded for *Centrolobium tomentosum*, *E. speciosa*, *E. falcata* and *Holocalyx balansae* (Almeida, 2010; Almeida, 2011; Urbano & Duarte, 2012; Ló & Duarte, 2011). Greater stomatal density on the abaxial surface is common in species that occur in xeromorphic environments, a fact explained as a feature that minimizes water loss by ostiolar evapotranspiration (Esau, 1974; Cutter, 1986).

Sinuuous anticlinal walls were observed in *E. falcata*, *E. speciosa* and *E. cristagalli* (Almeida, 2010; Almeida, 2011; Gratieri-Sossela, 2005), similar to that found in *E. velutina* in this study.

Cells containing crystals inside it in the cortical and/or medullar parenchyma is classified as crystalliferous, according to Metcalfe & Chalk (1950). Crystalliferous' cells are found in the internal and external cortical and medullar parenchyma of *E. velutina*, always predominant in the styloid type. The genus *E. velutina*, unlike other species, as well as in *E. speciosa*, *E. falcata* and *E. verna* presents a sheath of crystalliferous cells involving the fibres over the vascular bundles, along the veins (Almeida, 2011).

The presence of secretory structures is indicative of substance production by the plant (Fahn, 1990) and, in the case of *E. velutina* is associated with the production of resins according Metcalfe & Chalk (1950). The secretory ducts are associated with the phloem in the vascular bundles of the stem in *E. velutina*; the same was not observed for other species of the *Erythrina* genus and even for species of other Fabaceae genus (Almeida, 2010; Almeida, 2011; Urbano & Duarte, 2012, Ló & Duarte, 2011). Secretory structures in Fabaceae are mainly related with fruits (Paiva et al., 2008), extrafloral nectaries (Paiva & Machado, 2006) and trichomes (Paiva, 2009); secretory cavities in leaves were described by different authors (Fahn, 1979; Metcalfe, 1983; Metcalfe & Chalk, 1983; Langenheim, 1967).

Dorsiventral mesophyll type is also recorded in the literature for the *Erythrina* genus, as in *C. tomentosum* and *H. balansae*, with the exception of *E. cristagalli*, for which some authors classified as isobilateral (Almeida, 2010; Almeida, 2011; Gratieri-Sossela, 2005).

The type of vascular bundle found in *E. velutina* (bicollateral in closed arc form) was similar to that found in *E. falcata* and different from a semi closed arc, as found in *E. cristagalli* (Almeida, 2010; Almeida, 2011; Gratieri-Sossela, 2005). It is characteristic of these species have

layers of achlorophyllous cells or with little chloroplasts between the palisade and spongy parenchyma, or between the palisade layers as in the *E. cristagalli*. The presence of secretory cavities for these species was not cited. Fiber caps on the xylem and phloem as well the crystals into the cells of vascular bundle sheaths also have been described for *E. cristagalli* (Gratieri-Sossela, 2005). Great grooves on the abaxial epidermis can be a diagnostic character to *E. velutina*, since it is the most evident anatomical feature that distinguish it from others.

Leaves of *E. falcata* and *E. cristagalli* show the presence of prismatic crystals was also reported inside the phloem (Almeida, 2010; Almeida 2011; Gratieri-Sossela, 2005), differing from *E. velutina* that not have a sheath around the sclerenchyma fiber caps.

Histochemical tests evidenced phenolic compound in secretory cavities inside the phloem and branched trichomes. The phenolic compounds are heterogeneous groups of substances that are present in almost all plants, inside the vacuole, cytoplasm or consisting the wall cell (Fahn 1979).

The Sudam reaction detected total lipids in cuticle and branched trichomes. The presence of alkaloids in parenchyma cells and secretory cavities in the stem, in glandular, tector and capitate glandular trichomes in the leaf are according to the literature findings to the genus *Erythrina* (Virtuoso, 2005). The same was true to indolic alkaloids, mainly the erythrinic type (Almeida, 2010). The occurrence of alkaloids is a chemotaxonomical character in the Papilionoideae subfamily. There are registers of quinolizidic, dipiperidinic and pirrolizidinic types, isolated and identified at approximately 60 genera of this subfamily (Kinghorn & Smolenski, 1981).

The presence of secretory cavities in the stem and leaf, types of trichomes and stomata, its location and distribution in the leaf epidermis constitute diagnostic characters that are important for an accurate and safe identification for *E. velutina*.

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Authors contributions

MMBS (MSc student) and ASCOS (undergraduate student) contributed in plant sample identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. RMMP, FCLS, KPR contributed to data analysis

and discussion. LALS contributed to discussion and critical reading of the manuscript. All laboratorial activities were supervised by KPR and LALS. All the authors have read the final manuscript and approved the submission.

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