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Vol. 8(27), pp. 947-952, 17 July, 2014
DOI: 10.5897/JMPR2014.5460
Article Number: E72E8C646454
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Secretory structures in *Cochlospermum regium* (Schrank) Pilg. (Bixaceae): Distribution and histochemistry

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Received 27 April, 2014; Accepted 29 June, 2014

Cochlospermum regium **(Schrank) Pilg., known as the yellow cotton tree, is a small shrub with a number of pharmacological properties. A series of chemical compounds produced by the plant may be associated with secretory cells and tissues, such as idioblasts, trichomes and ducts. Thus, the aim of this study was to identify the secretory structures and major classes of compounds in vegetative organs of *C. regium*. Leaf, stem and root samples were collected, and transverse and longitudinal sections were examined by routine anatomical and histochemical methods. The results revealed that the leaves, roots and stems of *C. regium* are rich in ducts and idioblasts with a variety of secondary metabolites, justifying its wide use in phytotherapy.**

Key words: Yellow cotton tree, ducts, idioblasts.

INTRODUCTION

The use of medicinal plants for the treatment and prevention of diseases is one of the oldest practices in human history. Because of studies that have demonstrated their safety and efficacy, at present, medicinal plants and their products are sold in pharmacies and health stores, bearing commercial labels (Veiga Junior et al., 2005). Due to the growing demand for herbal medicines, the number of companies in this field has increased in Brazil, resulting in the issuing of the Presidential Decree No. 5813, of June 22, 2006, which approved the National Policy on Medicinal Plants and Herbal Medicines. This decree regulates the production, distribution and rational use of

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medicinal plants in a sustainable manner, thus ensuring the safety, efficacy and quality of these products, in addition to expanding the therapeutic options and user access to this therapeutic modality (Brazil, 2006).

Cochlospermum regium (Schrank) Pilg., known as the yellow cotton tree, is a small shrub that displays plasticity in flowering time, blooming several times over the year (Camillo et al., 2009; Inácio, 2010). This plant is known to have several pharmacological actions of medicinal interest, such as the treatment of gastritis, ulcers, gynecological infections, (Oliveira et al., 2007; Camillo et al., 2009), intestinal infections, skin disorders, arthritis (Camillo et al., 2009), urogenital disorders, and as a purgative (Vila Verde et al., 2003; Guarim-Neto, 2006). Examples of the many uses of *C. regium* include tea made from roots and bark, topical use of the decoction in sitz baths, white wine *garrafada* (single or mixed plant species macerated in white wine and sold in bottles) called *enóleo*, and aqueous infusion for the treatment of various diseases (Nunes et al., 2003; Vila Verde et al., 2003; Guarim Neto, 2006; Sólon et al., 2009). However, if the genus *Cochlospermum*, as a whole, is considered, not only the species, pharmacological properties including hepatoprotective, antimalarial, antibacterial, antitumor, antiviral, detoxifying, antileishmanial, antihypertensive, antidiabetic, anti-inflammatory, anti-schistosomiasis, and antidiarrheal activities, among others, have also been reported (Antunes, 2009).

A number of chemical compounds produced by the plant may be associated with secretory cells and tissues, such as idioblasts, trichomes, emergences, cavities, and ducts. This study aimed to identify the secretory structures and the major classes of substances in vegetative organs of *C. regium*.

MATERIALS AND METHODS

Plant

Leaves, stems and roots of *C. regium*, Bixaceae, were collected in the "Cerrado" (Brazilian savannah) of the Environmental Protection area of the University of Rio Verde (UniRV), more specifically at the "Fazenda Fontes do Saber" in the city of Rio Verde, Goiás, Brazil (17° 47' 53'' S; 51° 55' 53'' W). The identification of *C. regium* was performed by a specialist (first author) and the voucher specimen number HRV 362 was deposited at the Herbarium of Rio Verde in the Goiano Federal Institute (IFGoiano) Campus Rio Verde.

Assays

Samples for anatomical study were fixed in formalin, acetic acid and 70% alcohol (FAA_{70}). Transverse and longitudinal sections of the fixed plant organs were then cut using a microtome (model LPC, Rolemberg and Bhering, Belo Horizonte, Brazil) with disposable steel blades and stained with toluidine blue (O'Brien et al., 1965) or astra-blue/safranin stain (Bukatsch, 1972). For analysis of the leaf surface the blade sections were cleared (Kraus and Arduin, 1997), stained with safranin and mounted in 50% glycerol. Fresh samples of vegetative organs of *C. regium* were used for histochemical analysis. Sections of the vegetative organs of the plant were also cut with the microtome. The sections were stained with Sudan IV (red) for lipids (Pearse, 1980); Xylidine Ponceau for proteins (O'Brien and McCully, 1981); periodic acid-Schiff reagent (PAS) for total carbohydrates (Maia, 1979); ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for phenolic compounds; Nadi reagent for essential oils and oleoresins (David and Carde, 1964); vanillin-HCl for tannins (Mace and Howell, 1974); Wagner and Dittmar reagents for alkaloids (Furr and Mahlberg, 1981); and phloroglucinol for lignins (Johansen, 1940). The images were captured using a digital camera (Leica DC 50, Leica Microsystems, Germany) attached to a Leica DM500 microscope.

RESULTS

The leaf of the *C. regium* showed a single-layered epidermis with anomocytic stomata confined to the abaxial surface, characterizing the leaf as hypostomatic (Figure 1a and b). In cross-section view, the epidermis exhibited upright cells on the adaxial side, and cells with rectangular shape on the abaxial side. In frontal view, the epidermal cells had a polyhedral shape with straight walls (Figure 1c and d). The midrib of the *C. regium* leaf contained a collateral vascular bundle. Immediately below the epidermis, a thin layer of angular collenchyma was observed and fundamental parenchyma delimited numerous ducts distributed in the cortical region of the midrib (Figure 1c). A large number of cells just underneath the phloem, intensely stained with safranin, were identified as idioblasts, which together with ducts, constitute the secretory structures of *C. regium* (Figure 1c). The mesophyll was dorsiventral with 1 or 2 layers of palisade parenchyma cells followed by 3 to 4 layers of spongy parenchyma cells delimiting small intercellular spaces (Figure 1d). The stem of *C. regium* had a single-layered epidermis and a phellogen layer throughout its length (Figure 2a), indicating a secondary growth of the organ. The vascular cambium can be observed between the secondary xylem and phloem (Figure 2b). External to the phloem, there were several layers of fibers with walls of thin to medium thickness. Ducts, such as those present in the leaf, were observed in the medullary region (Figure 2b).

Secondary growth was also observed in the root of *C. regium*. The root surface had a thick periderm. The phloem layer was visible underneath the periderm as well as numerous layers of vascular cambium cells. Ducts were distributed in the root cortex and near the phellogen differentiation region, and delimited by fundamental parenchyma (Figure 3a and b). In the xylem, there was a predominance of elements of solitary vessels of simple perforation plates with a helical secondary wall deposition pattern (Figure 3c to f). Thin-walled fibers and axial

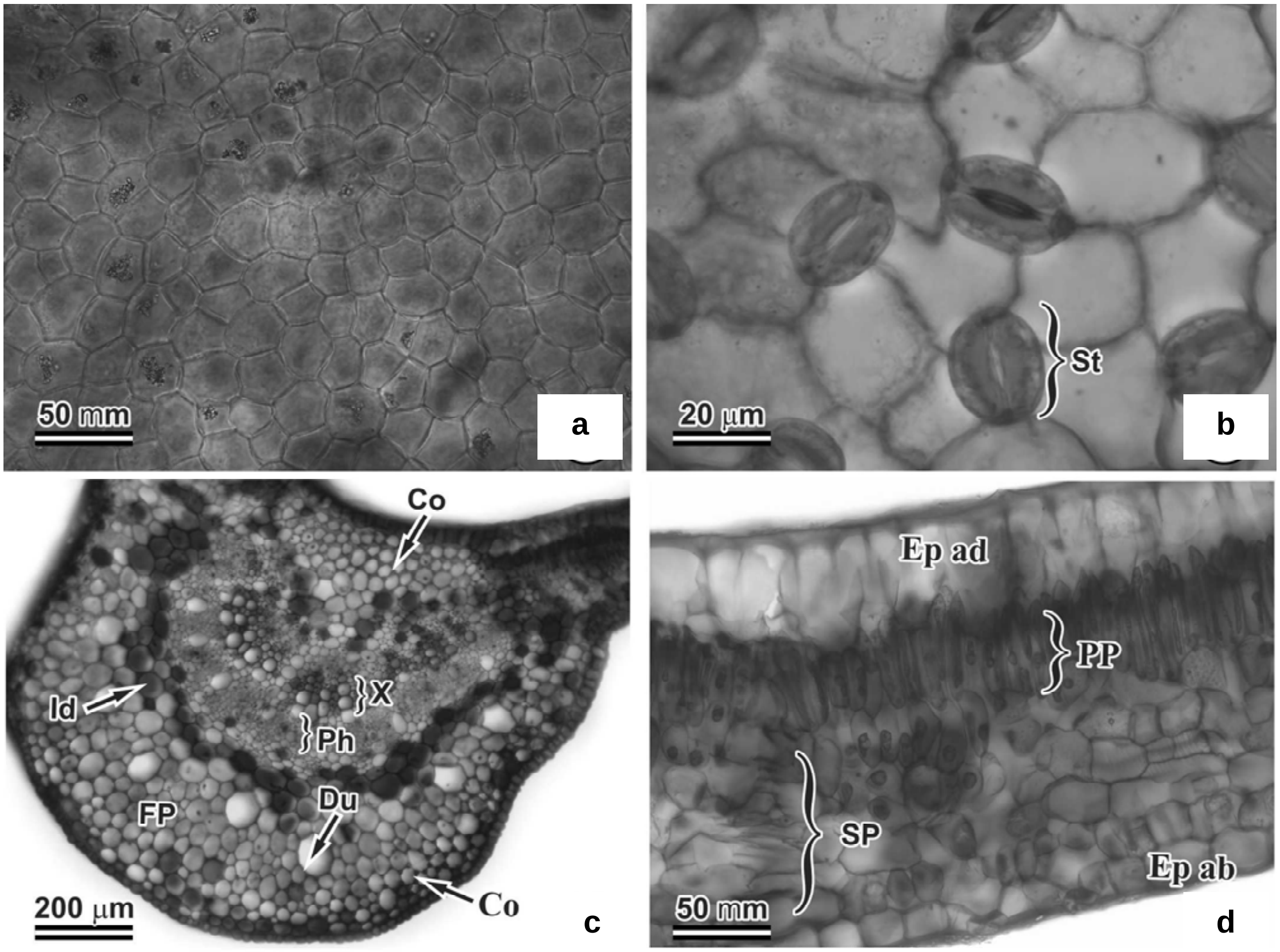

Figure 1. Anatomical structures of the *C. regium* leaf. 1, 2. Front view of leaf cleared and stained with safranin; adaxial (a) and abaxial surfaces (b). Cross-sections of the midrib (c) and mesophyll (d) stained with safranin and astra blue. Co, collenchyma; Du, duct; Ep ab, abaxial epidermis; Ep ad, adaxial epidermis; St, stomata; Ph, phloem; Id, idiobast, SP, spongy parenchyma; PP, palisade parenchyma; FP, fundamental parenchyma; X, xylem.

parenchyma were present in association with vessels (Figure 3e). Storage parenchyma (radial and axial) rich in starch grains and a small amount of thin-walled fibers were observed below the cambium (Figure 3e). Histochemistry revealed phenolic compounds (including tannins and lignin), starch, lipid compounds, alkaloids and essential oils in the different plant organs.

The leaves of *C. regium* had many cells distributed in the fundamental parenchyma containing phenolic compounds, which were detected using potassium dichromate and ferric chloride. The test with vanillin-HCl confirmed that these cells were tannin idioblasts. The same phenolic content was found in chlorenchyma cells. The phloroglucinol reaction indicated the presence of lignin only in the vessel elements, confirming the absence of fibers, sclereids or any other cells with lignified walls. Essential oils were found in some idioblasts. Proteins were widely distributed in the phloem and fundamental parenchyma. Total carbohydrate was observed in some idioblasts, ducts and parenchyma. Reserve lipids were detected in idioblasts and inside the ducts. Alkaloids were not found in the leaves. Likewise, several tannin idioblats were observed in the cortex and pith of the *C. regium* stem. A large number of fibers with lignified walls were present outside the phloem and xylem cells (vessel elements) and had a positive reaction to phloroglucinol. Essential oils occurred in isolated form in a few idioblasts. Proteins and carbohydrates were widely distributed in the parenchyma, cortex, and pith. In addition to lipid reserves, a thin cuticle layer was revealed with Sudan IV.

The *C. regium* root had a large number of cells containing phenolic compounds. A large number of tannin

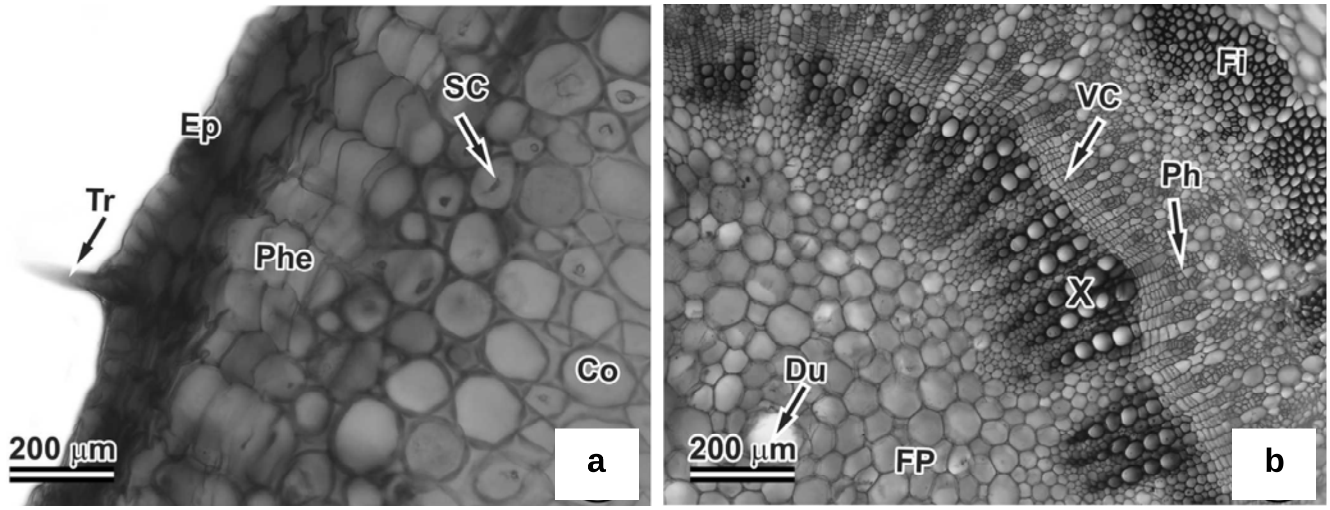

Figure 2. Transverse sections of the stem of *C. regium* stained with safranin and astra blue. (a) Cortex region. (b) Transport vessels and pith. Co, collenchyma; VC, vascular cambium; Du: duct; Ep, epidermis; Sc, sclereid; Ph, phloem; Phe, phellogen; Fi, fiber; FP, fundamental parenchyma; Tr, trichome; X, xylem.

idioblasts were found in the fundamental parenchyma of the cortex and radial parenchyma cells were observed in the secondary xylem. Starch grains were abundant in both the axial and radial parenchyma in the roots of *C. regium*. Essential oils were detected with Nadi reagent in single cells (idioblasts) dispersed among fundamental parenchyma cells; many of these cells had globules of essential oils and resin acids. The analysis of *C. regium* performed in the present study revealed that this species has a rather complex structural organization, and a rich and varied chemical composition, confirming the great potential for research in this understudied plant. The leaves, roots and stems of *C. regium* have a complex tissue organization and are rich in secretory structures, such as ducts and idioblasts. A varied composition of secondary metabolites, especially phenolic compounds and a mixture of lipophilic compounds are found in these organs. The wide variety of compounds present in *C. regium* is evidence of its pharmacological potential, as well as of the possible applications of this plant in various industrial sectors. This perspective points to *C. regium* as a promising medicinal plant and should be the basis for continuing research on this so important and yet so little known species.

DISCUSSION

Various idioblastic cells associated with secretory structures were observed on *C. regium* leaves. Categorically, idioblasts are individual cells with a different chemical composition from that of the surrounding cells; they vary in shape and are classified according to the substances that they synthesize (Castro and Demarco, 2008) and part of the bioactive compound precursor synthesis and storage may exist within the idioblast vacuoles (Mahroug et al., 2006). Several classes of phenolic compounds are synthesized and stored in a large central vacuole or multiple cells of varying sizes present in the idioblastic cytoplasm. These cells are involved in the secretion of mucilage, gum and phenolic compounds, and epithelial cells that delimit ducts can secrete heterogeneous material with mixed composition (Appezzato-da-Glória and Carmello-Guerrero, 2006).

The immunohistochemical analysis revealed phenolic compounds (including tannins and lignin), starch, lipid compounds, alkaloids and essential oils in different organs of the plant. Firstly, essential oils were detected only in leaves, but later found in plant roots by Solon et al. (2009) in a study on the antimicrobial properties of these compounds. Inácio (2010) reported the presence of essential oils, mainly terpenes and oxygenated derivatives in leaves and roots of *C. regium*. The same author also highlighted the high potential of *C. regium* for use in perfumes, the aromatherapy industry, and pharmaceutical applications. Unlike other vegetative organs, the *C. regium* root had several conduits that contain alkaloids, especially in the cortical region. Solon et al. (2009) reported that the roots of *C. regium* contain a variety of chemical compounds such as tannins and other phenolic compounds, mucilage and terpenes.

According to Appezzato-da-Gloria and Carmello-Guerreiro (2006), idioblasts are responsible for the secretion of various compounds, including lipophilic substances

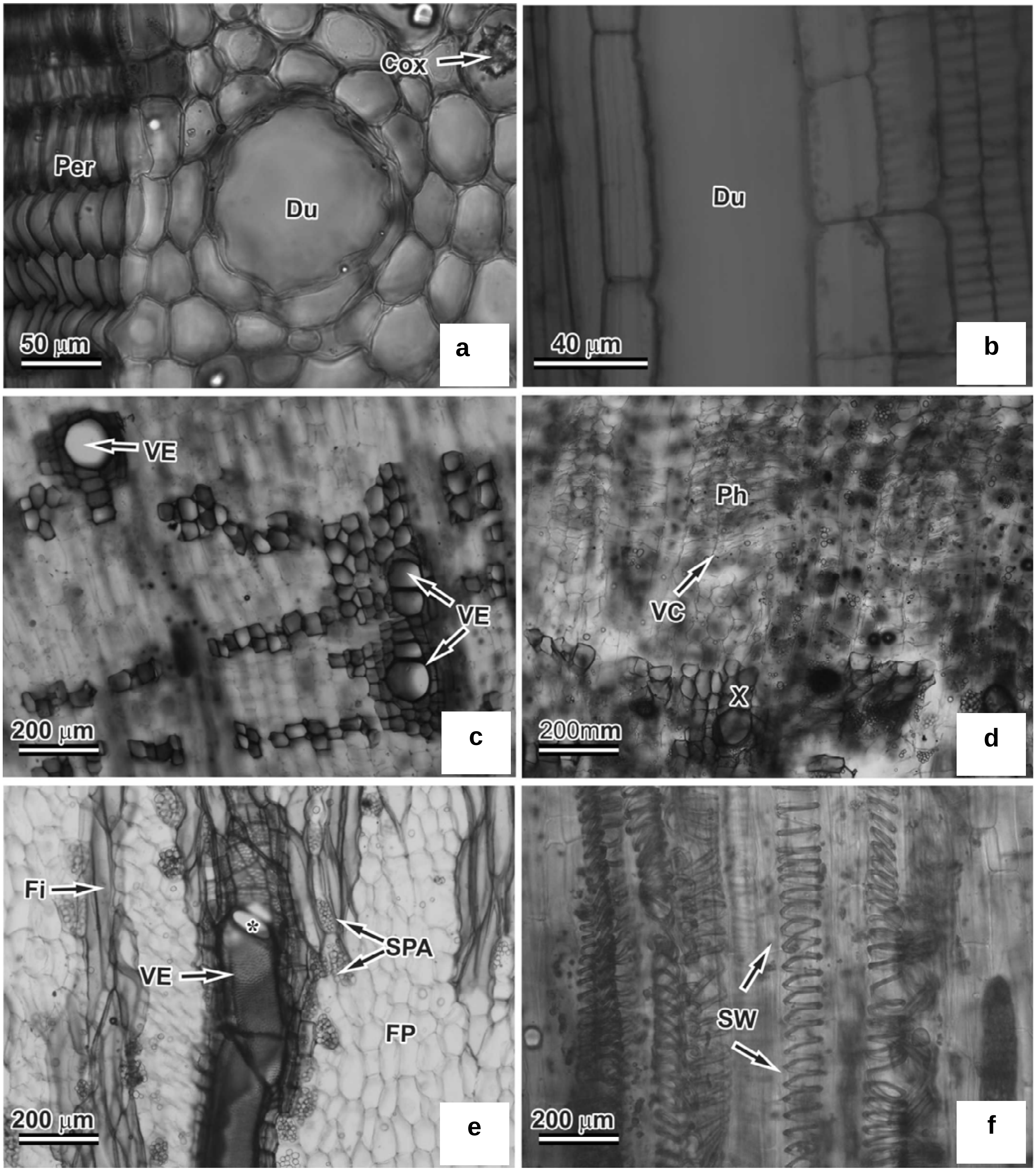

Figure 3. Transverse and longitudinal sections of the root of *C. regium* stained with toluidine blue. Transverse sections of the periderm and cortex (a). Longitudinal sections of the duct (b). (c, d) Transverse sections showing transport vessels. (e, f) Longitudinal sections of vessels and fibers. Cox, calcium oxalate crystal; VC, vascular cambium; Per, periderm; Du, duct; VE, vessel elements; Ph, phloem; Fi, fiber; SPA, storage parenchyma (axial); FP, fundamental parenchyma, SW, secondary wall of vessel elements; X, xylem; *, perforation plate.

(terpenes, free fatty acids, phenolic compounds and waxes), mucilage, proteins, amino acids and essential oils, among others. Other studies have reported that the roots of *C. regium* contain saponins, triterpenoid compounds, flavonoids, tannins and other phenolic compounds (Andrade et al., 2008). The analysis of essential oils from the *C. regium* roots by gas chromatography and mass spectrometry led to the identification of various substances (Solon et al., 2009).

The ducts are secreting structures composed of an epithelium terminating in an elongated lumen in which the secreted material has a varying chemical nature (Fahn, 1990). Aguiar-Dias and Cardoso-Gustavson (2011) reported the presence of secretory ducts in the angles of origin of the development of *Polygala angulata* rods under primary growth. In North American species, the resin ducts secret, while in *P. angulata*, a species found in the Brazilian "cerrado", the mucilage ducts secret, which is probably a xeromorphic adaptation to an arid environment. The *C. regium* root parenchymal tissue is rich in carbohydrates, with a lower content of proteins and lipids.

Author contributions

SCVF contributed to plant identification and supervision of sample collection, confection of herbarium, analysis and interpretation of data, and drafting of the manuscript. ALLF conducted laboratory procedures for anatomical and histochemical analyses. JMV contributed to data analysis and revision of the paper for important intellectual content. LSS conducted laboratory procedures for histochemical analysis. LCSP contributed to sample collection and laboratory procedures. All authors approved the final version of the paper.

Conflict of interests

The author(s) have not declared any conflict of interests.

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