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Short communication

Morpho-anatomy of native species used as substitute of quina (*Cinchona* spp.) in Brazilian traditional medicine: *Esenbeckia febrifuga*

Nádia S. Somavilla[a,*], Christopher W. Fagg[b], Maria G.L. Brandão[c]

[a] Departamento de Botânica, Universidade Federal de Juiz de Fora, Campus Universitário, Juiz de Fora, MG, Brazil
[b] Faculdade de Ceilândia e Departamento de Botânica, Universidade de Brasília, Brasília, DF, Brazil
[c] Centro Especializado em Plantas Aromáticas, Medicinais e Tóxicas. Museu de História Natural e Jardim Botânico, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

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ABSTRACT

Esenbeckia febrifuga (A.St.-Hil.) A. Juss. ex Mart., Rutaceae, is known by several popular names including quina-do-mato. This name is a reference to the use of its bark as febrifuge and in the past was employed as a substitute of *Cinchona* sp. for treatment of malaria symptoms. This confusion may have been reinforced by the fact that the bark of these plants are similar in appearance and have a bitter taste. In view thereof this study presents the description morphological and anatomical and the histochemistry of the stem bark and contributes to the pharmacobotanical study of plant drugs identified as Brazilian quinas, in sequence to two others studies. Compared with the *Cinchona* species, the prismatic shape of calcium oxalate crystals and the fibers with adornate end walls proved to be the main characteristics for differentiation.

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Introduction

Esenbeckia febrifuga (A.St.-Hil.) A. Juss. ex Mart. (basionym *Evodia febrifuga* A. St. Hil.) of the family Rutaceae, is a semi-deciduous tree growing to 5–11 m tall (outside of forest canopy it does not grow above 6 m). The trunk is very branched and tortuous with a diameter of 20–40 cm (Fig. 1A) with 3-foliolate leaves and capsule fruits (Fig. 1B) with 10.5–13 mm diameters, muricate, glabrous with loculicidal or septicidal dehiscence. This species is known in Brazil as “quina-do-mato”, “laranjeira-do-mato” and “três-folhas-vermelhas”. Its geographic distribution occurs in tropical rain forests of Brazilian states of the Northeast, Central-West, Southeast and South (Flora do Brasil 2020). In the Argentina this species is known as “ivirá-oví-guazú” and occur in the Misiones Rainforest (Chebez and Mazariche, 2010) and in the Paraguay its vernacular name is “guatambú-mi” or “ivirá-ñeti-(m)í (Grandtner and Chevrette, 2013). The bark of *E. febrifuga* is extremely bitter and astringent and considered as febrifuge in traditional medicine (Saint-Hilaire, 1824; Lindley, 1838; Albuquerque, 1968; Corrêa, 1969; Chebez and Mazariche, 2010; Cosenza et al., 2013; Grandtner and Chevrette, 2013) and was used in the past as a substitute of the true quina, *Cinchona* spp., Rubiaceae (Lindley, 1830; Peckolt, 1916). The bark resembles Angostura bark, and was imported into Europe about 1813 as “Brazilian China bark.” (Oberlin et Schlagdenhauffen, 1874; Kaastra, 1982). Infusion of bark was cited as antiparasitic agent in the fight against malaria (Brandão et al., 1985; Dolabela et al., 2008). Previous studies have confirmed the antiplasmodial activity of the aqueous extract of *E. febrifuga* bark (1 g/kg), with a 43% reduction in the multiplication of *Plasmodium berghei* in infected mice (Carvalho et al., 1991).

We have previously studied the stem barks of two Brazilian quinas (Somavilla et al., 2017) and they showed important characters that can be useful for differentiation. The objective of this study was to characterize the stem bark of *E. febrifuga*, contributing for its better knowledge and pharmacobotanical study.

Materials and methods

Plant material

Stem bark samples of *Esenbeckia febrifuga* (A.St.-Hil.) A. Juss. ex Mart., Rutaceae, were collected at the Campus Pampulha – Universidade Federal de Minas Gerais, Belo Horizonte, state of Minas Gerais, Brazil and registered as DAT-278 in the DATAPLAMT (http://www.dataplamt.org.br). A voucher specimen Fagg CW 2196 was deposited in the University of Brasilia herbarium (UB).

* Corresponding author.
E-mail: nadia.somavilla@ufjf.edu.br (N.S. Somavilla).

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Fig. 1. *Esenbeckia febrifuga*. (A) Overview of whole plant. (B) Detail of twig with leaves and fruits. (C–F) Stem bark. (C) Curved and quill aspect of the dry samples. (D) Outer surface. (E) Inner surface. (F) Maceration. Scales: B (2 cm), C (1 cm), D–F (2 mm).

Morphological, anatomical and histochemical analysis

External and internal aspects such as coloring and texture of the samples were described. For anatomical characterization part of these samples were fixed in solution of formaldehyde-acetic acid-ethanol 50 (1:1:18, Johansen, 1940), rinsed in ethanol 50% and then stored in ethanol 70%. After fixation, these samples were sectioned in microtome type Ranvier and stained with astra blue and fuchsin dyes (Kraus and Arduim, 1997) and mounted on slides with verniz vitral incolor 500® (Paiva et al., 2006). Fresh samples, after being cut with the Ranvier microtome, were submitted to histochemical tests: ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) to detect phenolic compounds, vanillin hydrochloric acid for tannins (Gardner, 1975), acid phloroglucin for lignin (Johansen, 1940), solution of lugol for starch (Johansen, 1940), Sudan III (Sass, 1951) and Sudan IV (Pearse, 1972) for lipids, Dittmar and Wagner reagents for alkaloids (Furr and Mahlberg, 1981); hydrochloric acid and acetic acid were used for crystals tests (Kraus and Arduim, 1997). Part of the samples was macerated for tissue components analysis. For that, the samples were placed in Franklin solution and maintained in an oven (60 °C) for 72 h (Franklin, 1945 modified by Kraus and Arduim, 1997). After this process, the macerated material was washed with distilled water to completely remove the Franklin solution and stained with ethanolic safranin 1%. The slides obtained from these preparations were analyzed by microscopy Axio Lab A1 (Zeiss) with a camera AxioCamERc 5S attached, and the acquisition, measurement and description of the photomicrography were obtained by ZEN 2012 (Zeiss) program. The botanical description followed the recommendations of Junikka (1994) and Richter et al. (1996). Mean and standard deviation of the length and width measurement of the phloem fiber cells were performed by Microsoft Excel® 2010 in 100 of these cells.

Results and discussion

Stem bark of *E. febrifuga* is very thin, ranging from 0.5 to 1.5 mm of thickness. When dried it is curved and single quill (Fig. 1C) and the outer surface is dark gray and lighter gray lichens may occur on its surface. The texture is rough with longitudinal wrinkles and transversal fissures and lenticels (Fig. 1D). Inner surface is yellowish and longitudinally striated (Fig. 1E). This description is similar to that Oberlin et Schlagdenhauffen (1878), however the coloring of the inner surface is described as reddish by those authors. The powder is yellowish with dark gray dots (Fig. 1F).

In transversal section, the bark is composed of periderm, parenchymatous cortex and phloem (Fig. 2A). Periderm has a variable number of layers of phellem (cork or suber), generally five to twenty layers, followed by one layer of phellogen and two to three layers of phelloderm (Fig. 2B). Cortical cells exhibit anticlinal divisions and tangential elongation (Fig. 2C) and this avoids the breakage of tissues with the increase in the stem circumference.

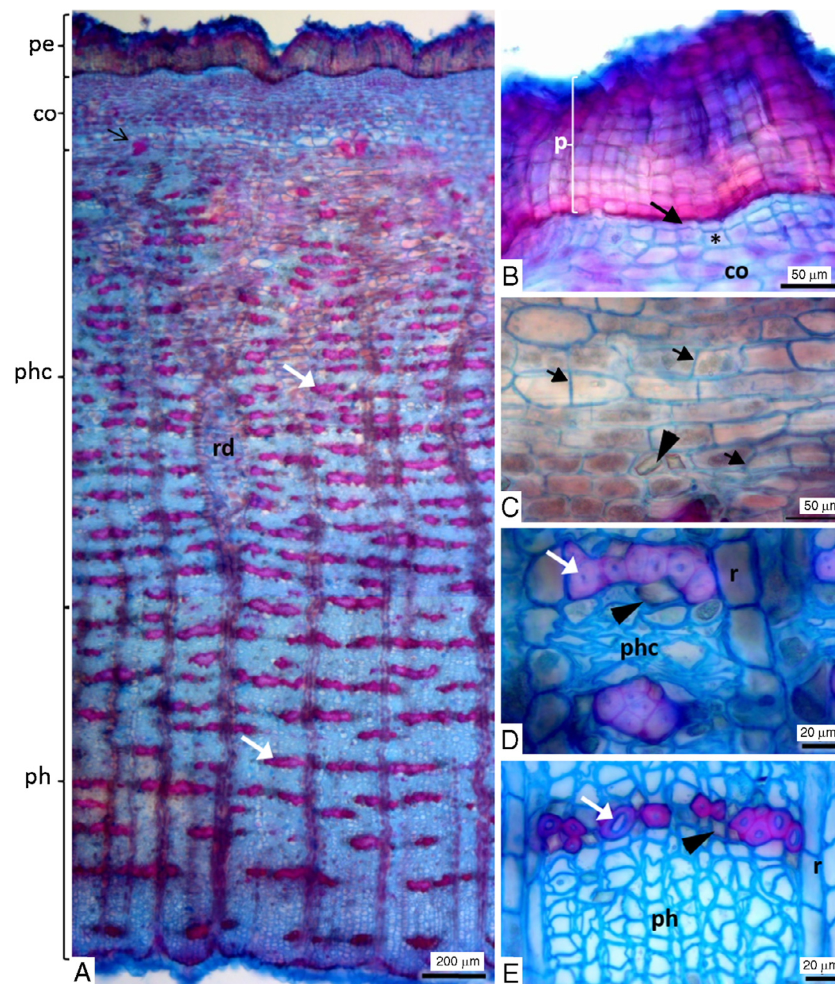

Fig. 2. Stem bark of *Esenbeckia febrifuga*. (A) Transversal section shown periderm (pe), cortex (co), collapsed (phc) and non-collapsed (ph) phloem and dilated ray (rd). White arrow: clusters of fibers. (B) Detail of periderm shown phellem (p), phellogen (arrow) and phelloderm (asterisk). (C) Detail of cortical cells with anticlinal division (arrow) and prismatic crystals (arrowhead). (D and E) Collapsed secondary phloem (phc) and non-collapsed secondary phloem (ph), respectively, shown fibers (white arrow), ray cells (r) and prismatic crystals (arrowhead).

Cortical cells have calcium oxalate prismatic crystals (Fig. 2C). Green color of the fresh bark is due to deposition of chloroplasts in the outer cortical cells and this feature was also found in the stem bark of another Rutaceae – *Phellodendron lavallei* Dode (Meskheli et al., 2010). Normally, in some Rutaceae species this photosynthetic cortex is replaced by a rhytidome in the older trunk (Kyriakis and Fasseas, 2010), however *E. febrifuga* maintains this tissue even with the secondary growth of the trunk. Secondary phloem is divided into collapsed phloem and phloem non-collapsed with clusters of fibers tangentially distributed (Fig. 2A, D and E). These clusters demarcate the limit of the phloem with the cortical parenchyma and Brocadet (1921) also emphasizes this feature in *E. febrifuga* when describing species of pseudo-quina. There is a deposit of prismatic crystals in the parenchyma cells adjacent to fiber clusters (Fig. 2E). These prismatic crystals were dissolved with hydrochloric acid and did not dissolve with acetic acid indicating calcium oxalate (Kraus and Arduim, 1997, p. 136).

In radial and tangential longitudinal section, the secondary phloem is not storied and displays sieve-tube elements with simple (Fig. 3A and B) and compound (Fig. 3C and D) sieve plates on inclined end walls and lateral sieve areas (Fig. 3C). Phloem rays are multiseriate and heterocellular with procumbent and upright cells (Fig. 3E and F). In the maceration the secondary phloem fibers (Fig. 3I–K) are elongated with length ranges from 383.28 to 831.19 μm (630.52 ± 102.97, mean ± standard deviation) and the width in the cell middle region ranges from 11.09 to 24.07 μm (16.41 ± 2.61). The fiber cell wall is not lamellar and usually has the adornate end wall (Fig. 3J–K). Phellem cells appear in the maceration as pieces of tissue with polygonal cells (Fig. 3K). The results of histochemical tests performed on the stem bark show lignin in the fibers and xylem elements, lipids in the cell wall of phellem and large amounts of oil droplets in the parenchyma (Fig. 3G) of the cortex and secondary phloem. Small starch grains appear in the secondary phloem but are very sporadically distributed. Phenolic compounds and tannin tests were negative. Tests for alkaloids was not conclusive because the large amounts of oil droplet in the fresh samples did not allow to verify the staining expected by the test in the cortex and phloem parenchyma, and there was no difference in the phellem and phelloderm cells between control test and that with reagents. Negative tests with reagents of alkaloids was highlighted by Dolabela (2007, p. 131) and the use of Draggendorff reagent in chromatographic plates (CCDS) containing extracts of this species. The author proposed that such result is due to the fact that the bases are amides and, therefore, will be less likely to give a positive result with Draggendorff reagent.

Since it presents a curved aspect when dried, bark pieces of *E. febrifuga* could be confounded with *Cinchona* species, however it is possible to use characteristics to differentiate them. In relation to the bark of *C. succirubra* Pav. ex Klotzsch (synonym of *C. pubescens* Vahl), the reddish color in the inner surface and in the

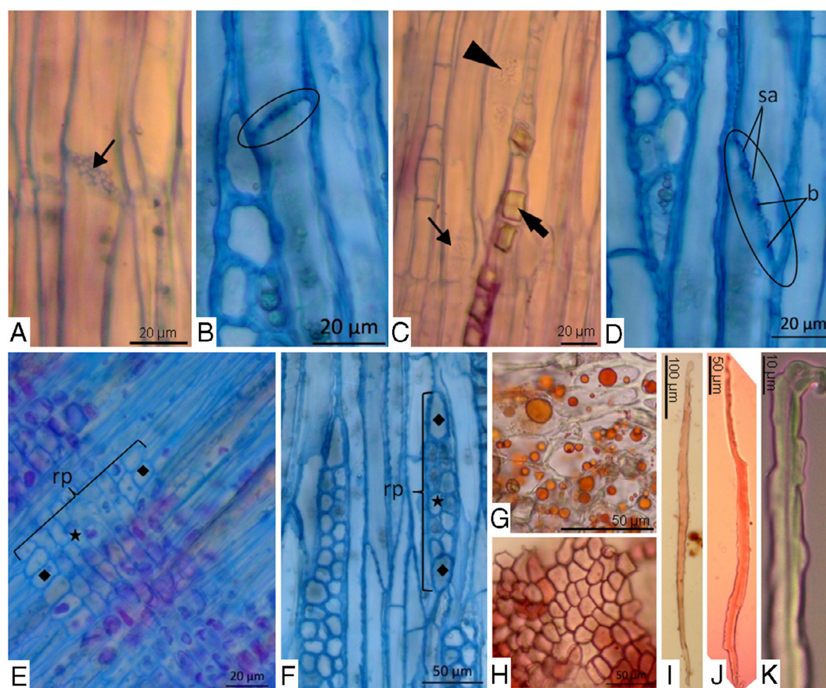

Fig. 3. Secondary phloem and phellem cells of the stem bark of *Esenbeckia febrifuga*. (A–F) Longitudinal section of secondary phloem. (A, C and E). Radial section. (B, D and F) Tangential section. (A and B) Simple sieve plate (thin arrow and ellipse, respectively). (C and D) Compound sieve plate (thin arrow and ellipse – showing sieve area (sa) and bars (b) of the compound sieve plate), lateral sieve area (arrowhead) and prismatic crystals (thick arrow) in parenchyma ray cells. (E and F) Heterocellular and multiseriate ray with procumbent (star) and upright (diamond) cells. (G) Transversal section shown oil droplets into the parenchyma cells (Sudan III test). (H–K) Maceration. (H) Phellem cells. (I and J) Fibers of secondary phloem. (K) Detail of fiber end cell.

powder (Wichtl, 2004) is the mean feature for distinction. However, other species such as *C. calisaya* Weed. (syn. *C. ledgeriana* (Howard) Bern. Moens ex Trimen) and *C. officinalis* L. (syn. *C. peruviana* Mutis) have yellowish or a pale color to the inner surface of the bark (Wallis, 1951), so the microscopy description is the most indicative. Although the anatomical descriptions are very similar between these species (Deutschmann et al., 1984) and *E. febrifuga*, these *Cinchona* species show parenchymatous idioblasts filled with microprisms (crystal sand) of calcium oxalate in the cortex and the spindle-shaped phloem fibers, without adornate end walls and larger in diameter and length (Claus, 1961; British and Pharmacopoeia, 2009), which are the main differing features from *E. febrifuga*. Crystal shape is a good feature for comparison because a particular species will form only a certain crystal type or subset of crystal morphologies (Franceschi and Nakata, 2005). Although *E. febrifuga* also had fibers without adorned end walls, the constant presence of fibers with adorned end walls in its macerate constitutes an important differential feature. The same anatomical information plus the color and morphological aspects of the bark can be used for avoid misidentification when compared to other Brazilian quinas: *Polyouratea hexasperma* (A. St.-Hil.) Tiegh (Somavilla et al., 2013), *Remijia ferruginea* (A. St. Hil.) DC. (Somavilla et al., 2017) and *Bathysa cuspidata* (A. St.-Hil.) Hook.f. ex K. Schum. (Coelho et al., 2012).

A phytochemical study carried out by Dolabela et al. (2008) found two coumarins (bergapten and isopimpinellin), four furoquinoline alkaloids (flindersiamine, kokusaginine, skimmiamine and γ-fagarine), one acridone (1-hydroxy-3-methoxy-*N*-methylacridone) and a limonoid (rutaevine) in the *E. febrifuga* stem ethanol extract. They also evaluated the *in vitro* antiplasmodial activity of the ethanol extract and concluded that only the furoquinoline alkaloids are related to this activity, specially skimmiamine and γ-fagarine. These two alkaloids, obtained from another Rutaceae species, proved to be promising therapeutical agents due to their efficacy and safety against promastigote forms of *Leishmania tropica* (Östan et al., 2007) and *Leishmania braziliensis* (Santos et al., 2011), causative agents of cutaneous leishmaniasis. Moreover, Napolitano et al. (2004) isolated one coumarin (aurapten) in the leaves of *E. febrifuga* with significant inhibition of the *in vitro* growth of *Leishmania major* promastigotes, another agent of cutaneous leishmaniasis. These data reinforces the importance of this species as a source of active principles with activity against these parasitic diseases, which are co-occurring in geographical regions (Gelb and Hol, 2002; Kvist et al., 2006) and considered as the most prevalent tropical diseases caused by protozoan parasites (WHO, 2015).

Authors' contributions

NSS contributed to anatomy and histochemical studies. CWF contributed in collecting plant sample and identification and confection of herbarium specimens. MGLB and NSS 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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