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# Morphoanatomical identification and physicochemical parameters of the drug *Erythrina verna* Vell. trunk bark

[Identificación morfoanatómica y parámetros físico-químicos de la corteza del leño de *Erythrina verna* Vell.]

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**Abstract**
"Mulungu" (*Erythrina verna* Vell., Fabaceae.) is an arboreal specie native to southeastern Brazil, used for medicinal purposes. This plant is characterized by the presence of alkaloids that have demonstrated anxiolytic activity. Due to this activity, this plant is listed by the Brazilian National Program of Medicinal Plants and Herbal Medicines. However, bibliographic information is lacking regarding this species. This study aims to describe *E. verna* barks, macro and microscopically, as well as some physicochemical parameters for the quality control of its raw material. In addition, the chromatographic profile of its alkaloid fraction and optimization of extraction methods for crude extract production has also been performed.

**Keywords:** *Erythrina verna*, Alkaloids, Quality Control, HPLC.

**Resumen**
"Mulungu" (*Erythrina verna* Vell., Fabaceae.) es una especie arborea nativa del sudeste de Brasil, utilizada con fines medicinales. Esta planta se caracteriza por la presencia de alcaloides que han demostrado actividad ansiolítica. Debido a esta actividad, esta planta se encuentra en la lista del Programa Nacional Brasileño de las Plantas Medicinales y Medicinas Herbarias. Sin embargo, la información bibliográfica es escasa con respecto a esta especie. Este estudio tiene como objetivo describir *E. Verna* corteza, macro y microscópicamente, así como algunos parámetros físico-químicos para el control de la calidad de la materia prima fresca. Además, el perfil cromatográfico de la fracción alcaloidica y la optimización de métodos de extracción para la producción de extracto crudo también fue realizada.

**Palabras Clave**: *Erythrina verna*, Alcaloides, Control de Calidad, CLAE.

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## INTRODUCTION

The standardization of herbal medicinal products is often a difficult task due to aspects related to lack of homogeneity in plant material, and the absence of botanical characterization, which is an important tool for authentication of genuine species and the distinction of adulterants. The quality of the raw material is also related to other aspects, such as chemical identification tests and physicochemical characters. Moreover, extracts with well defined constituents are a requirement for consistency in quality control and chemical profiles can be a useful tool for the standardization of these complex extracts, especially for those that have undetermined active constituents or specific chemical markers.

"Mulungu" (*Erythrina verna* Vell.), Fabaceae, is an arboreal specie native to southeastern Brazil, where it occurs in the States of São Paulo and Minas Gerais. It is also found in some forests near the basin of the Paraná river and in regions of the Brazilian cerrado (Lorenzi, 1992; Dantas *et al.,* 2004). Popularly, *E. verna* is known as mulungu, amansa-senhor, bico-de-papagaio, canivete, corticeira, flor-de-coral, suína-suinã, árvore-de-coral, mulungu-coral, capa-homem and tiriceiro. In folk medicine, decoctions of its barks and flowers are employed to treat health conditions such as agitation, asthma, inflammatory processes, cough and disorders of the nervous system, such as insomnia, anxiety and depression (Sarragiotto *et al.,* 1981; Patocka, 2009).

Among the pharmacological activities ascribed to *E. verna* (for its crude flower extract and isolated compounds, erythravine and hydroxyerithravine), its anxiolytic effect in mice models particularly stands out (Onusic *et al*., 2002; Onusic *et al*., 2003; Flausino *et al.,* 2007). In addition, other effects on central nervous system and anti-inflammatory activity have been reported for extracts obtained from *E. verna* (De Oliveira *et al.,* 2011; Faggion *et al.,* 2011; Rosa *et al.,* 2012) and *Eryhtrina velutina* Willd. (Vasconcelos *et al.,* 2011). Hydroalcoholic extracts from the stem bark have shown antinociceptive activity in three experimental models of pain in mice after intraperitoneal acute treatment (Vasconcelos *et al.,* 2003). In a subsequent investigation, oral and intraperitoneal acute treatments with water-alcohol extracts from stem bark of *E. verna* and *E. velutina* were found to display depressant effects on the CNS, characterized by a decrease in the locomotor activity of female mice in the open field and elevated T maze tests (Vasconcelos *et al.,* 2007).

The first chemical study of the *Erythrina* genus was published in 1937 (Folkers and Major, 1937). This study initiated a series of investigations that lead to the isolation and identification of several types of substances from the bark and leaves, including the alkaloids erythravine, erysothrine and erythrartine. Further work led to the identification of other groups of metabolites in *E. verna*, such as flavonoids and terpenes (Nkengfack *et al.,* 1997; García-Mateos *et al.,* 1998).

With regard to the national market for herbal medicines, it was noticed that the vast majority of suppliers of the raw material, *Erythrina* bark, sell the product only using the denomination, "mulungu". This constitutes a problem, since *E. velutina* is also known popularly as "mulungu", and presents different chemical constituents compared to *E. verna*. For example, in seeds of *E. velutina* were isolated erysodine, erysovine and erythraline whereas erysotrine, erysotrine N-oxide, erythrartine and erythrartine N-oxide were found in flowers of *E. verna* (Bisby *et al.,* 1994). Due to the scarce botanical and physicochemical studies of *E. verna*, it is essential to determine the specifications that regulate and insure the quality of such raw-material. As such, the present study was undertaken for the botanical characterization and physicochemical quality control tests of *E. verna.*

## MATERIALS AND METHODS

### *Plant Material*

Barks from young and older branches of *E. verna* were collected at the Botanical Garden of São Paulo (São Paulo-SP) in May 2009. A sample was identified and the voucher was deposited in the Herbarium of the Instituto de Botânica, as Cordeiro 3102.

### *Morpho-anatomical and histochemical description*

Young and older barks were softened in equal parts of water, ethanol and glycerin for about two weeks. Transverse, longitudinal and tangential sections were carried out with razor blades and the slices were mounted in glycerin jelly (Johansen, 1940). Barks were also powdered and slices prepared after clearing in a solution of chloral hydrate and glycerin. The illustrations were prepared with a Wild M5A stereomicroscope and an Olympus CB optic microscope equipped with a drawing apparatus.

Histochemical tests were obtained to detect cell walls of cellulose with toluidine blue (Gerlach, 1984), lignin with phloroglucinol (Sass, 1951), lipids with Sudan IV (Sass, 1951), starch grains with Lugol reactive (Foster, 1949), and mucilage with Chinese ink (WHO, 2011; Argueso, 2006; Farmamacopeia Brasileira, 2010; Kraus and Arduin, 1997).

### *Physicochemical characteristics*

The powdered barks of *E. verna* were tested to determine the water-soluble extract compounds and weight loss on drying. These tests were carried out according to the Brazilian Pharmacopoeia (2010) and Bundesvereinigung (1986), and weight loss was measured using a drying infrared balance. This assay was performed in triplicate for samples of 1 g, employing a Bel Mark Top Ray Page infrared balance, with a specified batch job of 105 ºC. The minimum temperature was set at 50 ºC at the beginning of the process. For analysis, the temperature was increased by 0.20 ºC every 10 seconds and results were automatically registered with the balance software. Extractive conditions were optimized for particle size, extractor solvent, extraction time and temperature.

### *Optimization of extractive conditions*

The material was ground and classified accordingly to its particle diameter. Subsequently, the samples were submitted to static maceration in methanol:water (70:30 v/v) and the yields were compared to establish the optimum particle size distribution. After the determination of the optimum particle size, different extractive methods were evaluated.

The choice of the extractive method was based on the conventional production of extractive solutions. The extracts were prepared in a ratio of 1:10 (w/v) plant material: solvent (methanol/water, 70:30 v/v) by static maceration (in an Erlenmeyer flask for 15 minutes), decoction (70 ºC for 15 minutes, coupled to a reflux condenser), infusion (solvent extractor at 70 ºC and cooling for 15 minutes in a closed flask with a watch glass), turbo extraction (for 15 minutes in a blender) and ultrasonication (15 minutes). In order to optimize the extraction process, the time and temperature parameters were compared. The effect of time was evaluated by comparing the extraction yields of the maceration process after 24, 48 and 72 hours. On the other hand, the influence of the temperature was evaluated by comparing maceration at room temperature with the decoction at 40 ºC and 60 ºC for 1 hour. All methods were performed in triplicate.

### *Chromatographic conditions*

HPLC analyses were performed on a Waters Alliance 2690 chromatograph using a photodiode array detector (DAD; UV/VIS Waters 996) and a Luna C18(2) column (Phenomenex; 5 µm, 250 x 4.6 mm) with a save guard column. UV spectra were recorded in the range of 200 to 400 nm. The mobile phase in channel (A) was water:trifluoroacetic acid (100:0.025;v/v) and in channel (B) acetonitrile. The gradient profile was: 0–10 min at 95:5% of (A:B); 10–30 min at 75:25%; 30–45 min at 65:35%; 45–60 min at 45:55% and 60–70 min at 100% acetonitrile with a flow rate of 0.8 $mL \cdot min^{-1}$. The injected volume was 10 µL. The chromatographic peaks were detected and their identities were confirmed by co-chromatography and by comparison of the UV spectra with standards of the alkaloids using a DAD detector.

### *Preparation of samples*

Samples of *E. verna* bark (1 g) were extracted with three portions of 10 mL of methanol/water (70:30) under static maceration in cycles of 6 hours, the resulting solution was then concentrated in vacuum to dryness. The dry crude extract obtained was resuspended in 150 mL 2 mol/L HCl, transferred to a separation funnel and washed with two portions of 25 mL hexane: ethyl acetate (1:1). For the selective extraction of alkaloids, the pH of the extract was adjusted to 9 - 10 with 25% ammonium hydroxide. The solution was then partitioned 5 times with 25 mL dichloromethane. The organic layer was evaporated to dryness under vacuum and with a temperature not exceeding 40 °C, resulting in a fraction of total alkaloids. The alkaloid fraction contained in the round bottom flask was diluted with 500 µL methanol, filtered through a PVDF membrane (0.45 µm) and then transferred to a vial for LC analysis.

## RESULTS AND DISCUSSION

### *Drug Description*

### *Macroscopical*

Currently, barks of only two native Brazilian medicinal plants are collected and marketed in Brazil by the herbal industry, one from *Erythrina verna* Vell. and the other from *Stryphnodendron adstringens* (Mart.) Coville (= *S. barbatimam* (Vell.) Mart.), Leguminosae-Mimosoideae, known nowadays as Fabaceae (APG III); the latter is popularly known as "barbatimão", which is listed in the fourth edition of the Brazilian Pharmacopoeia. No consistent botanical description for *Eryhtrina velutina* Willd is available;

however, a comparison of the *Erythrina verna* specie with the similar Stryphnodendron species is important for safely differentiating the material used to produce drugs. Other exotic barks marketed in Brazil belong to species from different botanical families, such as *Cinchona pubescens* Vahl (Rubiaceae), *Cinnamomum verum* J.S. Presl (Lauraceae) (Farmacopeia Brasileira, 2000) and *Rhamnus purshiana* DC. (Rhamnaceae) (Farmacopeia Brasileira, 1996). Macroscopically, the barks from the two Fabaceae species differ from each other in form, appearance, texture and color, although the bark of "barbatimão" in more developed stems may have a whitish appearance due to the presence of mosses.

The cortex of *E. verna* is sold in irregularly sized and shaped pieces (Figure 1A - E). The bark fragments of the branches are thin, curved, pale brown-gray, with a very irregular and furrowed outer surface, and a whitish fibrous inner surface. The bark fragments of the older branches are light and thicker, less curved, brownish-gray to black, with an irregular surface, showing deep longitudinal slits (Figure 1C - D). The old bark presents craters that form scars of up to 1.0 cm in diameter and verrucous prominences, arranged irregularly (Figure 1C); tapering (= conic) small and plain thorns may also occur. The inner surface of the latter fragments is often covered with woody, smooth and yellowish plates, interrupted by finely ribbed and whitish-cream regions (Figure 1D). The cross section shows the cortex with an outer region corresponding to the darker cork and an inner whitish region, corresponding to the cortical parenchyma. In the lateral view, the outer cortex has the same color as the outer surface and the inner cortex is similar to the inner surface (Figure 1E). Gray-green plaques of lichens can also be found on the outer surface of young and old fragments. The fracture is fibrous in young barks and smoother in old barks.

The bark of "barbatimão" is reddish and very woody-fibrous, whereas the bark of mulungu has a brown color and is very soft and light. Internally the "barbatimão" bark is striate and has a reddish-brown color, while the bark of mulungu has a smooth surface and a whitish-yellow color. The macroscopic description agrees with those reported by Oliveira *et al*., (1991) and the Brazilian Pharmacopoeia 1$^{ed}$ (1929) and 2$^{ed}$ (1959).

From the front view, the young cortex of *E. verna* presents cells that are organized in different manners; they are often square, thin-walled, occasionally with lipid droplets, and often aligned in rows with varying numbers of layers (Figure 1F). The phellogen is composed of up to four layers of small tangentially-flattened cells, with straight walls and dense protoplast.

In old barks, the cork cells present many forms, which are more voluminous, tangentially flat, with straight walls and a few lipid droplets (Figure 1G). In cross section, the cortex is formed by the cork, phellogen, chlorenchyma, cortical parenchyma and phloem (Figure 1H). The cork shows a variable number of layers of small square cells, tangentially flattened, thick-walled, arranged in radial rows, some of which contain lipid droplets and others showing dense protoplasts. The outer cells are generally smaller and with a more deformed organization. The phellogen has cells similar to those described for the front view, and is formed by a variable number of layers. According to the development of the bark, the cork shows several sets of alternating layers with the phellogen. More externally, several formations of phellogen are often seen. The inner cork has cells with a smaller volume. The chlorenchyma, when present, is formed by a few layers and presents polygonal cells with thick rounded, cell walls, lipid droplets and a few chloroplasts. The cortical parenchyma cells are, in general, polygonal, with a larger volume than the chlorenchyma cells and with thinner walls and larger intercellular spaces and with simple and/or composed starch grains that are small in size and with a few lipid droplets. More internally, the cortical parenchyma demonstrates cells with greater definition, often with rectangular walls, higher volume and regular arrangement. Stone cells occur scattered throughout this tissue, are most often grouped, with strongly lignified walls, a large lumen and with evident canaliculi and pits. Fiber bundles are also common and are usually accompanied by a crystal idioblast bundle sheath, containing calcium oxalate crystals with different sizes and shapes. These crystals also occur in parenchyma cells. The fibers sometimes present stratification of the parietal layers and lignified groups of cells and fibers, also accompanied by a crystal bundle sheath.

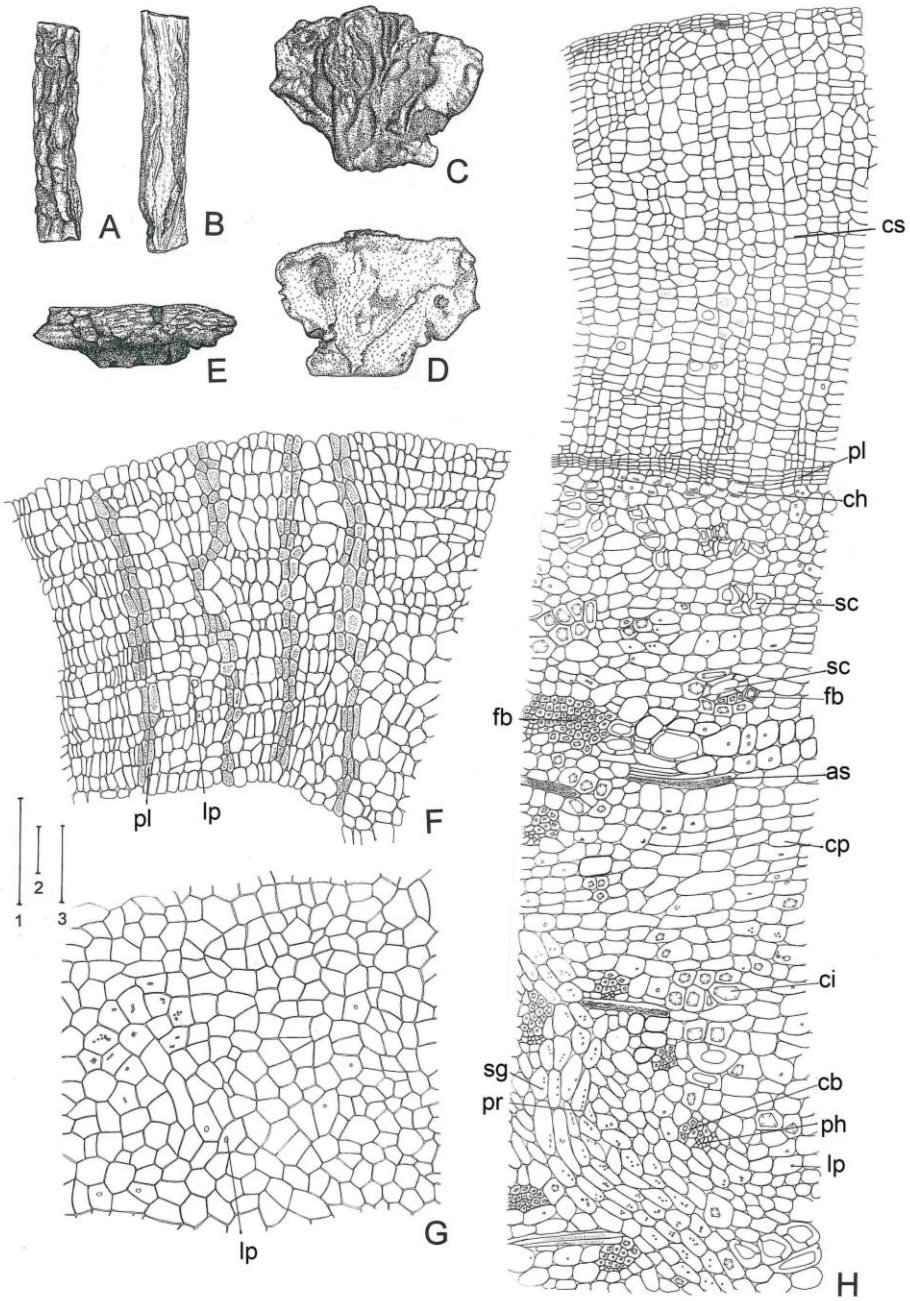


**Figure 1**
***Erythrina verna* Vell. - A. General appearance of the outer surface of young bark; B. general appearance of the inner surface of young bark; C. general appearance, from frontal view, of the outer surface of old bark; D general appearance, from frontal view, of the inner surface of old bark; E. general appearance, from lateral view, of old bark; F. frontal view of cork cells of young bark; G. frontal view of cork cells of old bark; H. partial cortex, in cross section, of old bark; as: amorphous substance; cb: crystal bundle; ch: chlorenchyma; ci: crystal idioblasts; cp: cortical parenchyma; cs: cork cells; fb: fiber; lp: lipid droplets; ph: phloem; pl: phellogen; pr: parenchyma ray; sc: stone cell; sg: starch grains. A-E: 3 cm (bar 1); F-H: 100 µm (F - bar 3 and G-H: bar 2).**

***Microscopical***

Parenchyma rays are irregularly distributed throughout the cortex and are formed in the outer limit by a row of rounded cells, with larger volumes than the internal cells and sometimes containing crystals. The internal cells are arranged in a regular and longitudinal manner, in two to five rows and are usually elongated, rich in starch grains and demonstrate a few lipid droplets (Figure 1H). Internally from the cortex, usually alternating with the parenchyma rays, regions are formed by phloem, bundles of fibers, parenchyma and an amorphous substance (Figure 1H). The bundles of fibers have a small caliber, a variable number of cells and are accompanied by a crystal bundle sheath with irregular distribution.

Internally from the phloem fibers are infrequent sieve tubes that are very small, with dense contents. The parenchyma cells are roundish, with thick walls and have a larger volume than the other cells of the cortex and are very rich in lipid droplets. The amorphous substance has a variable form, without clear definition, and usually has a parallel arrangement to the cork and a yellow to orange content (Figure 1H). Rarely orange, voluminous and thick-walled isolated cells occur in this parenchyma. In the longitudinal tangential view, the cork cells are rectangular, with straight walls and arranged in rows, often with oblique end walls (Figure 2D).

Crystal bundle sheaths appear intercalated with the cork cells and generally bound to the parenchyma rays. The crystal bundle sheath is formed by up to four layers of square cells, with straight and slender walls and almost all cells usually have large solitary crystals with different forms, centrally located or disposed along one side of the cell walls (Figure 2D). The parenchyma rays, composed of 2 - 6 cell rows, are ovoid-fusiform, with rounded cells, the outer one with a larger volume and the inner with a smaller and denser protoplast, rich in starch grains and with more developed intercellular spaces. Fiber bundles of a small caliber are accompanied by a crystal bundle sheath (Figure 2D). In a radial longitudinal section, the cortex presents a cork, as described for the other sections, with the largest number of layers in the older cortex (Figure 2A). The cortical parenchyma cells present various forms, with thin walls, starch grains and occasionally lipid droplets. Isolated crystal idioblasts occur scattered in the cortex, as well as clusters of stone cells and crystal bundle sheaths, parenchyma rays, fiber bundles, among other characteristics, as described above (Figure 2.B and Figure 2C).

Microscopically, in cross section, the bark cells of “barbatimão” present a tabular form and a regular arrangement, with reddish and thickened cell walls, while in mulungu the cells are of different shapes; additionally, the cell walls are thin and show no staining. However, Metcalfe and Chalk (1950), for different *Erythrina* species, reported that the cells of the outer cork are cubic and with thin walls. In this study, we observed different cellular forms in the cork; Oliveira *et al*., (1991) related the same features. The cortex is highly developed and parenchymatous in the “mulungu” and “barbatimão” barks. Metcalfe and Chalk (1950), describing the *Erythrina* genus, affirmed that the bark cortex is formed by abundant parenchyma. The occurrence of parenchyma rays for *E. verna* was mentioned by Oliveira *et al*., (1991) and by Brazilian Pharmacopoeia 2$^{ed}$ (1959). Metcalfe and Chalk (1950) reported, for different species of *Erytrhina*, the occurrence of multiseriate rays, usually with 4 - 10 or more rows, and no less than 4, with no sclerified cells, which is consistent with findings presented herein for the mulungu bark, which has rays formed of 2 - 6 cell rows with thin walls.

However, the Brazilian Pharmacopeia 2$^{ed}$ (1959) mentions the presence of only 2 - 3 cell rows for this species. The presence of the crystal sheath is in agreement with the report from Oliveira *et al.*, (1991), and with Metcalfe and Chalk (1950), who stated that the crystals are found in the phloem sheath and in the sclerenchyma pericycle, and also often in isolation. In the “barbatimão” and “mulungu” barks, the crystal sheath accompanies the phloem and the parenchyma rays; however, in the mulungu bark, they also occur as crystal idioblasts scattered in the parenchyma. The same authors reported that the rhombohedral-type crystals are common in the sheath cells near the parenchyma rays, which was confirmed herein for “mulungu”.

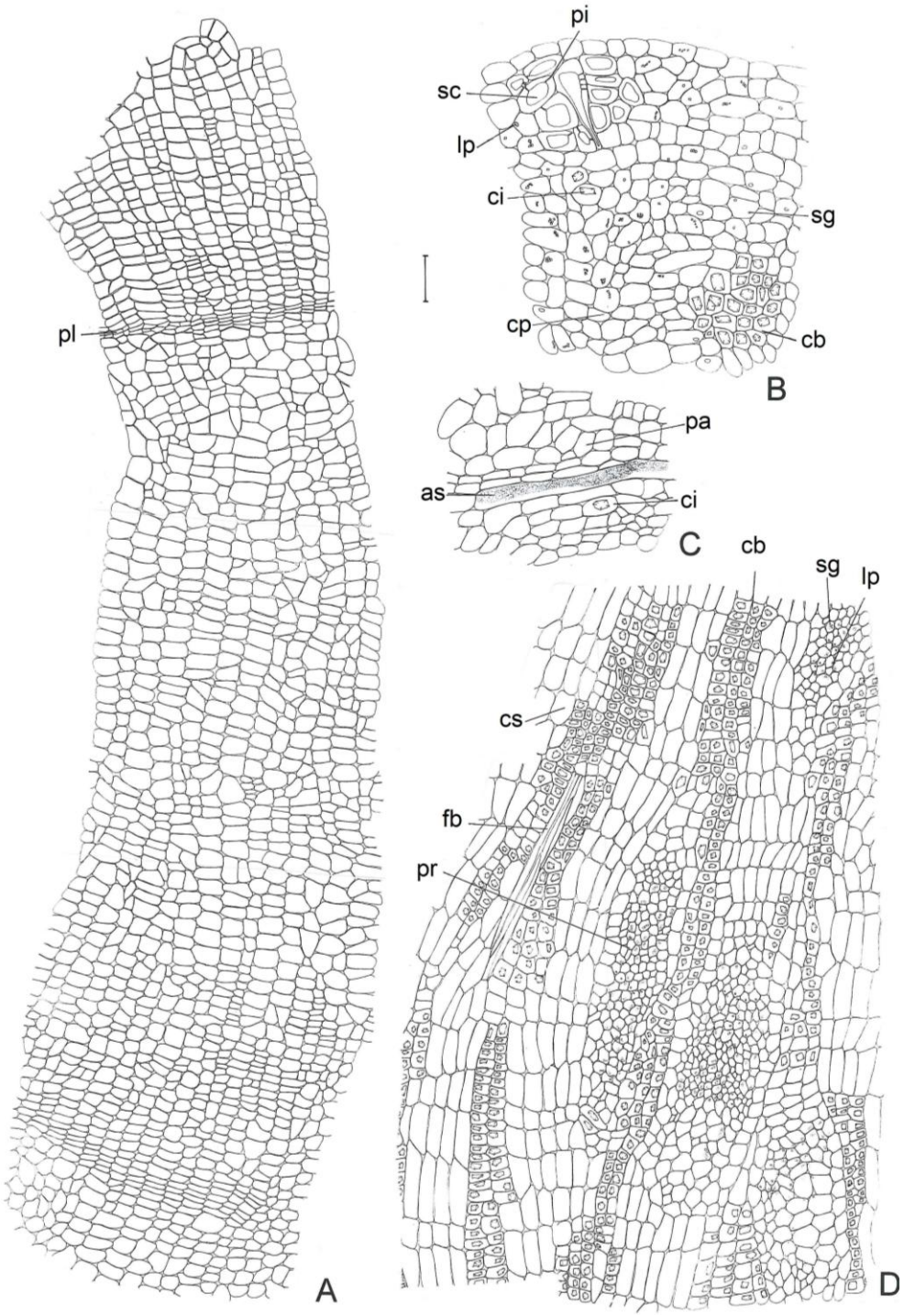


**Figure 2**

***Erythrina verna* Vell. - A. Detail of outer portion of old bark in radial-longitudinal section; B. detail of outer portion of old bark in radial-longitudinal section showing clusters of stone cells and clusters of crystal bundle sheath; C. detail of outer portion of old bark in radial-longitudinal section, showing amorphous substance; D. detail of outer portion of the old bark in tangential-longitudinal section; as: amorphous substance; cb: crystal bundle; ci: crystal idioblasts; cp: cortical parenchyma; cs: cork cells; fb: fiber; lp: lipid droplets; pa: parenchyma; pi: pit; pl: phellogen; pr: parenchyma ray; sc: stone cells; sg: starch grains. Scale: 100 µm.**

Importantly, the crystal shapes are different in "barbatimão", presenting a polyhedral type. The secondary phloem is formed by fibers, mucilage cells and a rhombohedral crystal sheath, as reported by Metcalfe and Chalk (1950). The Brazilian Pharmacopoeia 1ed (1929) reports that no crystals and starch grains are found in "mulungu" bark, but in the second edition of the same Pharmacopoeia, starch grains are cited in the parenchyma rays and in the internal cortex, as found in this present study (Farmacopeia Brasileira, 1929; Farmacopeia Brasileira, 1959). The occurrence of stone cells in the cortical parenchyma is also reported by Oliveira *et al*., (1991) and Brazilian Pharmacopoeia 1ed (1929) and 2ed (1959); Metcalfe and Chalk (1950) mention that sclerenchyma cells are unevenly distributed in the cortex in Papilionaceae (Fabaceae). Clusters of stone cells are found in the cortical parenchyma of "mulungu" and "barbatimão" barks; however, in "barbatimão", such groups can be very dense and the stone cells can also form continuous layers, which are not observed in the "mulungu" bark.

Metcalfe and Chalk (1950) related different types of secretory cavities for Papilionaceae, and for *Erytrhina* secretory cells distributed in the cortex and in the phloem. However, for *E. verna*, we found only mucilage cells in the cortical region. The same authors reported that secretory cells were brown in color, due to tannins, proteins, mucilage or other materials found in the primary and secondary phloem, and that these are rare in the primary cortex in Papilionaceae (Fabaceae). Authors also described the presence of mucilage in the fibers or in scattered cells of the cortex, as well as the occurrence of tanniniferous cells, in agreement with the mucilage found in "mulungu". For "barbatimão" bark, there is no information regarding the presence of mucilage.

***Microscopical description of the impurity***

The xylem, when present as an impurity, is not continuously distributed and is composed of well-developed vessel elements of distinct caliber, and parenchyma cells that are rich in lipid droplets.

***Organoleptic characteristics***

The drug has a bitter taste and an unpleasant odor, which diminishes following desiccation.

***Microscopical characteristics of powdered bark***

The powdered bark of *E. verna* presents a pale-brown color and presents all the requirements for this specie, with the exception of its macroscopic characteristics. Following the addition of chloral hydrate, the powders characteristics are: fragments of cork, in the frontal view (Figure 3A); fragments of cork with cells filled with starch grains in the tangential longitudinal section (Figure 3B); fragments of cork cells, in cross section (Figure 3C); fragments of isolated cork cells, in cross section (Figure 3D); fragments of the cortical parenchyma, containing lipid droplets, in cross section (Figure 3E) and fragments of cortical parenchyma in cross-section, containing starch grains (Figure 3F), and fragments of cortical parenchyma in cross-section, with crystal idioblasts (Figure 3G); fragments of cortical parenchyma accompanied by a crystal bundle sheath portion, in the tangential longitudinal section (Figure 3H); isolated parenchyma cells containing starch grains (Figure 3I); clusters of stone cells, in cross section (Figure 3J); isolated stone cells, in cross-section (Figure 3L); fragments of parenchyma rays, accompanied by fibers, in the tangential longitudinal section (Figure 3M); bundles of fibers partially surrounded by a crystal bundle sheath, in the longitudinal section (Figure 3N); bundles of fibers, in the longitudinal section (Figure 3O); fragments of bundles of fibers, in the longitudinal section (Figure 3P); isolated fibers (Figure 3Q); fragments of crystal bundles, in the longitudinal section (Figure 3R); isolated crystal idioblast (Figure 3S); simple and compound starch grains in isolation and/or grouped (Figure 3T); isolated oxalate crystals, with different forms and sizes (Figure 3U).

***Physicochemical parameters and chromatographic profile***

Herbal medicines are used in developed and developing countries, as dried or fresh plants or raw material for the pharmaceutical industry, and represent a substantial proportion of the global drug market. The acceptance of a plant-derived drug as a pharmaceutical raw material presupposes the existence of well-established quality specifications (Alves *et al.*, 2010). The preliminary analysis of plant material requires physicochemical characteristics, and the employment of techniques such as volumetric analysis, gravimetric determinations, gas chromatography, high-performance liquid chromatography and spectroscopic methods (WHO, 2011).

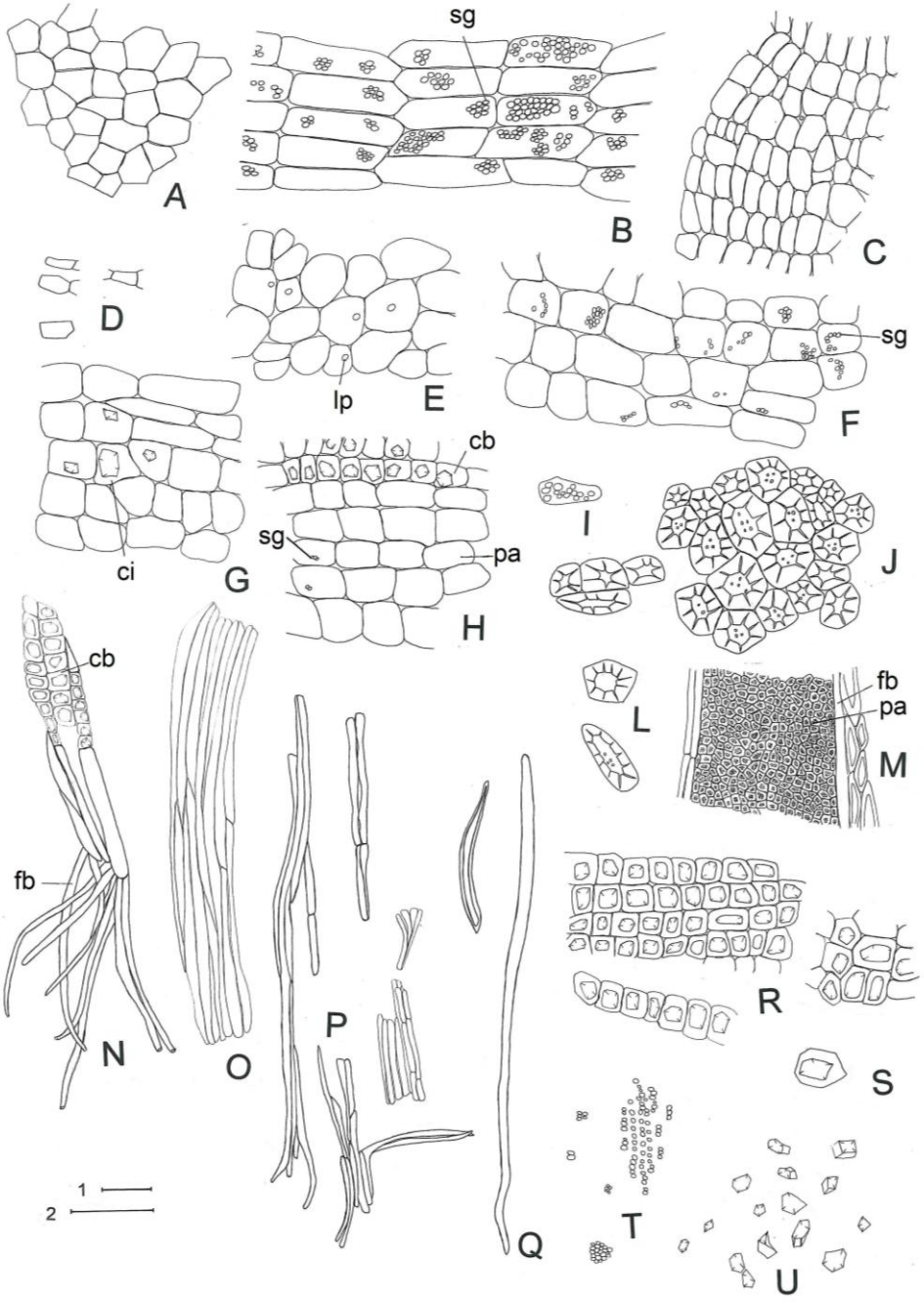


**Figure 3**

***Erythrina verna* Vell. - Powdered drug: A. fragments of cork, from frontal view; B. fragments of cork with cells filled with starch grains in tangential longitudinal section; C. fragments of cork cells, in cross section; D. fragments of isolated cork cells, in cross section; E. fragments of the cortical parenchyma in cross section, containing lipid droplets; F. fragments of cortical parenchyma in cross section, containing starch grains; G. fragments of cortical parenchyma in cross-section, with crystal idioblasts; H. fragments of cortical parenchyma accompanied by crystal bundle sheath portion, in tangential longitudinal section; I. isolated parenchyma cell containing starch grains; J. clusters of stone cells, in cross section; L. isolated stone cells, in cross section; M. fragments of parenchyma rays accompanied by fibers, in tangential longitudinal section; N.**

**bundles of fibers partially surrounded by a crystal bundle sheath, in longitudinal section; O. bundles of fibers, in longitudinal section; P. fragments of bundles of fibers, in longitudinal section; Q. isolated fibers; R. fragments of crystal bundle, in longitudinal section; S. isolated crystal idioblast; T. simple and compound starch grains, in isolation and/or grouped; U. isolated oxalate crystals, with different forms and sizes; cb: crystal bundle; ci: crystal idioblasts; fb: fiber; lp: lipid droplets; pa: parenchyma; sg: starch grains. Scales: 100 µm (A-M e Q-U - bar 2 and N-P - bar 1).**

The moisture of a plant drug is an important parameter for the evaluation of the physicochemical quality control of the raw plant. High levels of moisture can lead to microbial growth, and promote the action of enzymes, which can lead to the degradation of the plant constituents. Weight loss after drying is a technique that measures not only the loss of residual water, but also the loss of volatile substances, and may indicate the efficiency of the drying operation for productions of phytopharmaceuticals (Alves *et al*., 2010).

Analyses of *E. verna* by the gravimetric test and infrared determination demonstrated moistures of 10.54 ± 0.009% and 10.39 ± 0.07%, respectively, in the samples. These values are appropriate for the limits set by the different pharmacopoeias consulted (8 to 14%, unless exceptions) (Farmacopéia Brasileira , 2010). The sample must present moisture at a minimum, and these levels may vary according to the part of the plant. *E. verna* displays levels of extractable matter of 10.67 ± 1.41%; a positive feature with regard to the production of dry plant extracts.

The optimization of extractive variables for maceration demonstrated the influence of particle size. The bark particles with sizes of 710 µm or greater presented the best yield for extract production than smaller sizes of less than 180 µm and those particles in the range of 350 - 180 µm. The worst yield was obtained with particle sizes of between 710 - 500 and 500 - 350 µm (Table 1).

**Table 1**
**Particle size and extractive content of *E. verna* barks**.

| Particle Size (µm) | Extractive content (g/%) |
|---|---|
| > 710 -- | 12.03 ± 0.81 |
| 500 - 710 | 11.29 ± 0.45 |
| 350 - 500 | 11.22 ± 0.25 |
| 180 - 350 | 10.40 ± 0.41 |
| < 180 | 8.59 ± 0.15 |

The choice of the extractor solvent is a key step in the extractive process and the aim of this optimization was to establish the conditions for the production of extracts, preferably using water-alcohol mixtures that allow the production of dried extracts, especially using a spray drier. The best yield was observed with a ratio of 70:30 (MeOH:$H_2O$), a proportion that is commonly employed for the production of plant extracts, since more than 80% of the chemical constituents have some solubility in ethanol or methanol blends (Falkenberg *et al*., 2003).

When choosing a method of extraction, the efficiency, cost, time required for extraction, quality of the product, and chemical composition must be taken into account amongst other factors (Henriques *et al.*, 2003). The method of extraction that presented the best results was decoction, followed by ultrasonication and turbo extraction. These results confirmed previous reports in the literature that indicated a more effective extraction for processes using heat, stirring and/or still decreasing particle size. For testing optimum time and temperature, an increase in the extract production was observed when the time and temperature of extraction were increased, considering the limit of time required for the saturation of the solvent in question and the possibility of degradation of active principles at temperatures exceeding 60 ºC (Gião *et al.*, 2009).

Among the techniques of quality control of products and extraction processes, the chromatographic methods have been greatly

emphasized since they allow both qualitative and quantitative analyses (Marston, 2007). LC-fingerprinting can be a useful tool for the quality control of herbal extracts, since it demonstrates a chemical pattern and ensures the identification of a specific sample. The alkaloid extract of *E. verna* bark has also been tested by LC-DAD and compared with the reference alkaloid (erythravine). The chromatogram of the standard solution is represented in Figure 4. A peak may be observed with a retention time of 12.34 min and with an ultraviolet spectrum with maximum absorptions at 229.7 and 281.9 nm. A representative chromatogram for the alkaloid extract is shown in Figure 5, and shows two major peaks at 12.97 min and 13.39 min with an ultraviolet spectrum that is characteristic of erythrinian alkaloids (Sangster & Stuart, 1964). Both compounds presented the same UV profile with absorptions at 229.7 and 281.9 nm. Co-injection of the reference substance and the sample confirmed the presence of this alkaloid in the extract with a peak at 12.97 min. This finding is of importance, since this compound has not yet been established as a chemical marker for *E. verna* and may facilitate the quality control of this raw-material.

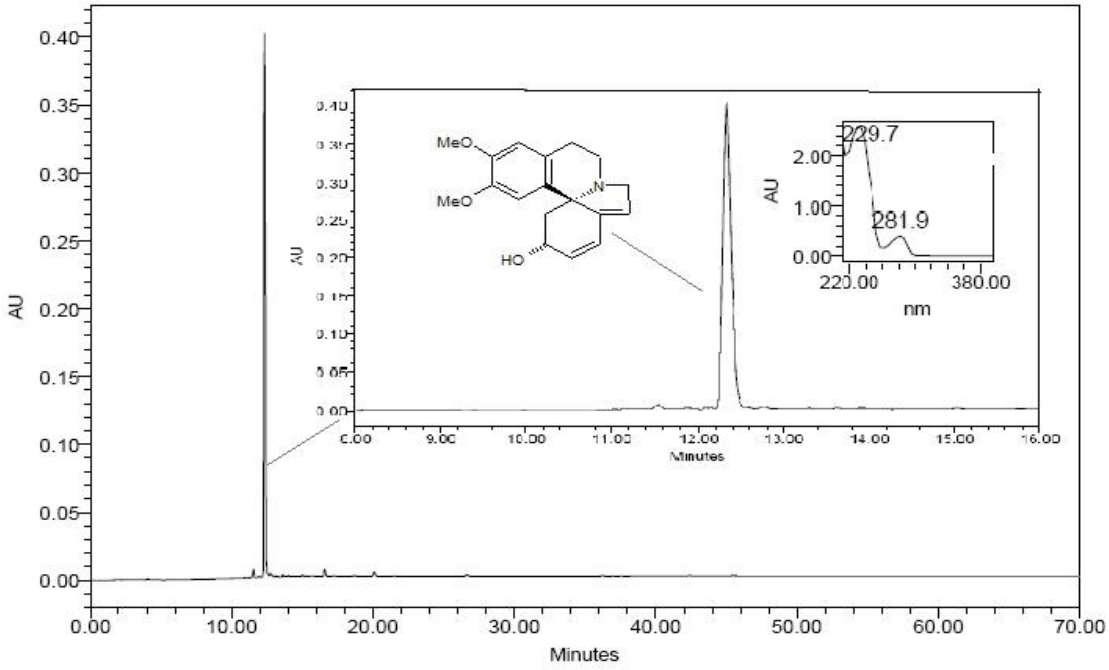


**Figure 4**
**Chromatogram of erythravine reference solution. Mobile phase water:acetonitrile, Luna C18 column (5 µm, 250 x 4.6 mm), 10 µL injection volume, λ 280 nm detection, peak at 12.34 min.**

## CONCLUSION

This study may contribute to the understanding of parameters that could be useful for the authentication of herbal drugs derived from *E. verna* trunk bark, avoiding the presence of adulterants and other closely-related species.

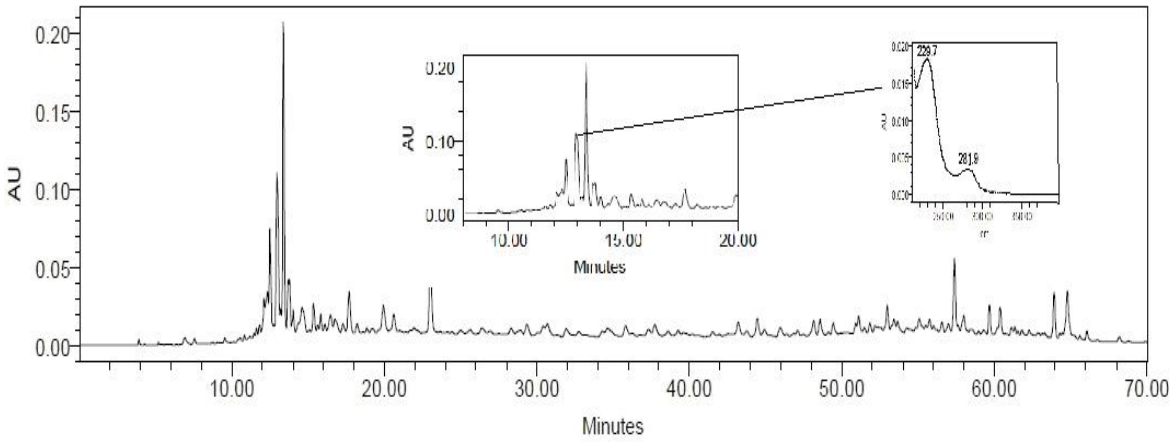


**Figure 5**
**Representative LC chromatogram of the alkaloid extract from *E. verna*, mobile phase water:acetonitrile, Luna C18 column (5 µm, 250 x 4.6 mm), 10 µL injection volume, λ 280 nm detection. Arrow shows the alkaloid erythravine.**

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