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Original Article

Morpho-anatomy and chemical profile of native species used as substitute of quina (*Cinchona* spp.) in Brazilian traditional medicine. Part II: *Remijia ferruginea*

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ABSTRACT

This research is part of a larger study of the Brazilian species that are commonly referred to as "quinas" and used as substitute of *Cinchona* species. In this study, we have performed the botanical characterization of the stem bark of *Remijia ferruginea* (A. St.-Hil.) DC., Rubiaceae, by morphological and anatomical description, and the analysis of its chemical profile. Stem bark is thin and has the color and the texture of its external and internal surfaces as diagnostic features. Types and sizes of sclerified cells in the cortical parenchyma and in the secondary phloem are important features for analysis of the transversal sections and in the macerate. Alkaloids, flavonoids and chlorogenic acid were detected in the chemical analysis for TLC. These standard references can be used in the quality control of the bark of quinas.

Introduction

Quina (or china) is the traditional name attributed to *Cinchona calisaya* Wedd. and *C. succirubra* Pav. ex Klotzsch, Rubiaceae, species native from Peru that produces the antimalarial quinine (Kaur et al., 2009; Dondorp et al., 2009). In Brazil, species from different botanical families are used for centuries as substitute of these true quina. They have its name linked to the bitter taste of its stem bark and the medicinal use as febrifuge (Cosenza et al., 2013). Recently, we are focusing in to performer morphological, anatomical and chemical profile that can be useful in the quality control of quina barks. In the part I of these work, we have studied the barks of *Polyouratea hexasperma* (A. St.-Hil.) Tiegh. (sin. *Ouratea hexasperma* (A. St.-Hil.) Baill.) (Somavilla et al., 2013).

Stem barks of *Remijia ferruginea* (A. St.-Hil.) DC., Rubiaceae, are known as "quina-da-serra", "quina-de-remijo", "quina-mineira" (Corrêa, 1984; Botsaris, 2007; Saint-Hilaire, 2014). This shrubby species (Fig. 1A and B) is endemic of Brazil, occurring mainly in rocky outcrops (INCT, 2014). The geographic distribution including the states of Minas Gerais, Mato Grosso, Mato Grosso do Sul, Bahia and Espírito Santo (Delprete and Cortés, 2006; Zappi, 2014). These barks were widely used in 19th century to treat fevers and malaria (Cosenza et al., 2013). Lindley (1838), for example, cited this species in his book Flora Medica as one of the most important plant used in the world and, despite having an inferior efficacy could be considered as a substitute Peruvian quina. Due its use also in conventional medicine, monographs for the barks of *R. ferruginea* were included in the first edition of the Brazilian Pharmacopeia (Silva, 1926; Brandão et al., 2009). Extracts from the barks are ingredient of the traditional formula Ierobina®, used to treat dyspepsia (Botion et al., 2005). On the other side, in our recent study, in which we identified barks of quina sold in popular market by DNA barcode, we observe a decline in use barks from *R. ferruginea* as quina (Palhares et al., 2014).

Study shown that high doses of the bark extracts of *R. ferruginea* induced reduction of the parasitaemia and mortality in mice infected by *Plasmodium berghei*, and indicating moderate antimalarical activity (Andrade-Neto et al., 2003) although in this alkaloids-producing species has not been detected the presence of quinine.

The aim of our work is to describe the botanical features and to analyze the chromatographic profile of stem bark of the *R. ferruginea* in order to provide support in the identification, analyses and standardization of this raw material.

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Fig. 1. *Remijia ferruginea* (A. St. Hil.) DC. (A) Shrubby habit on rocky soil of rock outcrop. (B) Detail of inflorescence and leaves. (C) General external view of stem bark with highlighting for presence of lichens (arrows). (D) Samples of stem bark shown external (left) and internal (right) aspect. Bars: 1 cm.

Materials and methods

Plant material

The samples of the stem bark of *Remijia ferruginea* (A. St.-Hil.) DC., Rubiaceae, for analysis were collected in São Gonçalo do Rio das Pedras, Serro, Minas Gerais (S 18°25′41″W 043°30′03″) and registered as DAT-134 in the DATAPLAMT (http://www.dataplamt.org.br).

Morphological, anatomical and histochemical analysis

The samples were described as to external and internal aspects such as coloring, texture and tests organoleptic. For purposes anatomical characterization part of these samples were fixed in solution of formaldehyde–acetic acid–ethanol 70 (1:1:18, Johansen, 1940), rinsed in distilled water and stored in ethanol 70. After, 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 were submitted to histochemical tests: ferric chloride (Johansen, 1940) and potassium dicromate (Gabe, 1968) to detect phenolics compounds, vanillin hydrochloric acid for tannins (Gardner, 1975), acid phloroglucin for lignin (Sass, 1951), solution of lugol for starch (Kraus and Arduim, 1997), Sudan III (Sass, 1951) and Sudan IV (Gerlach, 1984) for lipids. Part of sample was submitted to maceration process for dissociation and tissue components analysis. For that, the samples were placed in Franklin solution and maintained in a kiln (60 °C) for 72 h (Kraus and Arduim, 1997). After this process, the macerate was washed with distilled water to complete removal of Franklin solution and kept in 50% ethanol. For staining was employed ethanolic safranin 1%. The slides obtained from these preparations were analyzed and described by Olympus CX31 optical microscope and photographed with a digital camera Olympus C-7070, with wide zoom. The botanical description followed the recommendations of Junikka (1994) and Richter et al. (1996).

Chromatographic profile for phenolic and alkaloids by TLC

Preparation of fractions enriched in phenolic substances

Briefly, 1 g of dried *R. ferruginea* bark was extracted under reflux conditions with 20 ml 70% ethanol for 30 min. The sample was then

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