Revista Brasileira de Farmacognosia 27 (2017) 153–157

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

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*

Nádia S. Somavilla[a,b,*], Gustavo P. Cosenza[c,d], Christopher W. Fagg[e], Maria G.L. Brandão[c,d]

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

ARTICLE INFO

Article history:
Received 29 May 2016
Accepted 5 September 2016
Available online 26 October 2016

Keywords:
Stem bark anatomy
Quality control
Quinine derivatives

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.

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

http://dx.doi.org/10.1016/j.bjp.2016.09.005

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

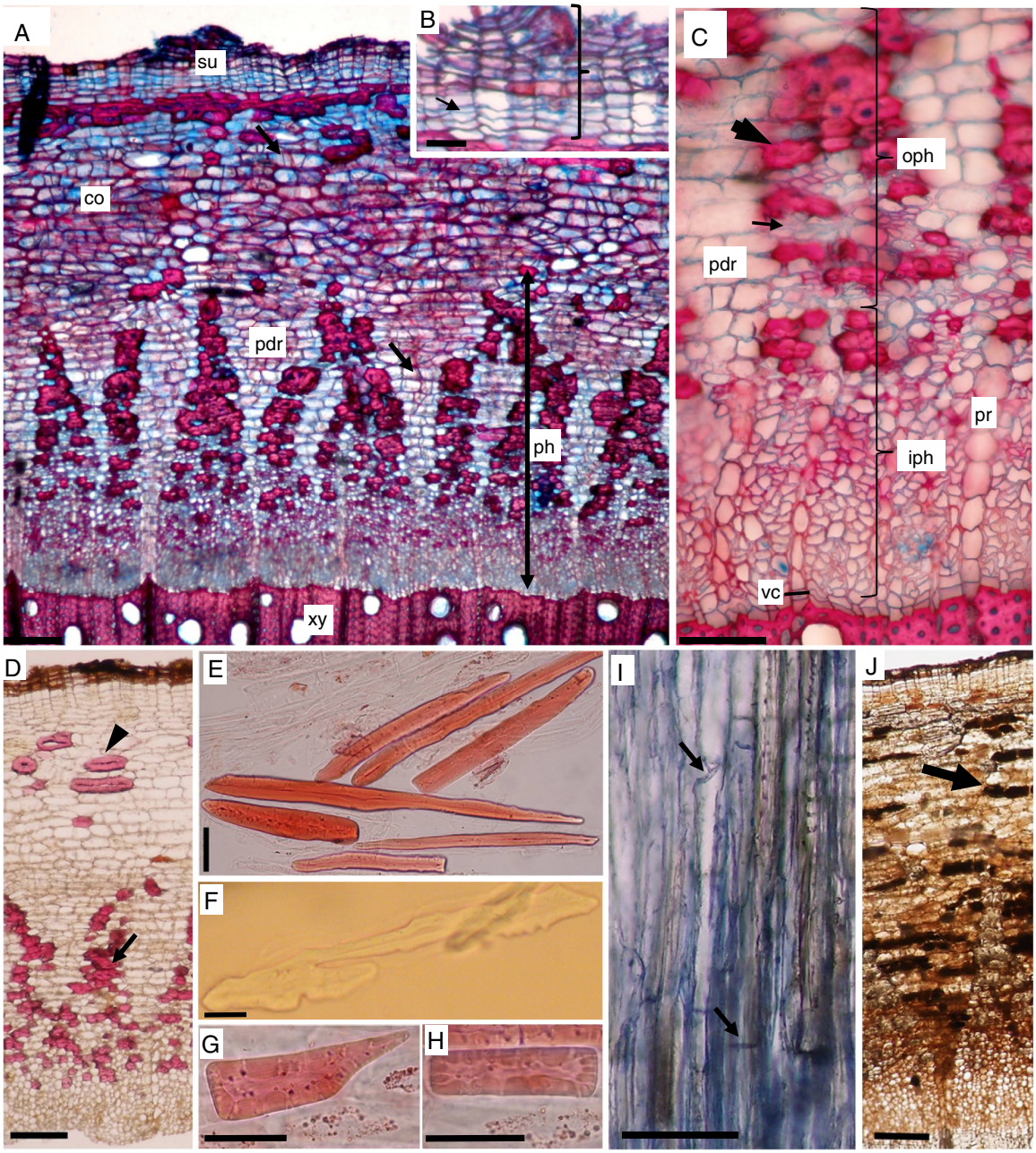

Fig. 2. Stem bark of *Remijia ferruginea* (A. St. Hil.) DC. (A) General aspect of transversal section. Arrow indicates division and expansion of parenchyma cells. (B) Magnification of the periderm (bracket) with destac for phellogen (arrow). (C) Magnification of secondary phloem region highlighting the sclerified cells (arrow) and collapsed cells of outer phloem (oph). (D) Histochemical test with phloroglucin acid shown the sclerified cells: cortical cells (arrowhead) and phloem cells (arrow). (E and F) Elongated sclerified cells of phloem. (E) Fibers. (F) Fiber-sclereids. (G and H) Cortical sclerified cells (stone cells). (I) Phloem longitudinal section shown sieve plates (arrows). (J) Transversal section shown cells (arrow) with phenolics compounds by potassium dichromate histochemical test. Legend: co, cortex; iph, inner secondary phloem; oph, outer secondary phloem; pdr, phloem dilated ray; ph, secondary phloem; phe, phellem; pr, phloem ray. Bars: 200 μm (A, D, J); 100 μm (B and C, I); 50 μm (E, G and H); 20 μm (F).

filtered through filter paper and concentrated and dried using a rotary evaporator (crude extract). The resulting residue was diluted with 50 ml water and extracted 3 times with 30 ml ethyl acetate and 30 ml of butanol. The fractions were concentrated to dryness and the aqueous lyophilized. Each dried extract and fraction were dissolved in 1 ml methanol for subsequent analysis.

Preparation of alkaloid fraction

Briefly, 3 g of dried *R. ferruginea* bark was extracted under reflux conditions with 20 ml 0.1 M HCl for 30 min. The solution was alkalized with NH_4OH to pH 9 and extracted three times with 20 ml ethyl ether. The organic phase was concentrated to dryness, and the resulting residue was dissolved in 2 ml methanol for subsequent analysis.

Chromatographic analysis

TLC was performed on silica gel plates (Macherey-Nagel Alugram®Xtra SIL G UV 254) using solvents and reagents specific for the detection of phenolic substances, including flavonoids and alkaloids. For the detection of phenolic substances, an ethyl acetate, toluene, formic acid and water (60:20:20:15) mixture was used, followed by spraying with NP/PEG. The rutin hydrate (Sigma-Aldrich, BCBH6323V), chlorogenic acid (Aldrich, SLBB6914V) and quercetin (Sigma-Aldrich, SLBD8415V) flavonoids were used as

reference standards. For the detection of alkaloids, a toluene, methanol, and diethylamine (80:10:10) mixture was used, followed by spraying with Draggendorf reagent. Samples of quinine hydrochloride (Sigma, BCB3224V), quinidine (Sigma, BCBF1345V), cinchonine (Aldrich, STBB1223), and cinchonidine (Sigma-Aldrich, BCBD9930V) were used as reference standards.

Results and discussion

The stem bark of *R. ferruginea* measure between 1 and 2 mm thick, the outer surface is slightly striated, whitish to light brown color and may have grayish color due the presence of lichens (Fig. 1C); inner surface is light brown color, smooth and slight glossy texture (Fig. 1D). The bark does not have a remarkable smell and the taste is bitter and astringent. In transversal section the stem bark is made up of periderm, cortex and secondary phloem (Fig. 2A). There is a one periderm and the phellem made up of 4–12 cells layers and outer layers detach from surface, the phelloderm show 1–2 cells layers and the phellogen is inconspicuous (Fig. 2B). Most parenchyma cells of the cortex have tangential division and expansion line with the improved in circumference of the stem (Fig. 2A, arrow). Some cortical cells show tangential expansion and lignification of its cells wall and are more clustered in the periphery of cortex (Fig. 2A and D, arrowhead). In the secondary phloem is possible to differentiate between an innermost and younger non-collapsed phloem where sclerified cells occur in reduced number or lack from an outer and oldermost collapsed phloem with numerous clustered sclerified cells (Fig. 2C and D). The cells of ray undergo tangential division and expansion toward the periphery giving rise to dilated rays and the outer cells of dilated ray anastomose with cortical parenchyma cells (Fig. 2A and C). Sclerified cells of phloem are different from sclerified cells of the parenchyma cortical. In the longitudinal section and in the macerate it is possible identify the sclerified cells of phloem as fiber or fiber-sclereids (Fig. 2E and F) with length ranging from 396.85 to 1547.73 μm (832.64 ± 320.68 μm, mean ± standard deviation) and width ranging from 26.61 to 98.04 μm (47.73 ± 19.13 μm). These cells are axially elongated and exhibit non-lamelar cell wall with simple pits and intrusive growth. During the intrusive growth the cell can form lateral projections and shown different formats (Fig. 2F). Sclerified cells of the cortical parenchyma are usually shorter sclereids (stone cells) with branched pits and lamellar walls (Fig. 2G and H), are tangentially elongated and can show intrusive growth (Fig. 2G). Length of these cells varies from 180.28 to 535.59 μm (321.57 ± 110.59 μm) and its width ranging from 44.21 to 123.11 μm (81.0 ± 22.84 μm). In the phloem the sieved plate are simple and straight or slightly oblique (Fig. 2I).

Histochemical tests were positive for phenolic compounds inside of the phellem cells and parenchyma cells located in the ray and axial system of the phloem as well as at the cortex (Fig. 2J). Probably this deposition refers to brown-yellowish substance mentioned in the description of this species by the Brazilian Pharmacopeia (Silva, 1926). Starch was identified inside of cortical and phloem rays parenchyma cells. Lignin occur in the cell wall of sclerified cells (Fig. 2D).

The analysis performed to phenolic compounds showed the presence of rutin in R*f* of 0.5 and chlorogenic acid in R*f* of 0.6 in the fractions enriched these substances, spraying NP/PEG reagent followed by UV 365 nm (Fig. 3). The chromatographic profile for alkaloids in TLC, obtained for a purified fraction showed the presence of bands in the same R*f* of cinchonine (0.65) (Fig. 4). Cinchona alkaloids occur most notably in genus *Cinchona* and the related genera *Remijia* and *Ladenbergia* and cinchonine is one of the four principal alkaloids of a total of 35 cinchona alkaloids known (Hofheinz and Merkli, 1984). Ruiz-Mesia et al. (2005) and Arana et al. (2011) observed the presence of alkaloids of the quinine type in *Remijia peruviana* highlight the richness of these alkaloids group in the genus *Remijia*. Díaz et al. (2004) detected bioactive *Cinchona* alkaloids in the leaves of *R. peruviana* indicating the importance of researches involving plant organs which obtaining the raw material is more profitable and less harmful to the conservation of the species.

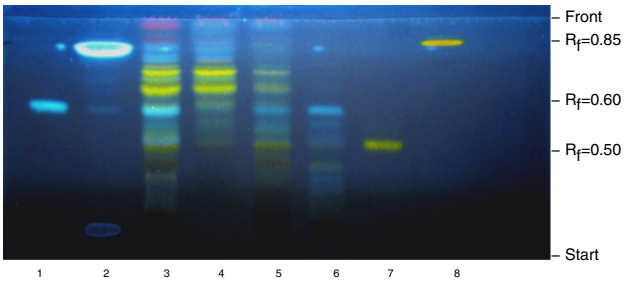

Fig. 3. TLC for identification of phenolics substances in *Remijia ferruginea* barks.

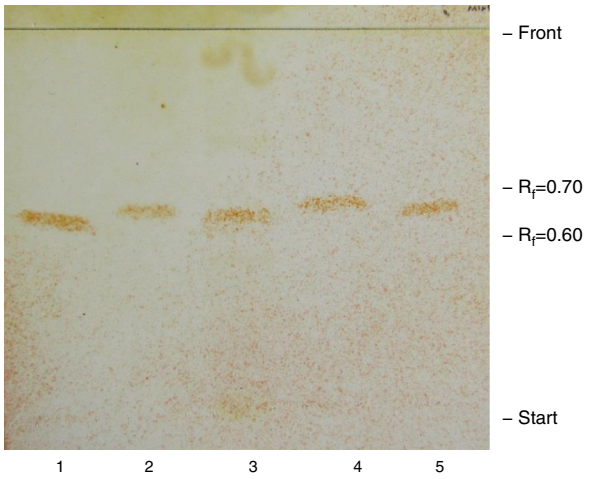

Fig. 4. TLC for identification of alkaloids from *Remijia ferruginea*.

Bathysa cuspidate (A. St. Hil.) Hook. f. ex K. Schum is a Brazilian flora Rubiaceae also known as quina and its stem bark had bitter taste and the folk medicine use this specie for the malaria treatment (Corrêa, 1984; Botsaris, 2007). In the phytochemical prospection by TLC in the stem bark were found terpen, flavonoids, tannins, cumarins and alkaloids (Coelho et al., 2012; Gontijo et al., 2012). Anatomical description and histochemical tests also shown resemblance to *R. ferruginea* and may cause misidentification (Coelho et al., 2012). However some morphological and anatomical characteristics enables us differentiate structurally the stem bark of both species take into account the color not reddish of inner surface, the absence of crystals in the secondary phloem and cortex cells, the most elongated sclereids in the cortex and only one periderm (lack of rhytidome) in *R. ferruginea*. In relation to *Cinchona* spp. stem bark (Gilg and Brandt, 1926; Costa, 1982; British Pharmacopoeia, 2009) the reddish color of outer surface, rare sclereids in cortical parenchyma, presence of calcium oxalate microprisms in some parenchymatous cells, the occurrence of exclusively fusiforms fibers in the phloem and phloem rays non-dilated are the features used for differentiate of *R. ferruginea*. These features may also be used in the quality control in relation to other specie known as quina, *P. hexasperma* (A. St.-Hil.) Tiegh. Ochnaceae (sin. *Ouratea hexasperma* (A. St.-Hil.) Baill.) (Somavilla et al., 2013), and in relation to this specie the presence of cristarque cells in the phelloderm,

the fibers format and the thickness and color of the bark are the principal diagnostic characteristics.

Authors' contribution

NSS contributed in anatomy and histochemical studies. MGLB is the coordinator of the research and GPC has done the chromatographic analyses. CWF contributed in collecting plant material, identification and herbarium confection. All the authors have read the final manuscript and approved the submission.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are grateful to CNPq for financial support (563311/2010-0, 563563-2010-9) and fellowships (150523/2011-4 and 150453/2012). Special thanks to PhD Augusto César Franco of the Physiology Laboratory of Botanical Departament/University of Brasília for use of photomicroscopy during the Post doctoral of first author in this institution.

References

Andrade-Neto, V.F., Brandão, M.G.L., Stehmann, J.R., Oliveira, L.A., Krettli, A.U., 2003. Antimalarial activity of *Cinchona*-like plants used to treat fever and malaria in Brazil. J. Ethnopharmacol. 87, 253–256.

Arana, G.V., Mesía, L.R., Mesía, W.R., Reina, M., 2011. Estudio químico de la corteza de *Remijia peruviana* "Cascarilla". Cienc. Amaz. 1, 41–49.

Botion, L.M., Ferreira, A.V.M., Côrtes, S.F.C., Lemos, V.S., Braga, F.C., 2005. Effects of the Brazilian phytopharmaceutical product Ierobina® on lipid metabolism and intestinal tonus. J. Ethnopharmacol. 102, 137–142.

Botsaris, A.S., 2007. Plants used traditionally to treat malaria in Brazil: the archives of Flora Medicinal. J. Ethnobiol. Ethnomed. 3, 18–21.

Brandão, M.G.L., Cosenza, G.P., Grael, F.F., Netto Junior, N.L., Monte-Mór, R.L.M., 2009. Traditional uses of American plant species from the 1st edition of Brazilian Official Pharmacopoeia. Rev. Bras. Farmacogn. 19, 478–487.

British Pharmacopoeia, 2009. Her Majesty's Stationary Office, London.

Coelho, V.P.M., Leite, J.P.V., Nunes, L.G., Ventrella, M.C., 2012. Anatomy, histochemistry and phytochemical profile of leaf and stem bark of *Bathysa cuspidata* (Rubiaceae). Austral. J. Bot. 60, 49–60.

Corrêa, M.P., 1984. Dicionário de plantas úteis do Brasil e das exóticas cultivadas, vol. 6. Rio de Janeiro, Di Giorgio.

Cosenza, G.P., Somavilla, N.S., Fagg, C.W., Brandão, M.G.L., 2013. Bitter plants used as substitutive of *Cinchona* spp. (quina) in Brazilian traditional medicine. J. Ethnopharmacol. 149, 790–796.

Costa, A.F., 1982. Farmacognosia, vol. 3. Calouste Gulbenkian, Lisboa.

Delprete, P.G., Cortés, B.R., 2006. A synopsis of the Rubiaceae of the states of Mato Grosso and Mato Grosso do Sul, Brazil, with a key to genera, and a preliminary species list. Rev. Biol. Neotrop. 3, 13–96.

Díaz, J.G., Sazatornil, J.G., Rodríguez, M.L., Mesía, L.R., Arana, G.V., 2004. Five new alkaloids from the leaves of *Remijia peruviana*. J. Nat. Prod. 67, 1667–1671.

Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J., Lwin, K.M., Ariey, F., Hanpithakpong, W., Lee, S.J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., An, S.S., Yeung, S., Singhasivanon, P., Day, N.P.J., Lindegardh, N., Socheat, D., White, N.J., 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. N. Engl. J. Med. 361, 455–467.

Gardner, R.O., 1975. Vanillin–hydrochloric acid as a histochemical test for tannin. Biotech. Histochem. 50, 315–317.

Gabe, M., 1968. Techniques histologiques. Masson &Cie, Paris.

Gerlach, D., 1984. Botanische Mikrotechnik. Thieme, Stuttgart.

Gilg, E., Brandt, W., 1926. Farmacognosia: matéria farmacéutica vegetal y animal. Labor, Barcelona.

Gontijo, D.C., Nunes, L.G., Souza, C.J.A., Fietto, L.G., Leite, J.P.V., 2012. Evaluation of the genotoxic potential of ethanolic extracts of stem bark and leaves of *Bathysa cuspidata* (A. St.-Hil.) Hook. Rev. Cienc. Farm. Basica Apl. 33, 355–359.

Hofheinz, W., Merkli, B., 1984. Quinine and quinine analogues. In: Peters, W., Richards, W.H.G. (Eds.), Antimalarial Drug II: Current Antimalarial and New Drug Developments. Springer-Verlag, Berlin.

INCT, 2014. Herbário Virtual da Flora e dos Fungos, http://inct.splink.org.br [accessed 23.11.14].

Johansen, D.A., 1940. Plant Microtechnique. Macgraw-Hill Book Company, New York.

Junikka, L., 1994. Survey of English macroscopy bark terminology. IAWA J. 15, 3–45.

Kaur, K., Jain, M., Kaur, T., Jain, R., 2009. Antimalarials from nature. Bioorg. Med. Chem. 17, 3229–3256.

Kraus, J.E., Arduim, M., 1997. Manual básico de métodos em morfologia vegetal. EDUR, Rio de Janeiro.

Lindley, J., 1838. Flora Medica; a botanical account of all the more important plants used in medicine, in different parts of the world. Longman, Orme, Brown, Greensand Longmans, London, pp. 429.

Paiva, J.G.A., Fank-de-Carvalho, S.M., Magalhães, M.P., Graciano-Ribeiro, D., 2006. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. Acta Bot. Bras. 20, 257–264.

Palhares, R.M., Drummond, M., Brasil, B.S.A.F., Krettli, A.U., Oliveira, G.C., Brandão, M.G.L., 2014. Molecular identification associated to chemical and biological activity to evaluate species of Brazilian quina, used as substitute of *Cinchona* spp. J. Ethnopharmacol. 155, 815–822.

Richter, H.G., Mazzoni-Viveiros, S.C., Alves, E.S., Luchi, A.E., Costa, C.G., 1996. Padronização de critérios para a descrição anatômica da casca: lista de características e glossário de termos. IF-Sér. Reg. S. Paulo 16, 1–25.

Ruiz-Mesia, L., Ruiz-Mesia, L.W., Reina, M., Martínez-Dias, R., Guadanõ, A., Gonzalez-Coloma, A., 2005. Bioactive cinchona alkaloids from *Remijia peruviana*. J. Agric. Food Chem. 53, 1921–1926.

Saint-Hilaire, A., 2014. Plantas usuais dos brasileiros. FinoTraçoEditora Ltda, Belo Horizonte.

Sass, J.E., 1951. Botanical Microtechnique. The Iowa State College Press, Ames.

Silva, R.A.D., 1926. Farmacopéia dos Estados Unidos do Brasil, 1ª.ed. Indústria Gráfica Siqueira, São Paulo, http://www.anvisa.gov.br/hotsite/farmacopeiabrasileira/publicacoes/1_edicao.pdf.

Somavilla, N.S., Cosenza, G.P., Fagg, C.W., Brandão, M.G.L., 2013. Morpho-anatomy and chemical profile of native species used as substitutes of quina (*Cinchona* spp.) in Brazilian traditional medicine. Part I: *Polyouratea hexasperma*. Rev. Bras. Farmacogn. 23, 592–599.

Zappi, D., 2014. Remijia in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro, Available in http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB26089 [accessed 20.11.14].