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Original Article

Morphoanatomical and physicochemical profile of *Piper callosum*: valuable assessment for its quality control

Rolf J.F. Silva[a,*], Ana C.A. de Aguiar-Dias[b], Kelson do C.F. Faial[c], Maria S. de Mendonça[d]

[a] *Programa de Capacitação Institucional, Museu Paraense Emílio Goeldi, Coordenação de Ciências da Terra e Ecologia, Belém, PA, Brazil*
[b] *Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil*
[c] *Laboratório de Toxicologia, Seção de Meio Ambiente, Instituto Evandro Chagas, Ananindeua, PA, Brazil*
[d] *Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Manaus, AM, Brazil*

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ABSTRACT

Piper callosum Ruiz & Pav., Piperaceae, popularly known as "elixir-paregórico" and "matricá" in Brazil, is used in folk medicine to treat gonorrhea, general pain, and digestive disorders, and has repellent, astringent, diuretic, depurative, and haemostatic properties. Despite the fact that this plant is sold as a traditional phytotherapeutic product, we did not find reports on its quality control. We, therefore, performed macroscopic, microscopic, histochemical, and physicochemical analyses using standard methods to establish botanical authentication and purity degree parameters for leaves and stem of this species in two forms: medicinal plant and herbal drug. We observed the size, shape, color, texture, fracture surface and transection characteristics, leaf venation patterns, and calluses are valuable diagnostic characters to identify the herbal drugs when they are not ground or powdered. Since medicinal plants and herbal drugs did not differ anatomically, the following key anatomical characters for *P. callosum* can be used for diagnostic purposes of both types raw plant materials: epicuticular wax and cuticular flanges patterns; collenchyma features; fibers in the midrib; arrangement pattern of the vascular bundles of the midrib and petiole; shape of the midrib, leaf margin, petiole, and stem; occurrence of raphides; and morphology of the starch grains. Acid lipids, essential oils, oleoresins, steroids, tannins and flavonoids were histochemically identified. Total ash (leaves: 11.25%; stem: 5.25%), sulphated ash (leaves: 68.02%; stem: 12.50%), acid-insoluble ash (leaves: 2.82%; stem: 0.27%), moisture (leaves: 8.60%; stem: 6.10%), loss on drying (leaves: 11.08%; stem: 8.58%), and pH (leaves: 5.57, stem: 5.28) values were determined. The order of analyzed metal levels in leaf and stem herbal drugs was Al > V > Cu > Mn > Cr > Ni. Similar levels of Cd and Co and low levels of Hg were found. The results obtained can be used as quality control parameters for medicinal plants and herbal drugs of *P. callosum*.

Introduction

Piper callosum Ruiz & Pav., Piperaceae, popularly known as "elixir-paregórico," "óleo-elétrico," "ventre-livre," "erva-de-soldado," "panquilé," "matricá" and "joão-brandin" in Brazil (Andrade et al., 2009), is a shrub native to Bolivia, Brazil, Peru, and Colombia. In Brazil, it occurs in Acre, Amazonas, Amapá, Pará, Rondônia, Distrito Federal, Mato Grosso, Espírito Santo, Rio de Janeiro, and Paraná States (Guimarães et al., 2014).

In Brazilian folk medicine, *P. callosum* leaves and young stem are used in the form of infusion or poultice to treat dysmenorrhea, intestinal colic, diarrhea, nausea, toothache, rheumatic and muscular pain, mosquito bites, and gonorrhea, and have repellent, astringent, haemostatic, digestive, diuretic, and depurative properties (Andrade et al., 2009). At open-air markets in northern Brazil, vegetative aerial parts of *P. callosum* are sold fresh, dried, ground, and rarely powdered or as an ingredient in artisanal preparations called "garrafadas" for medicinal purposes. The plant is also cultivated in backyards and medicinal gardens (authors' observations).

A number of volatile and fixed phytoconstituents have been isolated from *P. callosum*, including alkaloid amides; terpenes, such as hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and steroids; and phenolics, such as oxygenated flavonoids and phenylpropanoids (Parmar et al., 1997; Facundo et al., 2004; Andrade et al., 2009). Studies of essential oils obtained from *P. callosum* have demonstrated antifungal, insecticidal, and larvicidal activities (Andrade et al., 2009).

* Corresponding author.
E-mail: rolfsilva@museu-goeldi.br (R.J. Silva).

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P. callosum, currently being traded as a traditional phytotherapeutic product, represents a promising medicinal plant for phytopharmaceutical development due to the ethnopharmacological evidence for the numerous popular medicinal uses attributed to this plant and of the pharmacological potential of its phytoconstituents. Despite this, we did not find any systematic reports of its quality control parameters. The quality of raw plant materials represents the first step for the establishment of minimum criteria of acceptance and is a pre-requisite for the production and registration of phytomedicines (Couto et al., 2013; Anvisa, 2014). Hence, the present work aimed to establish parameters of botanical authentication and purity degree for the quality control of *P. callosum* leaves and stem as raw plant materials in forms of medicinal plant and herbal drug.

Materials and methods

Plant material

Fertile samples ($n = 14$ specimens; 7 specimens per sampled area) of *Piper callosum* Ruiz & Pav., Piperaceae, were collected from natural populations of two Brazilian states: Manaus-AM, and Belém-PA. A voucher specimen (MG 206892) was deposited at the João Murça Pires (MG) Herbarium of the Emílio Goeldi Paraense Museum. The taxonomic identity was confirmed by Elsie Franklin Guimarães, specialist in Piperaceae (Rio de Janeiro Botanical Garden Research Institute).

Preparation of the herbal drugs

Aerial parts of *P. callosum* (leaves from the 1st to 4th nodes and stem up to the 4th internode) were washed in 70% (v/v) ethanol and dried at 40 °C in a hot-air oven (Sterilifer SX 1.5 DTMS) until reaching a constant weight (Silva et al., 2016). Part of the leaf and stem herbal drugs were ground to a powder in a knife mill (Marconi MA580). The whole and powdered herbal drugs were stored at room temperature in airtight, light-resistant containers (WHO, 1998).

Pharmacobotanical analysis

Macroscopic and organoleptic characterization was performed on the whole and powdered herbal drugs using standard methods (WHO, 1998; Oliveira and Akisue, 2003; Farmacopeia Brasileira, 2010). The leaf herbal drugs were rehydrated, clarified, and stained for observation of the leaf venation (Silva et al., 2016). The photomacrographs were obtained using a digital camera (Nikon D 3100). The stereoscopic photomicrographs by reflective light (RL) and by differential interference contrast (DIC) were captured with a digital camera (Motic 2500) attached to a stereoscopic microscope (Motic SMZ-168) using Motic Images Plus 2.0 software.

Microscopic characterization was performed on the herbal drugs and fresh plant materials. For the latter, leaf (fully expanded mature leaves from the 4th node) and stem (from the 1st to 4th internodes) samples were obtained according to Silva et al. (2014), fixed in NBF-neutral buffered formalin (Lillie, 1965) and buffered glutaraldehyde/osmium tetroxide (Potiguara et al., 2013), and preserved (Johansen, 1940). NBF and glutaraldehyde/osmium tetroxide-fixed samples were used for light microscopy (LM) and scanning electron microscopy (SEM) observations, respectively.

Epidermal peels of the leaf blade were obtained through maceration in Jeffrey's solution, stained with astra blue, and mounted on glass slides with glycerol jelly (Johansen, 1940). Samples were infiltrated and embedded in methacrylate resin (Historesin, Leica®), and sectioned in a rotary, auto-advance microtome (Leica® RM 2245). The histological sections (transverse and longitudinal, 1.5–3.5 μm thick) were stained with citrate-buffered toluidine blue, pH 4.7 (O'Brien et al., 1964), and mounted on glass slides with synthetic resin (Permount-Fisher®) for structural characterization. Histological sections from fresh plant materials were made by hand with a steel razor and used for histochemical screening (Table 1). For all tests, standard control procedures were carried out simultaneously using the same procedures, and untreated sections were used to verify the natural coloration of the analyzed tissues (white). The photomicrographs by transmitted and polarized light were obtained with a digital camera (Motic 2500) attached to an optical microscope (Motic BA 310) equipped with an epifluorescence unit.

The SEM analysis followed the procedures described by Silva et al. (2014). Samples boiled in chloroform for one hour for partial or total removal of waxy deposits were also used. A Leo 1450 VP scanning electron microscope was used for the observations and capture of images.

Microscopic characterization of the herbal drugs was performed by LM and SEM. The whole herbal drugs were rehydrated and submitted to the above-mentioned methods, apart from histochemical screening. The powdered herbal drugs were processed according to WHO (1998) and Farmacopeia Brasileira (2010) for LM observations. For SEM observations, samples were mounted on SEM metal stubs, following procedures described by Silva et al. (2014).

Pharmacognostical analysis

Pooled samples of the herbal drugs were used for the physicochemical analysis. The total ash, acid-insoluble ash, sulphated ash, pH, moisture (Azeotropic method) and loss on drying (INFRATEST) were determined using standard procedures (WHO, 1998; Farmacopeia Brasileira, 2010). The analytical method to determine the selected metals (Al, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Ti, V, Hg and As) followed Pratsmoya et al. (1997), and the measurements were performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Varian model VISTA-MPX spectrometer for Al, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Ti and V, and a Thermo model ICAP 6000 spectrometer for Hg and As. Standard Reference Material (SRM 1547: peach leaves) from the National Institute of Standard and Technology (NIST) was used for validation of the applied analytical method using the same procedures.

All reagents were of analytical grade. Ultrapure water (18.2 MΩ-cm at 25 °C) from a Milli-Q system (Merck Millipore) was used. All determinations were performed in triplicate, and the results were expressed as mean ± standard deviation (mean ± S.D.).

Results

Pharmacobotanical characterization

The herbal drugs of whole leaves are complete, *i.e.* with leaf blade, petiole, and leaf sheath; *ca.* 3.4–8.7 cm long and 1.2–4.6 cm wide; wrinkled or folded; friable in texture; greenish in color on both faces, somewhat bright on the adaxial face; characteristic aromatic odor; taste predominantly characteristic aromatic, turning slightly bitter, and ending slightly spicy. The herbal drugs of powdered leaves are dark-green in color and have the same odor and taste as the whole herbal drugs (Fig. 1A and Q).

Leaf blade is *ca.* 5–9.6 cm long and 2.5–4.9 cm wide; symmetric; ovate-elliptical; entire margin; acuminate apex; cuneate base with callus in basilaminar position on each side of the adaxial face; surface rough to the touch on abaxial face; surface glabrous to the eye on both faces; smooth-granular fracture surface; prominent veins on both faces, mainly on the abaxial face; eucamptodromous major

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