Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

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

Original Article

Qualitative and quantitative analysis of the phenolic content of *Connarus* var. *angustifolius*, *Cecropia obtusa*, *Cecropia palmata* and *Mansoa alliacea* based on HPLC-DAD and UHPLC-ESI-MS/MS

Fernanda B. Pires[a], Carolina B. Dolwitsch[a], Valéria Dal Prá[a], Henrique Faccin[b], Débora Luana Monego[b], Leandro M. de Carvalho[a,b], Carine Viana[a], Osmar Lameira[c], Fernanda O. Lima[d], Lucas Bressan[b], Marcelo B. da Rosa[a,b,*]

[a] *Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil*
[b] *Programa de Pós-graduação em Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil*
[c] *Laboratório de Agrobiotecnologia, Embrapa Amazônia Oriental, Belém, PA, Brazil*
[d] *Curso de Química, Universidade Federal da Fonteira Sul, Realeza, PR, Brazil*

ARTICLE INFO

Article history:
Received 2 November 2016
Accepted 28 March 2017
Available online xxx

Keywords:
Barbatimão-do-pará
Red-embaúba
White-embaúba
Cipó-d'alho
Phenolic content
HPLC-DAD
UHPLC-ESI-MS/MS

ABSTRACT

The phenolic content of the medicinal species *Connarus perrottetti* var. *angustifolius* Radlk., Connaraceae, *Cecropia obtuse* Trécul, *Cecropia palmata* Willd., Urticaceae; and *Mansoa alliacea* (Lam.) A.H.Gentry, Bignoniaceae, collected in three different years was evaluated. Plant infusions and hydroalcoholic, butanol and ethyl acetate extracts were analyzed by high-performance liquid chromatography with diode array detection. In order to endorse these results, analysis by electrospray mass spectrometry was also performed. Were identified: gallic acid, catechin, caffeic acid, ferulic acid, rutin, quercitrin and resveratrol. *C. perrottetti* showed greater diversity of polyphenols. *M. alliacea* had the higher concentration of caffeic acid even though it was found in all species. Catechin was the major antioxidant, but was not detected in *M. alliacea*. However, we discuss the popular use of these species, as well as their phenolic constitution and the interannual distribution of phenolic compounds.

Introduction

The use of medicinal plants for prevention and recovery of infections as well as for health promotion is an ancient practice (Veiga et al., 2005; Schmitz et al., 2005; Alvim et al., 2006). This activity grew out of popular knowledge and for a long time was the only alternative treatment for health problems (Alvim et al., 2006).

Although modern medicine has been greatly advancing in the past decades, phytotherapy is still widely employed (Alim et al., 2006). The World Health Organization estimates that about 80% of the world population uses this traditional medicine in its primary health care needs (MS, 2006). According to the Institute for Applied Economic Research (IPEA, 2010), about 30% of commercially available drugs derive from natural sources, while Lahlou (2013) asserts that approximately 40% of all drugs are either natural products or their semi-synthetic derivatives.

Brazil holds an expressive plant biodiversity, along with the largest rain forest on the planet: the Amazon (Paracampo, 2011). Although the employment of plants for medicinal purposes is very widespread in Brazil, knowledge about their chemical composition is rather limited, as most of them are applied with little or no scientific proof of action (Campelo, 2006). In order to change this situation, relevant studies concerning the chemical composition of these species and the arising pharmacological properties are of considerable importance.

Different parts of the plant may be used in herbal medicine, such as roots, bark, leaves, fruits and seeds (Rezende and Cocco, 2002). Tea, usually obtained by infusion, is a very popular way of getting the active compounds of different plant products (Schmitz et al., 2005).

Polyphenols have been increasingly investigated and consumed in recent years due to their nutritional potential and therapeutic value (Ajila et al., 2011). However, numerous species of medicinal interest still need to be studied regarding this and other classes of compounds. Amongst them are *Connarus perrottetti* var. *angustifolius* Radlk., *Cecropia obtusa* Trécul, *Cecropia palmata* Willd., Urticaceae, and *Mansoa alliacea* (Lam.) A.H.Gentry, Bignoniaceae, all

* Corresponding author.
E-mail: marcelo.b.rosa@ufsm.br (M.B. Rosa).

http://dx.doi.org/10.1016/j.bjp.2017.03.004

Please cite this article in press as: Pires, F.B., et al. Qualitative and quantitative analysis of the phenolic content of *Connarus* var. *angustifolius*, *Cecropia obtusa*, *Cecropia palmata* and *Mansoa alliacea* based on HPLC-DAD and UHPLC-ESI-MS/MS. Revista Brasileira de Farmacognosia (2017), http://dx.doi.org/10.1016/j.bjp.2017.03.004

native species of the Amazon rainforest, where they are extensively used by the locals with limited scientific information.

The species *C. perrottetti* var. *angustifolius*, popularly known as "barbatimão-do-pará", is a member of the Connaraceae family. This plant present antidiarrheal, anti-bleeding, anti-inflammatory, antibacterial, antifungal, antiviral and healing activities (Paracampo, 2011), being commonly used macerated, or as tea and syrup, for the treatment of genitourinary infections in women, uterine bleeding, vaginal discharge, headache, gastric diseases and cough (Coelho-Ferreira, 2009).

The species *C. obtusa* and *C. palmata*, popularly known in Brazil as "red-embaúba" and "white-embaúba" respectively, are both members of the Urticaceae family (Beringhs et al., 2015). Plants of this genus are usually employed as tea, and exhibit several recognized activities, such as antidiabetic (Freitas and Fernandes, 2006), expectorant, mucolytic, antiseptic, laxative, antimicrobial (Lameira et al., 2004), diuretic, antitussive and anti-inflamatory (Freitas and Fernandes, 2006; Lameira et al., 2004; Costa et al., 2011).

In Brazil, especially in the state of Pará, *M. alliacea* is popularly known as "cipó-d'alho" due to the characteristic smell of garlic released by its leaves when macerated (Zoghbi et al., 2009). This plant belongs to the Bignoniaceae family and is used by its anti-rheumatic (Ribeiro et al., 2009), antimalarial (Pérez, 2002), antifungal and antiviral (Zoghbi et al., 2009), activities and to the treatment of respiratory diseases (Ribeiro et al., 2009). Maceration, infusion, tea preparation and decoction are the main processes popularly used in this plant to obtain its active compounds (Zoghbi et al., 2009).

Several factors can coordinate or change the rate of production of phenolic compounds and other secondary metabolites in plants. The period of collection is one of them, since the concentration and even nature of such compounds may vary considerably over the year (Gobbo-Neto and Lopes, 2007).

Phenolic compounds range from simple to highly polymerized structures, which can withal be complexed to various other plant components. Therefore, different methods of extraction combined with solvents of different polarities are required to obtain them (Naczk and Shahidi, 2004).

This paper aims to identify and to quantify phenolic content, namely, gallic acid, catechin, caffeic acid, rutin, ferulic acid, quercitrin, myricetin, fisetin, resveratrol, quercetin, kaempferol, chrysin and flavone, present in the species *C. perrottetti* var. *angustifolius*, *C. obtusa*, *C. palmata* and *M. alliacea*. The extracts were obtained by ultrasound extraction using ethanol/water, n-butanol and ethyl acetate as solvents. Water infusions were also analyzed. The separation, identification and quantification of the aforementioned compounds were carried out using high-performance liquid chromatography with diode array detection (HPLC-DAD). An ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) analysis was performed in order to endorse the results previously obtained by HPLC-DAD.

Materials and methods

Chemicals

Analytical standards of gallic acid, (+)-catechin, caffeic acid, rutin, quercitrin, myricetin, fisetin, quercetin, kaempferol, chrysin and flavone were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ferulic acid was purchased from Fluka (Buchs, Switzerland) and resveratrol from Tedia (Rio de Janeiro, RJ, Brazil). All standards used were of analytical grade (≥95% purity).

The mobile phase was prepared by diluting an 85% (v/v) phosphoric acid (PA) (F. Maya, Brazil) with ultrapure water (Milli-Q, Millipore Synergy UV, Bedford, MA, USA) to a concentration of 0.1% (w/w) in a volumetric flask. The solution was then filtered under vacuum system (Prismatec, 131, 2 V) through a 0.2 μm cellulose acetate membrane filter (Sartorius, Goettingen, Germany). Acetonitrile was also in the composition of the eluent and was obtained from Panreac ITW companis (Germany).

Stock solutions of 1000 mg/l were prepared by diluting each phenolic compound studied in HPLC grade methanol, supplied by Tedia (Rio de Janeiro, RJ, Brazil). They were stored in Falcon tubes at −30 °C. These solutions were then diluted in methanol to reach intermediate concentrations.

Plant samples were weighed on a digital analytical balance (Shimadzu/AUY 220) with a 0.0001 g precision.

Plant material

Barks of *C. perrottetti* var. *angustifolius* Radlk., *Connaraceae* (IAN 184393) and leaves of *Cecropia palmata* Willd. (IAN 185556), *C. obtusa* Trécul, Urticaceae (IAN 185555) and *M. alliacea* (Lam.) A.H.Gentry, Bignoniaceae (IAN 184394) were collected in April and May of 2012, 2013 and 2014, properly identified, dried and milled. All plants studied were provided by the herbarium IAN located at the Brazilian Agricultural Research Corporation (Embrapa) Eastern Amazon, Belém, PA, located at 1°27′21″ S and 48°30′14″ W, at an altitude of 10 m and an average annual temperature of 30 °C.

According to Ananias et al. (2010) the climate of the Amazon region is characterized by a dry period (July–October) and a rainy season (December–May), while June and November are considered transition periods. In addition, it is considered a hot and humid climate with very small temperature gradients. Therefore, samples studied were collected during the rainy season.

Extraction procedure

Plant extracts were prepared by ultrasound assisted extraction (Bandelin, Sonorex Super RK 510 H). Glass tubes containing approximately 0.2 g of the samples received 10 ml of the extraction solvent (70% hydroethanolic, butanol or ethyl acetate) and were placed in an ultrasonic bath for 4 h at room temperature. Then, the supernatant was withdrew and the remaining extract was filtered on a 0.22 μm membrane (Sorbline Tecnologie). Butanol and ethyl acetate were evaporated at 40 °C in a glass beaker. These extracts were later resuspended in HPLC grade methanol and filtered in a 0.22 μm membrane (Sorbline Tecnologie). The initial concentrations were maintained. The extracted samples remained stored at −30 °C until the analysis. Before injection into the chromatograph, all samples were diluted to 0.01 g/ml with HPLC grade methanol.

Infusions

A volume of 50 ml water at 90 °C were added to 1.5 g of dried plant. Thirty minutes later, the infusions were filtered, both by a paper filter and by a 0.22 μm membrane (Sorbline Tecnologie) and then stored at −40 °C until used. The samples injected were first diluted at 0.01 g/ml with HPLC grade methanol.

HPLC-DAD apparatus

The chromatographic analysis was performed in a Knauer chromatograph (Berlin, Germany) equipped with a manual Knauer injector (20 μl loop). The system consisted of a Smartline Pump 1000, a Smartline Manager 5000 and a Smartline UV detector 2600 with photodiode array technology. Instrument control and data acquisition and processing were managed by the ChromGate® V 3.3.1 Knauer software.

Please cite this article in press as: Pires, F.B., et al. Qualitative and quantitative analysis of the phenolic content of *Connarus* var. *angustifolius*, *Cecropia obtusa*, *Cecropia palmata* and *Mansoa alliacea* based on HPLC-DAD and UHPLC-ESI-MS/MS. Revista Brasileira de Farmacognosia (2017), http://dx.doi.org/10.1016/j.bjp.2017.03.004