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Original Article

Some triterpenic compounds in extracts of *Cecropia* and *Bauhinia* species for different sampling years

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ABSTRACT

The aim of this paper is to provide an overview on the chemical composition of triterpenes in widespread used folk medicine species, through the development and validation of eleven compounds using HPLC-UV detection. The compounds were separated using isocratic elution, on a reverse phase column (Kinetex C18, 250 mm × 4.6 mm, 5 μm) with mobile phase consisted of acetonitrile:tetrahydrofuran (90:10, v/v), flow-rate of 0.5 ml/min and detection in 210 nm. Diverse validation parameters were successfully evaluated. The samples of *Bauhinia variegata* L., *B. variegata* var. *candida* Voigt, Fabaceae, *Cecropia palmata* Willd. and *C. obtusa* Trécul, Urticaceae, collected in 2012, 2013 and 2014 from Amazon were treated with two different solvents (ethyl acetate and chloroform) and analyzed by the proposed method. Stigmasterol, lupeol, β-sitosterol, β-amirin and α-amirin were found in all the studied plants. Highlighting the presence of oleanolic acid, maslinic acid in *C. obtusa* and *C. palmata* extracts, erythrodiol only in *C. palmata*, stigmasteol in *B. variegata* and α-amirin in *B. variegata* var. *candida*. Overall, ethyl acetate showed better performance as the extractor solvent than chloroform. Moreover, it could be used for the quality control of medicinal plants and to assess potential marker compounds.

Introduction

Triterpenes, presents in vegetable oils, cereals, fruits and bark of trees are widespread in the human diet (Rhourri-Frih et al., 2009; Saleem, 2009; Siddique and Saleem, 2011; Szakiel et al., 2012) and one of the largest classes of secondary metabolites, with more than 30,000 different triterpenes reported (Muffler et al., 2011; Thimmappa et al., 2014).

This large number of compounds is related to the versatility of their structure, consisted of acycles, bi-, tri-, tetra- and pentacycles (Dias et al., 2011; Muffler et al., 2011). Among those, pentacyclic triterpenes presented promising pharmacological properties (Szakiel et al., 2012; Ghosh and Sil, 2013; Shanmugam et al., 2013) such as, anti-inflammatory (Saleem et al., 2008; Martelanc et al., 2009), hepatoprotector (Kumari and Kakkar, 2012; Pollier and Goossens, 2012), anti-tumor (Saleem, 2009; Shanmugam et al., 2013), anti-viral (Sánchez-Ávila et al., 2009; Kong et al., 2013), anti-HIV (Cheng et al., 2011; Wójciak-Kosior et al., 2013), antimicrobial (Pai et al., 2011), anti-fungal (Rocha et al., 2004), anti-diabetic (Manna et al., 2010), gastroprotective (Sánchez et al., 2006; Quílez et al., 2010), anti-hyperlipidemic (Claude et al., 2004), neuroprotector (Silva et al., 2011), antiarthritic (Siddique and Saleem, 2011), antioxidant (Allouche et al., 2010), cholesterol-reducing properties (Chauhan et al., 2013), cardioprotective (Somova et al., 2003) and trypanocidal activity (Ferreira et al., 2010).

Medicinal plants have been used for centuries in folk medicine associated with health promotion, prevention and cure of human diseases (Laszczyk, 2009; Romero et al., 2010). With more than 50,000 plant species, Brazil has the greatest levels of biodiversity in the planet (Giulietti et al., 2005) and Amazonia is a region with one of the richest flora in the world with a large potential discovery and research of new drugs (Giulietti et al., 2005; Coelho-Ferreira, 2009). Despite the diversity and the widespread use of phytotherapics in Brazil the scientific knowledge about this flora properties is limited (Figueredo et al., 2014).

Bauhinia variegata L., popular known in Brazil as "pata-de-vaca" or "unha-de-boi", member of the Fabaceae family, it has been

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used in popular medicine as a result of their hypoglycemic (Silva and Filho, 2002; Parekh et al., 2006; Murillo et al., 2007; Silva et al., 2007), anti-cholesterol, anti-elephantiasis (Silva et al., 2007), antibacterial (Silva and Filho, 2002; Parekh et al., 2006), anti-tumor (Rajkapoor et al., 2003), antifungal (Silva and Filho, 2002; Rajkapoor et al., 2003), diuretic, tonic, depurative activities (Pizzolatti et al., 2003), while also being useful against skin diseases and ulcers (Reddy et al., 2003) in bronchitis, leprosy (Rajkapoor et al., 2003), and in the management of inflammatory diseases (Silva and Filho, 2002; Rao et al., 2008).

Another medicinal plants included in this study is *Cecropia palmata* Willd. and *C. obtusa* Trécul, popularly known in Brazil as "embaúba-vermelha" and "embaúba-branca" from the Urticaceae family, traditional used as anti-rheumatic (Silva et al., 2007), anti-inflammatory (Rocha et al., 2007; Costa et al., 2011; Nicasio-Torres et al., 2012; Pelaez et al., 2013), anti-oxidant activities (Nicasio-Torres et al., 2012), anti-tumor (Rocha et al., 2007), act in central nervous system, including anxiolytic and antidepressant-like activities (Silva et al., 2007; Costa et al., 2011), against asthma, high blood pressure (Costa et al., 2011), as well used in the treatment of type 2 diabetes (Rocha et al., 2007; Nicasio-Torres et al., 2012; Pelaez et al., 2013).

This wide diversity of pharmacological properties reported in *Cecropia* and *Bauhinia* is related to secondary metabolites, as flavonoids, phenolic acids, carotenoids, tocopherols, alkaloids, lignans, tannins, salicylates, glucosinolates and triterpenes (Szakiel et al., 2012).

Several papers described the detection and separation of triterpenes in medicinal plants. Some methods include preparative thin-layer chromatography (TLC) (Martelanc et al., 2007; Martelanc et al., 2009), gas chromatography (GC) with derivatization step (Zhang et al., 2012), capillary electrophoresis (CE) (Cheung and Zhang, 2008; Li et al., 2011), evaporative light-scattering detectors (Lesellier et al., 2012), high-performance liquid chromatography (HPLC) (Martelanc et al., 2009; Li et al., 2011; Lesellier et al., 2012; Zhang et al., 2012) with UV detector or mass spectrometric detectors using atmospheric pressure chemical ionization (APCI) atmospheric pressure photoionization (APPI) and electrospray ionization (ESI) (Sánchez-Ávila et al., 2009). The simultaneous determination of triterpenes render a difficult task considering, their similar structure, lack of chromophores, very low UV absorption and similar polarity. According to the literature, there is only one published report that separates more than six triterpenes in a single HPLC run (Bedner et al., 2008; Martelanc et al., 2009; Sánchez-Ávila et al., 2009; Li et al., 2011; Slavin and Yu, 2012; Li et al., 2013).

In this study the development of a method for simultaneous determination of eleven triterpenes with isocratic elution and UV detection was proposed. The developed method was applied to the analysis of four different medicinal plants from the Amazon region (*Cecropia obtusa*, *C. palmata*, *B. variegata* and *B. variegata* var. *candida*) HPLC with UV detection.

Materials and methods

Chemicals

All the chemical standards used, α-amirin (98%), β-amirin (98.5%), β-sitosterol (85%), stigmasterol (95%), lupeol (90%), uvaol (95%), erythrodiol (97%), oleanolic acid (97%), betulinic acid (97%), arjunic acid (88%) and maslinic acid (95%) used were of analytical grade from Sigma–Aldrich (St. Louis, MO, USA). The solvents acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF) were of HPLC grade from Tedia (Fairfield, OH, EUA). Chloroform ($CHCl_3$) and ethyl acetate (EtOAc) were of analytical grade from Merck (Darmstadt, Germany). Stock solutions of α-amirin, β-amirin, uvaol, erythrodiol, oleanolic acid, arjunic acid and maslinic acid 1000 mg/l, stigmasterol 481 mg/l, lupeol 365 mg/l, betulinic acid 196 mg/l and β-sitosterol 873 mg/l were prepared in methanol. The working analytical solutions for analytical curve were obtained by diluting the analytical solutions in acetonitrile with the following concentrations 0.7, 6.5, 12.2, 18.0, 23.6, 29.3 and 35.0 mg/l. All the solutions were stored at −20 °C until analysis.

HPLC-UV analysis

Chromatographic measurements were performed on a Dionex® model P680 (Sunnyvale, CA, USA) liquid chromatograph equipped with a UV-vis detector model UVD170U, Rheodyne® injection valve model 8125 (Cotati, CA, USA) with loop of 100 μl. The analyses were carried out with a Kinetex reversed-phase C_{18} column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Torrance, CA, USA) which was preceded by a Security Guard C_{18}.pre-column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile:tetrahydrofuran (90:10, v/v) and the flow-rate was set at 0.5 ml/min. Spectrophotometry detection of analytes was performed at 210 nm wavelengths. Evaluation and quantification were made on a Chromeleon 6.7 Workstation. The same samples were previously studied also by performing an ultra-high-performance liquid chromatography – atmospheric pressure photoionization source mass spectrometry (UHPLC-APPI-MS/MS). Therefore, a comparison of the species detected using HPLC-UV was performed by UHPLC-APPI-MS/MS, described in details by Gobo et al. (2016).

Plant material

The medicinal plant species *Bauhinia variegata* L. (deposit n° IAN 185932), *B. variegata* var. *candida* Voigt (deposit n° IAN 185831), *Cecropia obtusa* Trécul (deposit n° IAN 185555) and *C. palmata* Willd (deposit n° IAN 185556) were obtained from the herbal collection of the Brazilian Agricultural Research Corporation, Embrapa Amazônia Oriental, Belém, PA, Brazil. The geographical location of the collection site is 1°27′21″ S latitude and 48°30′14″ W longitude.

The Amazon region has a hot and humid characteristic climate with small temperature gradients. There are two well establish seasons in that region, a dry-period (July–October) and rainy season (December–May); the months of June and November are considered transition periods (Ananias et al., 2010). According Gobbo-Neto and Lopes (2007) there is a positive influence of rainfall on the concentration of secondary metabolites (cyanogenic glycosides, glucosinolates, terpenes, anthocyanins and alkaloids) therefore, the samples studied were collected during the rainy season in three different years (2012, 2013 and 2014).

Sample preparation and extraction procedure

The fresh plant specimens were cleaned, dried at 40 °C for 12 h, ground into a fine powder in a laboratory mill and used as a dry powdered material. All plants were received as a fine powdered dried leaf material. Dried samples were stored in desiccators under vacuum at room temperature until sample treatment.

Ultrasound-assisted extraction was performed in a reactor thermostatic water bath (temperature accuracy of ±1.0 °C). The experimental setup consists of an ultrasonic bath USC 1800A (Unique Inc., Brazil, BR) equipped with a transducer with longitudinal vibrations. The ultrasonic unit has an operating frequency of 40 kHz and a maximum-rated ultrasound power output of 132 W. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath horizontally along the length of the bath (Dal Prá et al., 2015). Samples were weighed 0.5 g and placed into a conical flask, into which 10 ml of ethyl acetate or chloroform was added

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