Revista Brasileira de Farmacognosia 26 (2016) 451–458

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

Original Article

Chemical profiles of traditional preparations of four South American *Passiflora* species by chromatographic and capillary electrophoretic techniques

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ARTICLE INFO

Article history:
Received 17 November 2015
Accepted 23 February 2016
Available online 13 April 2016

Keywords:
Passiflora
C-glycosylflavonoids
Alkaloids
HPLC
UPLC
CE

ABSTRACT

Several species of the genus *Passiflora* are distributed all over South America, and many of these species are used in popular medicine, mainly as sedatives and tranquilizers. This study analyzes the chemical profile of extracts of four *Passiflora* species used in folk medicine, focusing on the flavonoids, alkaloids and saponins. We employed simple and fast fingerprint analysis methods by high performance liquid chromatography, ultra performance liquid chromatography and capillary electrophoresis techniques. The analysis led to the detection and identification of *C*-glycosylflavonoids in all the plant extracts, these being the main constituents in *P. tripartita* var. *mollissima* and *P. bogotensis*. Saponins were observed only in *P. alata* and *P. quadrangularis*, while harmane alkaloids were not detected in any of the analyzed extracts in concentrations higher than 0.0187 ppm, the detection limit determined for the UPLC method.

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Introduction

The genus *Passiflora* is the largest and most important genus of the family Passifloraceae, comprising about 500 species (Lewis and Elvin-Lewis, 1977). In North America and Europe, the main species, *P. incarnata*, is popularly known as passion fruit or passion-flower, while in South America, several others species of *Passiflora* are widely distributed and known by distinct names, such as 'maracujá', 'curuba', or 'badea', among others. (Arbelaez, 1996; Mors et al., 2000) Many of these species (*P. edulis* var. *edulis*, *P. edulis* var. *flavicarpa*, *P. tripartita* var. *mollissima* and others) are cultivated for their edible fruits or for the preparation of juices. Infusions of their leaves are also used in popular medicine in many countries, as a sedative or tranquillizer (Pio Corrêa, 1978; Arbelaez, 1996).

Different countries in South America have registered pharmaceutical preparations that use *Passiflora* species as the active component. In Colombia, for example, the leaves of *P. tripartita* var. *mollissima* are accepted as sedative and hypnotic component in phytopharmaceutical preparations (Invima, 2006). In Brazil, *P. alata* and *P. edulis* are included in the most recent version of the Brazilian Pharmacopeia (Farmacopeia Brasileira, 2010).

Regarding their chemical composition, the compounds more frequently reported for the genus are flavonoids, especially *C*-glycosylflavonoids, which are usually described as the main components (Ulubelen et al., 1982; Li et al., 2011; Zucolotto et al., 2012). These compounds have recently been associated with several pharmacological effects observed for distinct *Passiflora* species (Coleta et al., 2006; Santos et al., 2006; Sena et al., 2009; Zucolotto et al., 2009; Gazola et al., 2015). Harmane alkaloids are also frequently associated with *Passiflora* species, especially *P. incarnata* (Lutomski and Malek, 1975; Lutomski et al., 1975). Additionally, several saponins have been described for this genus, although their occurrence is restricted to certain species (Orsini et al., 1985; Reginatto et al., 2001; Doyama et al., 2005).

As part of our ongoing studies with species of the genus *Passiflora*, we evaluate, in this study, the variability of metabolite presents in the aqueous extracts of four South American *Passiflora* species: *P. alata*, *P. quadrangularis*, *P. bogotensis* and *P. tripartita* var. *mollissima*, focusing specifically on their *C*-glycosylflavonoid and alkaloid composition. The presence of saponins in these species was also evaluated. Chemical profiles were obtained by different analytical methods, such as high performance liquid

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http://dx.doi.org/10.1016/j.bjp.2016.02.005
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Table 1
Passiflora species, with their respective local common name, place of collection and identification.

Species	Local common name	Place of collection/time of year	Identification
P. quadrangularis Linn.	Badea, Maracujá-gigante	Rivera, Huila – Colombia (2°59′55″, −75°18′16″)/July 2011	Prof. Carlos Alberto Parra/Herbarium of the Universidad Nacional de Colombia (COL 572634)
P. alata Curtis.	Maracujá-doce	Urussanga, Santa Catarina – Brazil (−28°32′9″, −49°18′59″)/January 2011	Mrs. Ademar Brancher (EPAGRI/Urussanga-SC). Herbarium of the Universidade Federal de Santa Catarina (FLOR 37823)
P. tripartita var. *mollissima* Holm-Nielsen & Müller Jörgensen	Curuba-de-Castilla	Santa Sofia, Boyacá – Colombia (05°43′01″, −73°36′20″)/June 2011	Prof. Carlos Alberto Parra/Herbarium of the Universidad Nacional de Colombia (COL 564522)
P. bogotensis Benth	–	Nemocón, Cundinamarca – Colombia (4°35′60″, −4°4′60″)/May 2011	Prof. Carlos Alberto Parra/Herbarium of the Universidad Nacional de Colombia (COL 564523)

chromatography (HPLC), ultra performance liquid chromatography (UPLC) and capillary electrophoresis (CE).

Materials and methods

Chemicals

Acetonitrile, methanol and formic acid (HPLC-grade) were provided by Tedia® (Rio de Janeiro, Brazil). Water was purified with a Milli-Q system (Millipore®, Bedford, USA). For the CE analysis, stock solutions were prepared from the electrolytes sodium tetraborate (STB) and ammonium acetate ($AcNH_4$) at 100 mmol/l. Sodium hydroxide solution (NaOH) at 1 and 0.1 mol/l were also prepared. All the salts used were of analytical reagent grade, and were provided by Sigma-Aldrich® (St. Louis, USA). All the solvents and solutions were filtered through a 0.22 μm membrane before use. The reference standards orientin, isoorientin, vitexin, isovitexin, vitexin-2″-*O*-rhamnoside, harmol, harmane and harmine (all with purity ≥ 96%) were purchased from Sigma-Aldrich®. Swertisin was previously obtained from *Wilbrandea ebracteata* roots, and identified by NMR spectral data (Santos et al., 1996). The compound 4′-methoxyluteolin-8-*C*-6″-acetylglucopyranoside was previously isolated from *P. tripartita* var. *mollissima* leaves and provided by Prof. Dr. Freddy Ramos (Ramos et al., 2010). Quadranguloside was previously isolated from *Passiflora alata* leaves (Costa et al., 2013).

Plant material and preparation of extracts and samples

Leaves of adult individuals of species of *Passiflora* were collected from different regions of Brazil and Colombia (Table 1). Leaves of the different species were air-dried separately at 40 °C, powdered, and extracted by infusion with boiling water (95 °C, plant:solvent 1:10, w/v) for 10 min. The aqueous extract was then filtered, frozen and lyophilized. The samples for HPLC, UPLC and CE analysis were prepared by dissolving the lyophilized crude aqueous extracts or reference standards in methanol:water (1:1, v/v) and filtering through a 0.22 μm membrane before injection. The concentration of the sample extracts was 1000 μg/ml and for the reference standards, the concentration was 100 μg/ml.

HPLC analysis

The HPLC analyses were carried out in a PerkinElmer® Series 200 system, equipped with Diode Array Detection (DAD), quaternary pump, on-line degasser and autosampler. The data were processed using the software Chromera® Workstation. The chromatographic analyses for all samples were performed at room temperature (21 ± 2 °C), with an injection volume of 20 μl. The DAD spectra were acquired at the range of 190–450 nm. The peaks in the samples were characterized by comparing the retention time, UV spectra and co-injection with the reference standards. Vertical® VertSep C18 column (250 mm × 4.6 mm i.d.; 5 μm) was used as stationary phase. In the analysis of flavonoids, a gradient system of acetonitrile [solvent A] and formic acid 0.5% [solvent B] was used as mobile phase, in a single step: 15–35% A (0–20 min). The flow rate was kept constant at 1.2 ml/min and the chromatograms were recorded at 340 nm. For alkaloid analysis, the mobile phase used was composed of an aqueous buffer of sodium phosphate dibasic (50 mmol/l, pH 8.0) [A], methanol [B] and acetonitrile [C] at isocratic conditions of 56% A: 12% B: 32% C (0–20 min). The flow rate was kept at 1 ml/min and the UV detection at 245 nm. The chromatographic conditions for the analysis of saponins were previously described by our group (Costa et al., 2013).

UPLC analysis

An UPLC Waters Acquity® H Class system with DAD detector, quaternary pump, on-line degasser and autosampler was used for these analyses. The chromatographic parameters were converted from HPLC to UPLC using the software Empower®. Separations of both flavonoids and alkaloids were carried out in a PerkinElmer® BHE C18 column (100 mm × 2.9 mm i.d.; 1.8 μm). The analyses were also performed at room temperature (21 ± 2 °C), with DAD spectra acquired at the range of 190–450 nm. Flavonoid analysis used a two-steps gradient of acetonitrile [solvent A] and formic acid 0.5% [solvent B]: 15–35% A (0–8 min), followed by 35% A (8–10 min). The flow rate was kept constant at 0.25 ml/min. The injection volume was 3 μl. The chromatogram was recorded at 340 nm. For the alkaloids, the same mobile phase and isocratic system as the HPLC was used, with an analysis time of 7 min. The flow rate was determinate as 0.2 ml/min, with an injection volume of 2 μl. The UV detection was determined at 245 nm.

CE analysis

The analyses were performed on an Agilent 7100 capillary electrophoresis instrument equipped with DAD detector, temperature control device, and autosampler. For all the experiments, a fused-silica capillary (Agilent, model G1600-61232) of 60.5 cm (52 cm effective length), with 50 μm inner diameter and expanded detection window was used. The data were processed using the software Agilent ChemStation®. For the first use, the capillary was pretreated with a pressure flush with 1 mol/l NaOH solution (30 min). Each day, the capillary was conditioned with NaOH 1 mol/l (5 min), waiting time (1 min), Milli-Q water (5 min) and running buffer (5 min). In between runs, the capillary was flushed with running buffer (2 min). The DAD spectra were acquired at the range of 200–500 nm. A method for the analysis of flavonoids was developed using STB (50 mmol/l; pH 9.5, adjusted with NaOH 1 mol/l),

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