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Original Article

Development of a rapid and simple HPLC-UV method for determination of gallic acid in *Schinopsis brasiliensis*

Felipe H.A. Fernandes[a], Rayanne S. de A. Batista[b], Francinalva D. de Medeiros[a], Fábio S. Santos[b], Ana C.D. Medeiros[a,*]

[a] Laboratório de Desenvolvimento e Ensaios de Medicamentos, Centro de Ciências Biológicas e da Saúde, Universidade Estadual da Paraíba, Campina Grande, PB, Brazil
[b] Laboratórios Unificados de Desenvolvimento e Ensaios de Medicamentos, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, PB, Brazil

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ABSTRACT

The aim of this work was to develop and validate an analytical method for the identification of the chemical marker of *Schinopsis brasiliensis* Engl., Anacardiaceae. It would determine the total polyphenols and flavonoid content by spectrophotometric methodology in the dried extract of plant. The chromatographic profiles of *S. brasiliensis* were determined using HPLC-UV. The liquid chromatography method was conducted on a Phenomenex Gemini NX C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of 0.05% orthophosphoric acid: methanol. The flow rate was 1 ml/min and effluents were monitored at 271 nm. The retention time for gallic acid was 8.5 min. The described method has the advantage of being both rapid and easy. Hence it can be applied for routine quality control analysis of herbal preparation containing *S. brasiliensis*.

Introduction

Polyphenols are a group of secondary metabolites present in different plants. In general polyphenols are classified into four classes: phenolic acids, flavonoids, lignans and stilbenes. These are very important to the pharmaceutical, cosmetic and food industries, mainly for their antioxidant action and properties (Gharras, 2009).

Schinopsis brasiliensis Engl., Anacardiaceae, is a plant which belongs to the Anacardiaceae family, known as “baraúna” or “bráuna,” and can be found in the Brazilian semi-arid regions. Their shells have ethnopharmacological uses for the treatment of diarrhea and coughs, and can also be used as an antiseptic and analgesic (Chaves et al., 2013). Several studies have demonstrated the antimicrobial activity of *S. brasiliensis* against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* (Chaves et al., 2011; Saraiva et al., 2011; Silva et al., 2012); *Salmonella typhimurium* (Saraiva et al., 2011); *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus parasanguinis* and *Enterococcus faecalis* (Silva et al., 2012). Other studies included multi-drug resistant strains for tetracycline, gentamicin and oxacillin (Saraiva et al., 2011, 2013) and erythromycin (Silva et al., 2015).

The current list of compounds found in *S. brasiliensis* leaves includes the presence of tannins and polyphenols, such as methyl gallate, gallic acid, ellagic acid and 5,6,7,8,3′,4′ hexahydroxy-flavonol (Saraiva et al., 2011). New alkylphenol methyl 6-eicosanoyl-2-hydroxy-4-methoxybenzoate and steroid 5α,8α-epidioxy ergosta-6,22-dien-3β-ol are both compounds isolated from the bark of *S. brasiliensis* (Cardoso et al., 2005). The essential oil extracted from the leaves of *S. brasiliensis* has a good amount of myrcene and low amounts of other compounds such as β-caryophyllene, eucalyptol and guaiol (Donati et al., 2014).

One important aspect for the quality control of herbal medicine products is the determination of the chemical marker, which consists of a constituent (or groups of constituents) that is chemically defined and present in the plant product. These constituents may or may not be related to their pharmacological activity (Marques et al., 2013). While several different analytical techniques can be used to identify and quantify these substances, we used a high performance liquid chromatography (HPLC), which is the method most commonly used by the various official standards (Zollner and Schwarz, 2013).

The aim of this work was to develop and validate an analytical method using HPLC for the identification and quantification of the chemical markers of *S. brasiliensis*.

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* Corresponding author.
E-mail: anaclaudia@uepb.edu.br (A.C.D. Medeiros).

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Materials and methods

Raw material

The stem bark of *Schinopsis brasiliensis* Engl., Anacardiaceae, was collected in July 2013 from the region of Campina Grande, state of Paraiba (7°13′50″ S, 35°52′52″ W). The voucher specimen was prepared and identified by Professor Jayme Coelho de Morais Herbarium, Universidade Federal da Paraiba, under the EAN-14049.

The herbal drugs were dried at 40 °C using an air oven. After drying, the material was reduced in a knife mill (10 mesh outlet sieve) to a fine powder. The hydroalcoholic extract was obtained by maceration of the powder in a water:ethanol (30:70, v/v) solvent system for 72 h and then dried using Spray Dryer (LabPlant®), with onset temperature of 140 °C and under 3 ml/min flow rate. Colloidal silicon dioxide (Aerosil®200) was used as a drying agent at 20% (w/w). The drying extract was weighted, protected from light and stored in closed flasks at room temperature.

Determination of total polyphenols

The Folin–Ciocalteu method was used to determine the total polyphenols, using gallic acid as a standard (Chaves et al., 2013). A sample was dissolved in water with 0.5 ml of Folin–Ciocalteu solution (Sigma–Aldrich) and left at rest for 2 min. Then it was added to 1 ml of sodium carbonate solution 20% (w/w) and was left to stand for 10 min. The calibration curve was prepared with a gallic acid standard in different concentrations. The samples were measured using a UV–Vis Spectrophotometer Shimadzu UV-Mini 1240, with a wavelength of 757 nm. The results were expressed as micrograms of gallic acid/mg of extract (μg GAE/mg extract).

Determination of total flavonoids

The aluminum chloride method was used to determine the flavonoid content using a quercetin standard (Chaves et al., 2013). Initially an aliquot of the extract was dissolved in methanol. Then the extract was mixed with an aluminum chloride solution (2% in methanol) in a ratio of 1:1 and left to stand for 10 min. The calibration curve was prepared with a quercetin standard in different concentrations. We used a UV–Vis Spectrophotometer Shimadzu UV-Mini 1240, with a wavelength of 415 nm to analyze our sample. The results were expressed as micrograms of quercetin/mg of the extract (μg QE/mg extract).

Chemical marker

Chromatography analysis for the quantification of the chemical marker gallic acid in *S. brasiliensis* was conducted on a Shimadzu® HPLC model LC-10 controlled by Class VP Software and with quaternary pumps (LC-10AD), an on-line degasser (DGU-14A), and an injection valve (Rheodyne® LLC) with a 20 μl loop. The equipment included a UV–Vis detector (SPD-10A). The column used was a Phenomenex RP-Gemini NX C18 (250 mm × 4.6 mm, 5 μm) and it was protected by a pre-column Gemini NX C18 (10 mm × 4 mm).

The chromatographic separation was carried out using a mobile phase with phosphoric acid: water 0.05% as solvent A and methanol as solvent B at a flow rate of 1 ml/min. The gradient program was as follows: 90–10% B (10 min), 70–30% B (3 min), 40–60% B (5 min), 60–40% B (3 min), 80–20% B (3 min) and 90–10% B (6 min). The peaks were detected at 271 nm and were identified by comparing the retention time with standard gallic acid.

The validation of the analytical method has been made while taking into account the figures of merit such as precision, accuracy, linearity, limit of delectation and limit of quantification (Marques et al., 2013; Ribani et al., 2004; Pedroso and Salgado, 2014).

Table 1
Polyphenol and flavonoid contents of *Schinopsis brasiliensis*.

	Total polyphenols (μg/mg extract GAE)	Total flavonoids (μg/mg extract QE)
Concentration	24.52	1.43
%	15.08	1.44

The linearity was evaluated from the calibration curve, found = with a standard solution of gallic acid with a concentration between 2.5 and 15 μg/ml, and obtained the linear regression equation $y = ax + b$. According to Ribani et al. (2004), verification of the data fit can be observed by the correlation coefficient and determination. The Official Compendium recommends a value greater than 0.99.

Results and discussion

The total polyphenol content is quite high when compared to the total flavonoids (Table 1). Both these classes of compounds are present in various medicinal plants and are related to antioxidants, antimicrobial, and anti-inflammatory activities (Corradini et al., 2011). Although we used a quantitative method to determine these compounds, this technique cannot predict the composition of each polyphenol, since it may possibly also measure non-phenolic compounds (Roby et al., 2013).

The polyphenols were present in greater quantity in the plant when we analyzed the hydroalcoholic extract of *S. brasiliensis* using gallic acid as standard. Gallic acid (3,4,5-trihydroxybenzoic acid) is an important polyphenol present in plants and is a compound with important applications in the synthesis of the drug Trimethoprim, as a food preservative, and an antioxidant in oils and products rich in lipids (Battestin et al., 2004).

In the preliminary analysis, we detected the presence of these compound classes in large quantities. Based on these results, gallic acid was chosen as a marker and we proceeded to quantify and validate the methods by means of the standard addition. For this test, we used high performance liquid chromatography with a UV–Vis detector, as it is the method most suitable for this type of compounds (Arapitsas, 2012).

Fig. 1 was obtained from the chromatogram of the extract of *S. brasiliensis*, which shows the peak of gallic acid and its standard. The retention time was 8.5 min, in addition to peaks present in other compounds of the phytocomplex. Good linearity ($R^2 = 0.9984$) was achieved within the investigated ranges in the extract and was 99.5% of the explainable variance (Fig. 2).

Different studies are described in the literature (Arapitsas, 2012) analyzing gallic acid as the major compound; however, the difference between them is the type of mobile phase used. In a study of gallic acid by *Dendrophthoe falcata* L. f., Deshmukh and Prabhu (2011) found a retention time of approximately 12 min, using a mobile phase of water acidified with phosphoric acid (0.1%) and acetonitrile in the ratio of 4:6 and a flow rate 1 ml/min. In another work with *Schinus terebinthifolius* Raddi, gallic acid was quantified using two phases: one with water:methanol and the other with water:acetonitrile. In both cases, the water was acidified with formic acid to pH 2.7. The retention times under both conditions were 5 min, although the phase with acetonitrile was slightly shorter (Carvalho et al., 2009).

The choice of mobile phase (0.05% phosphoric acid and methanol) was determined using experiments described in the literature, which report that the acidified water prevents the ionization of gallic acid, causing it to have affinity for the stationary phase. Generally, the most widely used method is a reverse-phase column C-18. Although gallic acid absorbs at various wavelengths between 201 and 280 nm, we chose 271 nm due to its higher absorption and