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Antioxidant effect and characterization of South American *Prosopis* pods syrup

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ABSTRACT

The traditional syrup made of *Prosopis* pods, known as "algarrobina" or "arrope de algarrobo" in the Andean countries, is commonly used in confectionery and local cuisine to prepare sweets and cocktails. The polyphenolic content of four *Prosopis* pods syrup samples as well as from the phenolic-enriched Amberlite-retained fraction of the syrup, were analyzed using reversed phase high performance liquid chromatography-diode array detector coupled to electrospray ionization mass spectrometry (HPL-DAD-ESI-MS). The main phenolics in the syrups were apigenin-derived *C*-glycosyl flavonoids, including 6,8-*C*-pentoside-*C*-hexoside, 6,8-dihexoside and quercetin glycosides. The sugar derivative 5-hydroxymethyl furfural was present in most of the samples. All syrups were devoid of cytotoxicity towards human lung fibroblasts and human gastric AGS cells, with IC_{50} values >1000 μg/mL. The phenolic constituents of the syrups are *C*-glycosylflavonoids with known anti-inflammatory, antioxidant and other nutraceutical properties. The phenolic composition of South American algarrobo syrup is presented for the first time.

1. Introduction

The South American "algarrobo" trees (*Prosopis* spp.) are an important food resource in arid and semi-arid lands. Edible *Prosopis* pods were gathered, processed, stored, made into flour or dried, by all Amerindian groups living in places where these trees occur. In the Chaco phytogeographic region of South America, the preferred sources of the edible pods were *Prosopis alba* and *Prosopis nigra* (Schmeda-Hirschmann, 1994), while *Prosopis chilensis* and other species were consumed in Chile (Astudillo, Schmeda-Hirschmann, Herrera, & Cortés, 2000; Tapia et al., 2000). The polyphenol content, antioxidant activity and genotoxicity of Argentinian samples of *Prosopis* flour were recently reported (Cardozo et al., 2010) while samples of Brazil and Bolivia were analyzed by Galan, Correa, Patto de Abreu, and Barcelos (2008). *Prosopis* pods are also relevant as human food in Africa (Choge et al., 2007). A comprehensive review on the composition and food applications of *Prosopis* flour was recently published by Felker, Takeoka, and Dao (2013). The pods were also boiled with water to obtain a syrup known as "algarrobina" or "arrope de algarrobo". The syrup is widely commercialized in Peru, southern Ecuador and northern Chile to prepare cocktails, desserts and sweets.

Fruit syrups are part of the local cuisine worldwide, but mainly in dry areas. They are prepared by boiling the fruits in water, usually after several hours, until the consistency is considered to be appropriate. The syrup contains not only mainly sugars but also other constituents, including polyphenols. Recent work on traditional fruit syrups showed antioxidant, antimicrobial and cytotoxicity effect of date (Dhaouadi et al., 2011) and barbary-fig (Dhaouadi et al., 2013). The studies included identification of the main phenolic constituents by RP-HPLC-ESI-MS methods. The carbohydrate composition of Spanish plant syrups was reported by Ruiz-Matute, Soria, Sanz, and Martínez-Castro (2010). The syrups made from *Prosopis pallida* in Peru (Bravo, Grados, & Saura-Calixto, 1998) were analyzed for dietary fiber, sugars, condensed õtannins and phenolics such as catechins but no information is available on the identity of phenolic constituents other than catechin occurring in the syrup. The polyphenols are recognized for their nutritional value, since they may help to reduce the risk of chronic disease and have, in general, a positive effect on health. All these properties are strongly õdependent on the polyphenol chemical structure (Valls, Millán, Martí, Borrás, & Arola, 2009). Little is known on the identity of the phenolics in syrup made of *Prosopis* pods. Several bioactive constituents were reported from *Prosopis* pods than may have a beneficial effect on consumers (Schmeda-Hirschmann & Jakupovic, 2000; Tapia et al., 2000). The main aim of the present work was the characterization of the phenolic constituents in different algarrobo syrups commercialized

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in Peru, northern Chile and northwestern Argentina as well as to determine the antioxidant effect and cytotoxicity of the samples.

2. Materials and methods

2.1. Samples

The algarrobo syrups investigated were commercial "arrope" samples from Peru and Argentina. The Peruvian "algarrobina" was from Distrito de Lurín, Provincia de Lima, central coast of Peru (sample P1), Distrito de Chulucanas, Provincia de Morropon, Piura, northern coast of Peru (sample P2) and Lambayeque, northwestern Peru (sample P3). The Argentinean sample (AS) was from Caseritos de la Villa, Tucumán.

2.2. Chemicals

Folin–Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), $AlCl_3$, $NaNO_2$, NaOH, gallic acid and quercetin, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade MeOH was obtained from J.T. Baker (Phillipsburg, NJ, USA), and formic acid from Merck (Darmstadt, Germany). The purity of the chemicals used was as follows: $AlCl_3$ p.a. ≥ 99%, NaOH reagent grade, ≥98%, $NaNO_2$ ACS reagent ≥ 97.0%, gallic acid (purity ≥ 99%), quercetin (purity ≥ 97%), Folin–Ciocalteu phenol reagent (2 M, respect to acid, Sigma), 1,1-diphenyl-2-picrylhydrazyl radical (purity = 95% Sigma-Aldrich), and formic acid (≥99%).

2.3. General analysis

Total sugar content, in terms of °Brix was measured by the refraction index using an Abbe refractometer (Bausch & Lomb, model ABBE) at 20 °C. The instrument was calibrated with a 1% and 10% sucrose solution and water. The pH of the syrup was measured using a pH meter (Model Mettler Toledo MP 220, GmbH, Switzerland). Percent total acidity was determined by titration with 0.5 N NaOH solution according to AOAC 942.15-B 1990 methods (Heldrich, 1990). The formula used to calculate the total acid was: % Total acid (w/w) = N × V × Eq. wt × 100 / W × 1000, where N is the normality of NaOH, W is the mass of sample (g), V is volume of titrant (mL), 1000 is a factor relating mg to g, and Eq. wt is the equivalent weight of citric acid. The percent ash content was determined by placing 5 g of syrup sample in a crucible in a muffle furnace and heating at 500 °C for 5 h according to AOAC 900.02-G 1990 (Heldrich, 1990). The percent moisture content was determined by placing 10 g of syrup sample in an oven at 103 °C by 5 h until constant weight was achieved (AOAC 925.45-D 1990) (Heldrich, 1990). Determinations were performed in triplicate.

2.4. Total phenolic and total flavonoid content

The total phenolic content (TP) was determined by the Folin–Ciocalteu method as previously described (Simirgiotis, Caligari, & Schmeda-Hirschmann, 2009; Simirgiotis, Theoduloz, Caligari, & Schmeda-Hirschmann, 2009). The analyses were carried out using the lyophilized syrups and the Amberlite-retained phenolic enriched fraction of the syrups. All samples and gallic acid were dissolved in 50% (v/v) aqueous methanol. Samples (50 μL) were placed into test tubes and 250 μL Folin–Ciocalteu reagent was added. The mixture was left to stand for 5 min. Then, 750 μL of 20% sodium carbonate (20%, v/v, in water) was added and the solution was taken to a final volume of 5 mL with distilled water. After 30 min of incubation at room temperature (20 °C) the resulting absorbance was measured at 765 nm. The calibration curve was performed with gallic acid (concentrations ranging from 31.3 μg/mL to 500.0 μg/mL) and the results were expressed as mg of gallic acid equivalents per 100 g of dry sample (lyophilized syrup and Amberlite-retained fraction). The total flavonoid (TF) content of the samples (lyophilized syrup and Amberlite-retained fraction) was determined as follows. Briefly, 250 μL of the sample (1 mg of the dry extract per mL) was diluted with 1.25 mL of water. Then 75 μL of 5% $NaNO_2$ solution was added to the mixture. After 5 min, 150 μL of 10% $AlCl_3$–6 H_2O was added and the mixture was allowed to stand for 5 min. Then, 500 μL of 1 M NaOH solution and 275 μL of distilled water were added to make a total of 2.5 mL. The absorbance was measured immediately against the prepared blank at 415 nm. The results were expressed as mg quercetin equivalents per 100 g of sample (Simirgiotis, Theoduloz, et al., 2009). Determinations were carried out in triplicate.

2.5. Scavenging of DPPH radicals

The scavenging of DPPH radicals was assayed as previously reported (Simirgiotis et al., 2009) using the lyophilized Amberlite-retained phenolic enriched fraction of the syrups. All samples were dissolved in 50% (v/v) aqueous methanol to prepare stock solutions of 1 mg/mL. These stock solutions were serially diluted with methanol, mixed with an equal volume of DPPH solution (60 μM) and shaken vigorously. The mixture was incubated at room temperature for 30 min before the absorbance at 517 nm was read. Solutions of quercetin were used as a positive control. The scavenging activity was determined by comparing the absorbance with that of the blank (100%) that contained only DPPH and solvent. Antiradical DPPH bleaching activity is expressed as SC_{50} in μg/mL which denoted the concentration of sample required to scavenge 50% of DPPH free radicals. The lower the SC_{50} value, the higher the antiradical activity because less sample is needed to scavenge the free radical solution. SC_{50} values lower than 50 μg/mL are considered high, while values between 50 and 100 μg/mL are considered moderate. Determinations were performed in triplicate and are presented as mean values ± SEM.

2.6. Extraction of phenolic compounds from the syrups

The syrups (100 g each of samples P1–P3 and AS) were dissolved adding boiling water. The solution was allowed to reach room temperature and then filtered. The water-soluble dark brown solution was passed through an Amberlite XAD-7 column (column length: 150 cm, internal diameter: 6 cm). The column was rinsed with distilled water (2 L) and the adsorbed compounds were eluted with methanol (2 L) to afford the Amberlite-retained fraction. After concentration under reduced pressure, the extracts (methanol solutions) were lyophilized for analysis. The Amberlite-retained fraction was used for HPLC analysis of phenolic constituents as well as for TP, TF content and DPPH bleaching activity. Approximately 5 mg of each extract, obtained as explained above, was dissolved in 1 mL MeOH:H_2O, (1:1 v/v) filtered through a 0.45 μm PTFE filter (Waters) and submitted to HPLC-DAD and HPLC–MS analysis.

2.7. Isolation of the Amberlite-retained constituents

To isolate the main phenolics from the syrups, for spectroscopic analysis, one of the samples of Peru (P1) and the sample from Argentina (AS) were worked-up using chromatographic methods. The Amberlite-retained phenolic enriched fraction was used for the study. The Amberlite-retained fraction of the P1 and AS samples was separately permeated in a Sephadex LH-20 column (column length: 70 cm; i.d.: 2.5 cm), eluting with MeOH:H_2O 9:1 v/v. Fractions of 2 mL each were collected and pooled together according to the TLC patterns (SiO_2, *n*-BuOH:EtOAc:H_2O 3:2:1 v/v/v and revealed with diphenylboric acid ethanolamine complex). For the P1 sample, some 60 fractions were collected and pooled together into 9 groups as follows: 1–23, 24–27, 28–34, 35–39, 40–48, 49–50, 51–52, 53–55, and 56–60. Fractions 28–34 contained compound **1** (13.4 mg) while fractions 40–48 and 53–55 afforded *C*-glycosylflavonoids. The other fractions did not contain compounds of interest. For the AS sample, 50 fractions were collected,