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Gastroprotective effect and chemical characterization of a polysaccharide fraction from leaves of *Croton cajucara*

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ABSTRACT

Croton cajucara Benth. is a tree from the Amazon Forest, where it is known as *sacaca*. Its leaves and barks are used in medicinal preparations to treat different diseases, including gastric ulcers. The crude polysaccharide fraction (CCP), obtained from the hot aqueous extract of *C. cajucara* leaves, was able to promote gastroprotection on an ethanol induced gastric ulcer model. Therefore, a bioguided fractionation was performed to isolate the active polysaccharide fraction. After freezing-thawing, ultrafiltration and dialyses at 100, 50, and 25 kDa cut-off membranes, fraction 25R was obtained. It contained glucose, galactose, rhamnose, arabinose, galacturonic acid and mannose in a 7:5:5:3:1:1 molar ratio approximately, and had a M_w of 42,840 g/mol. Methylation analysis and NMR spectroscopy indicated that 25R is a very complex polysaccharide fraction containing type I rhamnogalacturonan, arabinan, type I arabinogalactan, type II arabinogalactan, rhamnan, starch and mannan. It was able to reduce ethanol-induced gastric ulcers in rats, through preservation of mucus and GSH levels.

1. Introduction

The use of plants for therapeutic purposes has been observed throughout the history of civilizations. Brazil has considerable biodiversity in flora and local populations consume plants as remedy to treat different diseases, especially in regions where people have less access to conventional manufactured medicines.

Peptic ulcer is a gastrointestinal disorder that consists in an excoriated area of the gastric or duodenal mucosa by action of the gastric juice. Natural products including extracts derived from plants have traditionally been used for the prevention and treatment of gastric ulcer [1,2]. Some chemical compounds, isolated or present in crude extracts from plants, have shown antiulcer activity [3]. Several studies have revealed the effectiveness of plant polysaccharides as gastroprotective agents, acting by different mechanisms, which depend on their chemical structure [4–9]. In aqueous extracts like teas, the presence of polysaccharides can be detectable, and thus they can contribute for the biological activities observed for plants.

The plant *Croton cajucara* Benth. is a tree native from the Amazon Forest, where it is popularly known as *sacaca*. Currently, ethnopharmacological information indicates that its leaves and barks are used in folk medicine to treat different health disorders, including gastric ulcers, inflammation, hepatic disorders, diabetes, fever and malaria [10–13]. Its leaves and barks are used to prepare teas by infusion or decoction [14].

Studies with *C. cajucara* barks show the chemical characterization of some terpenes. The main terpene is a clerodane-type-diterpene called *trans*-dehydrocrotonin (*t*-DCTN). Several studies suggest that the anti-inflammatory, antinociceptive, hypoglycemic, antiulcer, antispasmodic, antitumor and antiestrogen activities of the plant are related to the *t*-DCTN [14,15]. In *C. cajucara* leaves, *t*-DCTN was not yet detected [14,16,17]. A clerodane-type diterpene known as cajucarinolide was also extracted from barks of the plant and showed anti-inflammatory activity [18]. Phytochemical studies with apolar extracts of *C. cajucara* leaves revealed the presence of cajucarinolide, steroids – β-sitosterol, stigmasterol, sitosterol-3-*O*-β-glucoside, and flavonoids containing kaempferol as aglycone

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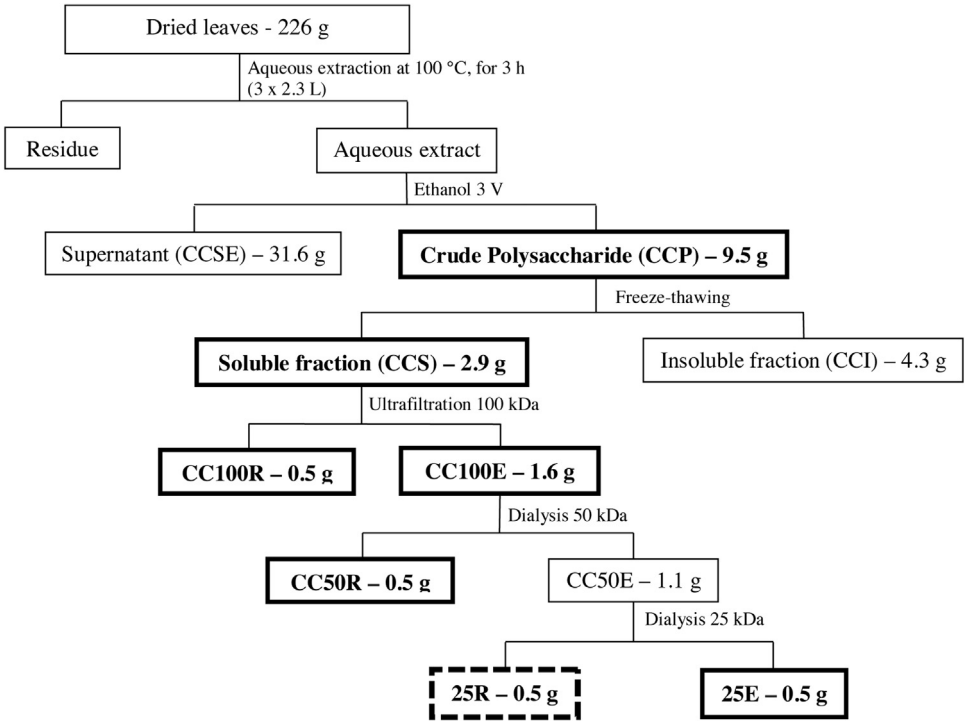

Fig. 1. Scheme of extraction and isolation of 25R fraction of leaves from *Croton cajucara*. The fractions in bold were evaluated for gastroprotective activity.

moiety [14]. The leaves also contain essential oils – linalool and 7-hydroxycalamenene, which showed antimicrobial and antileishmanial activities [19,20].

Considering the popular use of *C. cajucara* leaves teas to treat gastric ulcers and that polysaccharides can act as gastroprotective agents, the aim of this investigation was to verify if polysaccharides could be related with this biological property of the plant. They were obtained from hot aqueous extraction and submitted to a bioguided fractionation using the ethanol-induced gastric ulcer model in rats.

2. Materials and methods

2.1. Plant material

Leaves of *Croton cajucara* Benth. were collected in the Amazon Region (Cruzeiro do Sul, State of Acre, Brazil), in February 2014. The plant was identified by Inês Cordeiro PhD (Botanic Institute of São Paulo, São Paulo, Brazil), and it was compared with the existing voucher SP 319378.

2.2. Extraction and fractionation of the polysaccharides

Dried and milled leaves (226 g) were extracted with water (2.3 L) under conditions of reflux for 3 h (x 3). The aqueous extracts were obtained by filtration, combined, evaporated to a small volume (300 mL), and added to cold EtOH (x 3 vol.). The resulting precipitate was recovered by centrifugation (8000 rpm for 15 min), dissolved in water, dialyzed at a 6–8 kDa cut-off membrane and freeze-dried, to give the crude polysaccharide fraction (9.5 g). This fraction was dissolved in water (950 mL) at room temperature, and was submitted to freeze-thawing until no more precipitate appeared. The soluble portion was submitted to ultrafiltration at a 100 kDa cut-off membrane, yielding a retained and an eluted fraction. The latter was dialyzed at a 50 kDa cut-off membrane and then the eluted portion was dialyzed at a 25 kDa cut-off membrane (Fig. 1).

2.3. Uronic acids analysis

The uronic acid found in CCP and 25R was identified by thin layer chromatography (TLC). Each fraction (1 mg) was hydrolyzed with 1 M TFA (1 mL) at 100 °C for 16 h, the acidic solution was then evaporated, and the residue dissolved in water (1 mL). The resulting monosaccharide mixture was examined by silica-gel 60 TLC (Merck), the plates being developed with *n*-PrOH:H_2O (7:3, v/v) and stained with orcinol-H_2SO_4 at 100 °C [21].

The uronic acid found in CCP and 25R was quantified using the colorimetric *m*-hydroxybiphenyl method [22].

2.4. Carboxyl-reduction and methylation analysis

25R (10 mg) was per-*O*-methylated according to the method of Ciucanu and Kerek, using powdered NaOH in DMSO-MeI [23]. The product was hydrolyzed with 45% aqueous HCO_2H (1 mL) at 100 °C for 20 h. The acidic solution was evaporated, the mixture of partially *O*-methylated monosaccharides was dissolved in water (1 mL) and treated with $NaBD_4$ (2 mg). After 18 h, concentrated HOAc (0.5 mL) was added, the solution evaporated to dryness and the resulting boric acid removed as trimethyl borate by co-evaporation with MeOH. Then, acetylation was carried out with Ac_2O-pyridine (1:1 v/v, 0.6 mL) at room temperature for 18 h, and the resulting *O*-methylated alditol acetates were extracted with $CHCl_3$. These were analyzed by GC–MS (Varian Saturn 2000R–3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225 column (30 m × 0.25 mm i.d.) programmed from 50 to 210 °C at 40 °C/min, with He as carrier gas. They were identified by their typical retention times and electron impact spectra, compared to partially *O*-methylated alditol acetates prepared from standard monosaccharides (Sigma-Aldrich) according to Sassaki et al. (2005) [24]. The results were given as mol%, calculated according to Pettolino et al. (2012) [25].

An aliquot of 25R (20 mg) was carboxyl-reduced by the carbodiimide method, using $NaBH_4$ as the reducing agent [26]. After dialysis at a 6–8 kDa cut-off membrane, the material was freeze-