Carbohydrate Polymers 82 (2010) 714–721

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

An arabinogalactan with anti-ulcer protective effects isolated from *Cereus peruvianus*

Leonardo Yugo Abe Tanaka[a], Arildo José Braz de Oliveira[a], José Eduardo Gonçalves[b], Thales Ricardo Cipriani[c], Lauro Mera de Souza[c], Maria Consuelo Andrade Marques[d], Maria Fernanda de Paula Werner[d], Cristiane Hatsuko Baggio[d], Philip Albert James Gorin[c], Guilherme Lanzi Sassaki[c], Marcello Iacomini[c,*]

[a] *Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá, CEP 87.020-900 Maringá, PR, Brazil*
[b] *Centro Universitário de Maringá, CESUMAR, Maringá, PR, Brazil*
[c] *Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CEP 81.531-980, CP 19046, Curitiba, PR, Brazil*
[d] *Departamento de Farmacologia, Universidade Federal do Paraná, CEP 81.531-980, CP 19031, Curitiba, PR, Brazil*

ARTICLE INFO

Article history:
Received 1 April 2010
Received in revised form 20 May 2010
Accepted 21 May 2010
Available online 27 May 2010

Keywords:
Cereus peruvianus
Cactaceae
Arabinogalactan
Anti-ulcer activity

ABSTRACT

A type I arabinogalactan (CPP) was isolated from the viscous gum exuded by *Cereus peruvinus* (Cactaceae). It contained arabinose, galactose, galacturonic acid, and rhamnose in a 15:66:6:13 molar ratio and had M_w 9×10^5 g mol^{-1}. Methylation analysis and ^{13}C NMR spectroscopy indicated that CPP is composed of a (1 → 4)-linked β-D-Gal*p* main-chain with substituents of α-L-Ara*f* at O-2, O-3 and O-6, which are in turn substituted at O-2, O-3, and O-2,3. These are probably linked to O-4 of some rhamnosyl units of a type I rhamnogalacturonan (*RG1*). The main chain is formed by repeating (1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p* groups. The methylation analysis suggested the presence of (1 → 3)-linked β-Gal*p* structures, perhaps linked to the (1 → 4)-linked β-D-Gal*p* backbone. CPP significantly inhibited ethanol-induced gastric lesions in rats at an ED_{50} of 49 mg kg^{-1}, indicating that it has a gastroprotective effect. The gastric lesion inhibition by CPP suggests a potential use of this polysaccharide, or the crude plant extract, in phytotherapy.

1. Introduction

Arabinogalactans (AG) are essential structural polysaccharides in the cell wall of all higher plants (Fincher, Stone, & Clarke, 1983). They are also found as a main component in many gums and exudates (Delgobo, Gorin, Jones, & Iacomini, 1998; Delgobo, Gorin, Tischer, & Iacomini, 1999; Fincher et al., 1983; Menestrina, Iacomini, Jones, & Gorin, 1998), affording highly viscous solutions with a wide industrial use (Dong & Fang, 2001; Duan, Wang, Dong, Fang, & Li, 2003). Their chemical structures are very complex (Aspinall, 1982; Whistler, 1970) and members of this class of polysaccharides may exist as a pectin component (Carpita & Gibeaut, 1993) or linked to proteins (Fincher et al., 1983; Varner & Lin, 1989). The AG found in pectin fractions are type I arabinogalactans (*AG1*), which consist of a (1 → 4)-linked *β*-Gal*p* main-chain, with α-L-Ara*f* substituents at O-3, and can be linked to RG1 formed by repeating (1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p* groups (Carpita & Gibeaut, 1993).

Arabinogalactans have been frequently reported to be immunologically active (Mellinger et al., 2005; Wagner & Jordan, 1988) and also to act as an anti-ulcer protective agent (Cipriani, Mellinger, Gorin, & Iacomini, 2004; Cipriani et al., 2009; Nergard et al., 2005).

Cereus peruvianus Mill., a cactus species, provides various compounds of economic, pharmacological, and industrial interest. The viscous gum can be directly collected and has potential industrial applications, such as flocculation of impurities in drinking water (Nozaki, Messerschmidt, & Rodrigues, 1993), pollutant abatement from pulp and paper industry effluents (Barros & Nozaki, 2002), and cosmetic manufacture (Alvarez et al., 1992). Pectic acid can be used to prepare jam, jelly, gum, yogurt, and other products (Alvarez, Costa, Huber, Baron, & Fontana, 1995). The major gum fraction of *C. peruvianus* is an arabinogalactan (Alvarez et al., 1995), but fine details of its structure were not determined.

We now report the isolation, characterization, and gastric lesion inhibition of a type I arabinogalactan of *C. peruvianus* (CPP), since

* Corresponding author. Tel.: +55 41 3361 1655; fax: +55 41 3266 2042.
E-mail address: iacomini@ufpr.br (M. Iacomini).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.05.043

arabinogalactans have been reported to have protective anti-ulcer activity.

2. Materials and methods

2.1. Plant material

Stems of adult plants of *C. peruvianus* were used for extraction of polysaccharide, being collected from those maintained on the campus of the State University of Maringá, State of Paraná, Brazil).

2.2. General analytical methods

All solutions were evaporated at <40 °C under reduced pressure. Centrifugation was carried out at 8000 rpm for 25 min at 15 °C, and dialyses performed using a 12–14 kDa cut-off membrane.

2.3. Isolation, extraction, and purification of polysaccharide

Fresh stems of *C. peruvianus* (100 g; 10 g% of dry matter) were added to tap water (1 L) and mixed in a blender. The resulting viscous solution was filtered, the filtrate added to EtOH (×3 vol.), and the resulting precipitate was washed with acetone to provide a pigment-free powder. This was dissolved in water and freeze-dried to give polysaccharide (2.7 g), which was dissolved in hot water (200 mL) over 30 min. Filtration and centrifugation provided a viscous supernatant, which was treated with 20% aqueous trichloroacetic acid (TCA; 200 mL) to precipitate protein. The resulting solution was neutralized with aq. NaOH, dialyzed, and freeze-dried. The product (0.5 g) was dissolved in water (50 mL), which was submitted to freeze-thawing until no more precipitate appeared. The soluble component yielded a purified polysaccharide (CPP, 0.10 g). This process was repeated until the necessary quantities of CPP for future experiments were obtained.

2.4. Homogeneity and molecular weight determination

The homogeneity and average molar mass (M_w) of fractions were determined by high-performance size-exclusion chromatography (HPSEC) with Wyatt Technology equipment coupled to a refractive index detector Waters model 2410 and a multi-angle laser light scattering detector (MALLS) detector at 632.8 nm, using model Dawn DSP. Incorporated were four gel permeation ultrahydrogel columns in series, with exclusion sizes of 7×10^6, 4×10^5, 8×10^4, and 5×10^3 Da. Elution was carried out with 0.1 M aq. $NaNO_2$ containing 200 ppm aq. NaN_3 at 0.6 mL min^{-1}. The samples, previously filtered through a membrane (0.22 μm), were injected (250 μL loop) at a concentration of 1 mg mL^{-1}. The specific refractive index increments (dn/dc) were determined and the results were processed with software provided by the manufacturer (Wyatt Technologies).

2.5. Monosaccharide and uronic acid analyses

CPP (2 mg) was hydrolyzed in 2 M trifluoroacetic acid (TFA; 1 mL) at 100 °C for 8 h, the solution was evaporated, the residue then dissolved in water (1 mL) and further evaporated. The resulting monosaccharide and uronic acids were examined by silica-gel 60 thin layer chromatography (TLC; Merck), the plates being developed with ethyl acetate:*n*-propanol:acetic acid:water (4:2:2:1, v/v) and stained with orcinol-sulfuric acid (Sassaki, Souza, Cipriani, & Iacomini, 2008). The remaining hydrolyzate was also treated with $NaBH_4$ (2 mg), and after 18 h, HOAc was added, the solution evaporated to dryness, and the resulting boric acid removed as trimethyl borate by co-evaporation with MeOH. Acetylation was carried out with Ac_2O-pyridine (1:1, v/v; 2 mL) at room temperature for 12 h, and the resulting alditol acetates (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005). They were analyzed by GC–MS using a Varian Saturn 2000R-3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer). The column was DB-225 (30 m × 0.25 mm i.d.) programmed from 50 to 220 °C at 40 °C/min, with helium as carrier at a constant flow rate of 1 mL/min. The inlet temperature was kept constant at 250 °C, and the MS transfer line was set at 250 °C. MS acquisition parameters included scanning from m/z 50–550 in the electron impact (EI) mode. Components were identified by their typical retention times and electron ionization spectra.

Uronic acid content was determined by total conversion to their corresponding neutral monosaccharide by exhaustive carboxy-reduction of CPP, monitored by TLC analysis. CPP (10 mg) was carboxy-reduced by the carbodiimide method (Cipriani et al., 2009), using $NaBH_4$ as reducing agent. Carboxy-reduced material (CCP-CR) was submitted to successive hydrolysis, reduction, and acetylation and analyzed by GC–MS, as described above. The uronic acid content was calculated from the difference of corresponding neutral monosaccharide in CPP-CR and CPP, obtained by GC–MS analyses.

2.6. Methylation analysis

CPP and CPP-CR (5 mg) were each methylated according to the method of Ciucanu and Kerek (1984), using powdered NaOH in DMSO-MeI. The per-*O*-methylated derivative was hydrolyzed with 50% (v/v) aq. H_2SO_4 (0.5 mL) at 0 °C for 1 h, which was then diluted to 5.5% (v/v) and maintained at 100 °C for 17 h. Each resulting mixture of *O*-methyl aldoses was neutralized ($BaCO_3$), filtered, reduced ($NaBD_4$), and acetylated, as described above, to give a mixture of partially *O*-methylated alditol acetates. These were analyzed by GC–MS, under conditions identical to those described for alditol acetates, except that the final temperature was 215 °C. They were identified by their typical retention times and electron ionization spectra (Sassaki et al., 2005).

2.7. NMR spectroscopy

^1H and ^{13}C NMR spectra were obtained from samples in D_2O at 70 °C using a 400 MHz Bruker model DRX Avance spectrometer, incorporating a 5 mm inverse probe. Chemical shifts (δ) are expressed in ppm relative to acetone, at δ 30.2. Two-dimensional spectra (COSY and HMQC) were recorded using standard Bruker procedures (Cui, Eskin, Biliaderis, & Marat, 1996).

2.8. Partial acid hydrolysis

CPP (200 mg) was partially hydrolyzed with 0.2 M TFA (50 mL) at 100 °C for 4 h, which was evaporated to dryness and the residue dissolved in water (1.0 mL). Hydrolysis-resistant polysaccharide (CPP-HR, 42.3 mg, 21.1% yield) was precipitated with acetone (×3 vol.), centrifuged, and dried.

CPP-HR (5 mg) was also partially hydrolyzed, but under stronger conditions, with 0.5 M TFA (5.0 mL) at 100 °C, but for 2 h. The hydrolyzate was evaporated to dryness and the residue dissolved in water and freeze-dried to remove traces of TFA, yielding CPP-HR2. It was submitted to offline ESI-MS analysis for characterization of oligosaccharides containing uronic acid.

2.9. Electrospray ionization mass spectrometric analysis

The freeze-dried fractions obtained from hydrolysis were dissolved in 500 μL of 1:1 methanol–water solution to give a

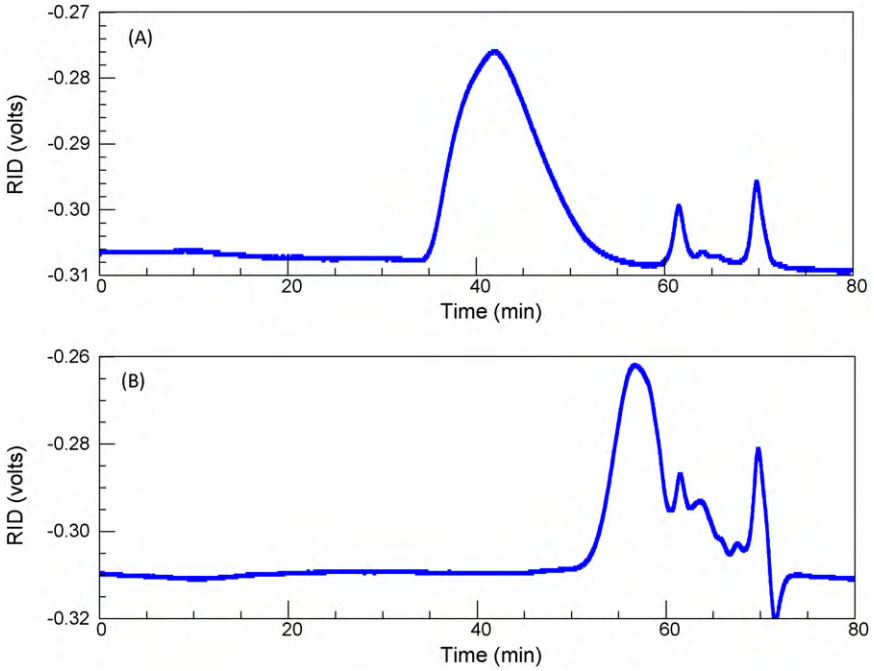

Fig. 1. Elution profiles obtained on HPSEC analyses of purified polysaccharide (CPP) from *C. peruvianus* (A) and hydrolysis-resistant polysaccharide CPP-HR (B).

concentration of 0.25 mg mL^{-1}. Samples were introduced into the mass spectrometer using a syringe pump, in offline ESI-MS analysis. ESI-MS^1 and MS^2 spectra were obtained in the negative ionization mode, using a triple quadrupole Quattro LC (Waters), setting the capillary voltage at 2300 V, cone voltage at 60 V and source at 100 °C. Each spectrum was produced by accumulating data over 1 min. MS^2 spectra of $[M-H]^-$ ions were obtained by collision induced dissociation (CID), using argon as collision gas with a variation of collision energy between 20 and 40 eV.

2.10. Animals

Female Wistar rats (180–200 g) from the Federal University of Paraná colony were maintained under standard laboratory conditions (12 h light/dark cycle, temperature 22 ± 2 °C). Standard pellet food (Nuvital®, Curitiba/PR, Brazil) and water were available *ad libitum*. The animals were deprived of food 24 h prior to the experiment. The experimental protocol for animals was performed according to the "Principles of Laboratory Animal Care" (NIH Publication 85-23, revised 1985) adopted by the Federal University of Paraná.

2.11. Induction of acute gastric lesions in rats

Rats were orally treated with vehicle (water, 0.1 mL 100^{-1} g of body weight), omeprazole (40 mg kg^{-1}) or CPP (10, 30, 100 mg kg^{-1}) 1 h before intragastric administration of pure ethanol (0.5 mL 200 g^{-1} of body weight). The animals were killed 1 h after ethanol administration (Robert, Nezamis, Lancaster, & Hauchar, 1979). Their stomachs were removed and the area of ulceration (mm^2) was measured by planimetry using an Image Tool 3.0 program.

2.12. Statistical analysis of gastric lesion rate

Data are expressed as mean ± SEM. The statistical significance of the results was determined using a one-way analysis of variance followed by the Bonferroni's test. Data were considered different at a significance level of $p < 0.05$. The effective dose 50 (ED_{50}) value was determined by nonlinear regression using nonlinear regression Graph-Pad software (GraphPad software, San Diego, CA, USA).

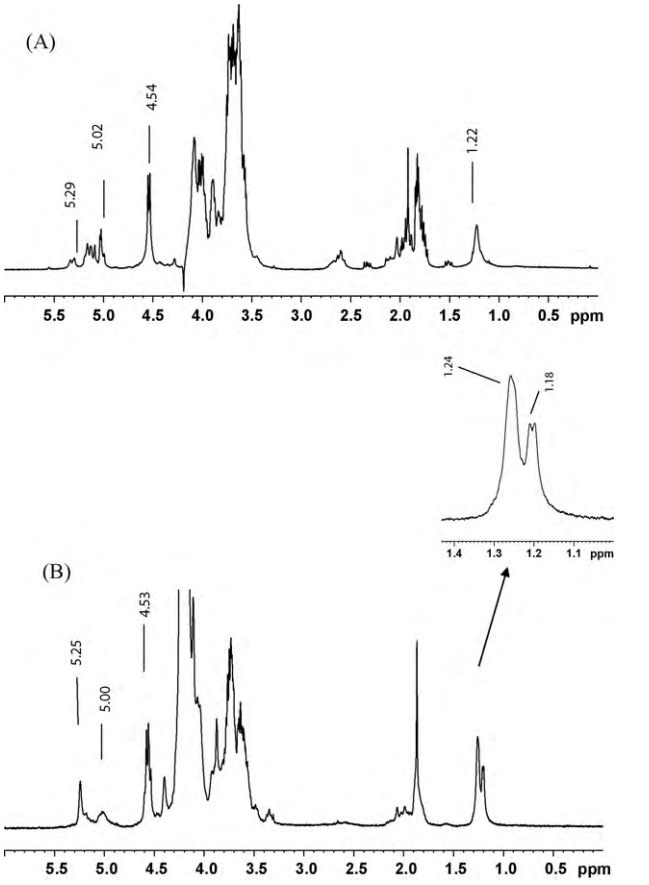

Fig. 2. ^{1}H NMR spectra of purified polysaccharide (CPP) from *C. peruvianus* (A) and hydrolysis-resistant polysaccharide (CPP-HR) (B). Solvent D_2O at 70 °C; numerical values are in δ ppm.

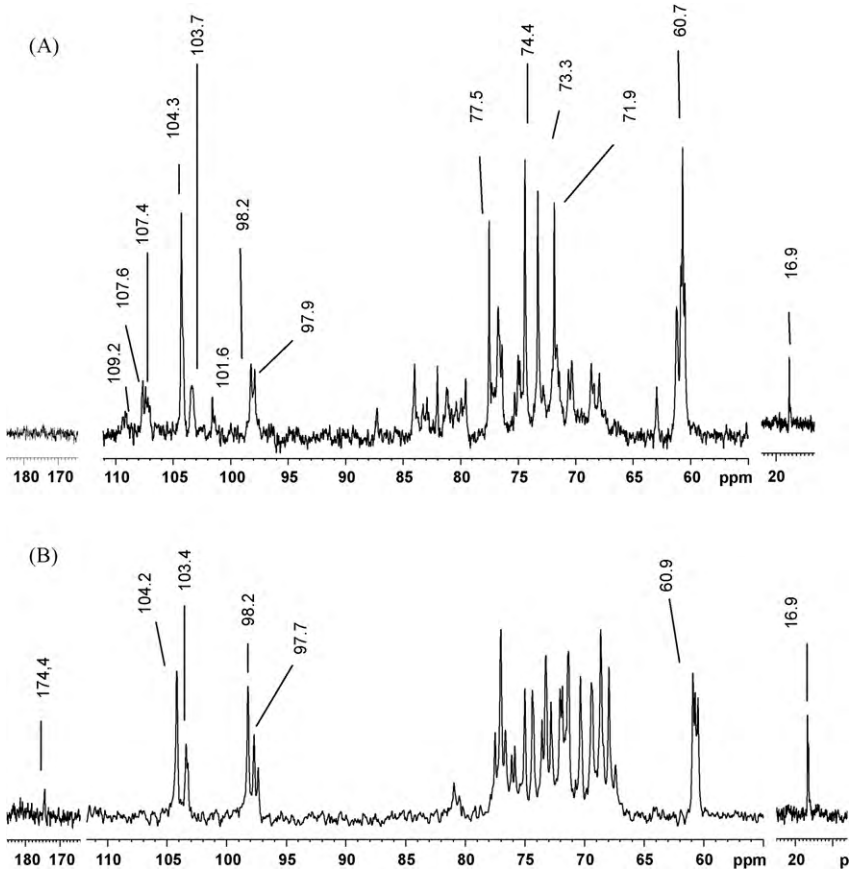

Fig. 3. ^{13}C NMR spectra of purified polysaccharide (CPP) from *C. peruvianus* (A) and hydrolysis-resistant polysaccharide CPP-HR (B). Solvent D_2O at 70 °C; numerical values are in δ ppm.

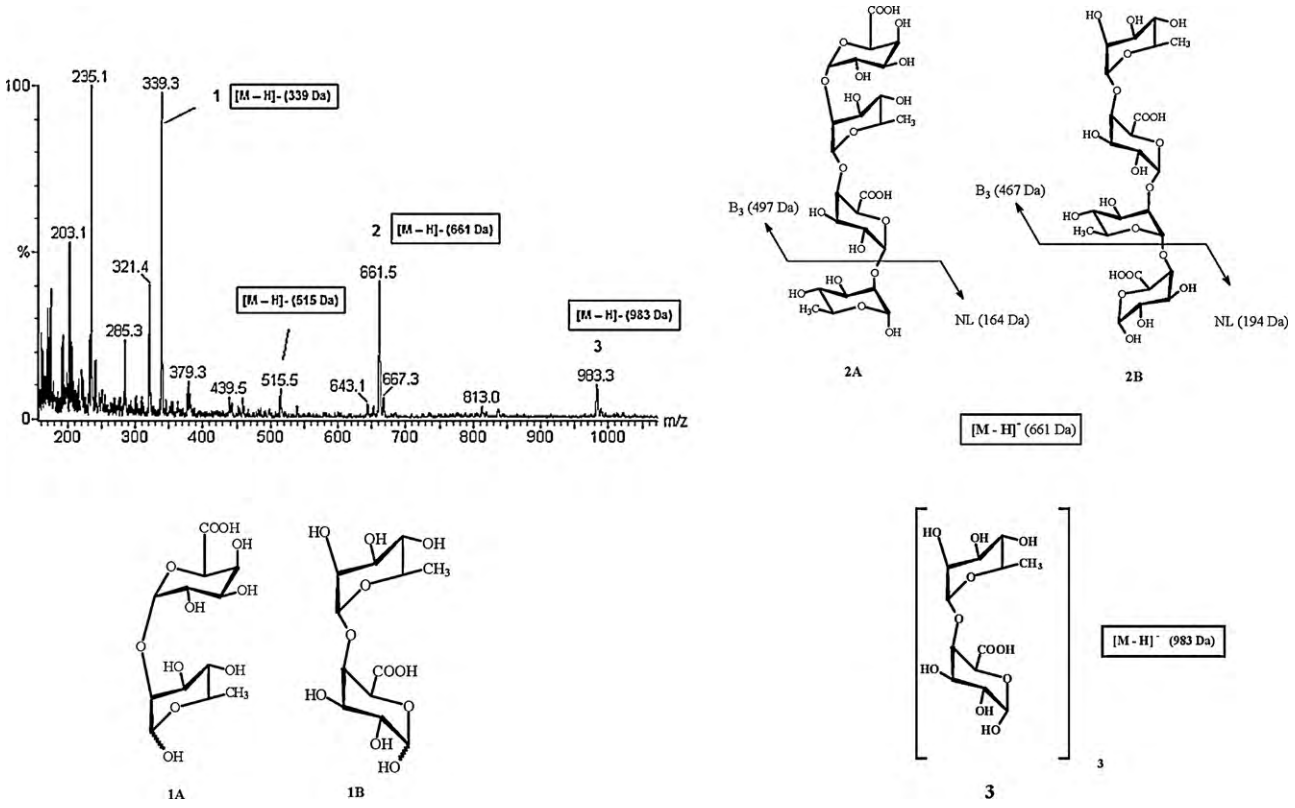

Fig. 4. Negative ESI-MS spectra of oligosaccharides present in fraction enriched with oligosaccharides containing a (1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p* group (CCP-HR2).

3. Results and discussion

3.1. Structural characterization of the arabinogalactan

Cereus peruvianus stems were extracted with water and the extract was treated with excess EtOH to obtain a precipitate of a polysaccharide-protein mixture (2.7%). This was freed from protein and submitted to a freezing-thawing process to give soluble polysaccharide (CPP, 0.1%). HPSEC showed it to be homogeneous, with M_w 9×10^5 g mol^{-1} and dn/dc 0.216 (Fig. 1A). It contained arabinose, galactose, galacturonic acid and rhamnose in a 15:66:6:13 molar ratio.

The ^{1}H NMR spectrum of CPP (Fig. 2A) contained signals at δ 5.0–5.4 and 4.5–4.6, from anomeric protons of α-arabinofuranose and β-galactopyranose residues. H-1 signals of α-rhamnopyranose and α-galacturonic acid were at δ 5.29 and 5.08/4.95, respectively. That at δ 1.22 ppm was from H-6 of CH_3 groups of the rhamnopyranosyl units (Cui et al., 1996; Habibi, Mahrouz, & Vignon, 2005).

The ^{13}C NMR spectrum of CPP (Fig. 3A) contained six main signals at δ 104.3 (C-1), 77.5 (C-4), 74.4 (C-5), 73.3 (C-3), 71.9 (C-2) and 60.7 (C-6) typical of a (1 → 4)-linked β-Gal*p* main-chain of an arabinogalactan (Cipriani et al., 2004, 2009). Although CPP contained 6% galacturonic acid, a signal at δ 174.0–175.0 for carboxyl groups did not appear, due to spectral conditions. The presence of CH_3-6 of Rha*p* units was shown by a signal at δ 16.9 (Gorin & Mazurek, 1975). Signals of C-1 of α-L-Ara*f* units were present at δ 109.2, 107.6, and 107.4.

Partial acid hydrolysis of CPP (0.2 M TFA at 100 °C for 4 h) was carried out to elucidate aspects of its core structure. From 200 mg of CPP was obtained 42.3 mg of a hydrolysis-resistant polysaccharide (CPP-HR), whose reduction of M_w was shown by HPSEC (Fig. 1B). It contained arabinose, galactose, galacturonic acid and rhamnose in a 1:24:46:29 molar ratio.

The ^{1}H and ^{13}C NMR spectra of CPP-HR (Figs. 2B and 3B) provided additional information on the CPP structure. In the proton spectrum (Fig. 2B), the signals of α-arabinofuranose were absent and those of β-galactopyranose became smaller.

Compared with these components, α-galacturonic acid and α-rhamnopyranose residues were largely retained, their H-1 signals appearing at δ 5.00 and 5.25, respectively, and signals arising from H-6 of CH_3 of rhamnopyranosyl units at δ 1.18 and 1.24 (Fig. 2B). These generally appeared as two partially superimposed doublets, due to the presence of two different rhamnose structures, which were respectively those linked to O-2 and others linked to O-2 and O-4 (Cui et al., 1996; Habibi et al., 2005).

The ^{13}C NMR spectrum of CPP-HR (Fig. 3B) contained signals compatible with a rhamnogalacturonan structure (RG1), with C-1 signals at δ 98.2 and 97.7 from α-L-Rha*p* and α-D-Gal*p*A units, respectively (Renard, Lahaye, Mutter, Voragen, & Thibault, 1997). A signal of the carboxyl groups of α-D-Gal*p*A units was present at δ 174.4. It was also possible to assign signals of C-1 of β-Gal*p* units at δ 103.4 and 104.2 (Cipriani et al., 2004), as well as the disappearance of those of α-Ara*f* units.

Methylation analysis of CPP showed that it contained non-reducing end-units of Ara*f* and Gal*p*, due to the formation of

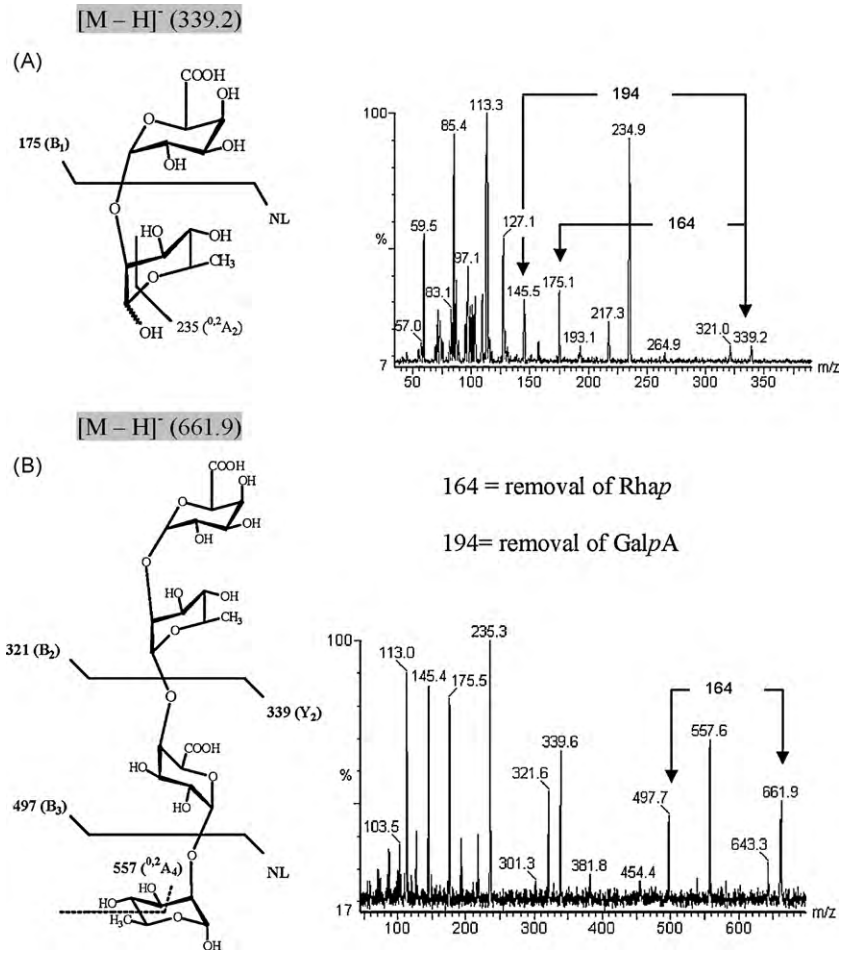

Fig. 5. Structure and spectra of MS^2 fragmentation by ESI in negative mode of ions at: (A) MS^2 spectrum of ion with *m*/*z* 339 ([M−H]$^-$) and (B) MS^2 spectrum of ion with *m*/*z* 661 ([M−H]$^-$).

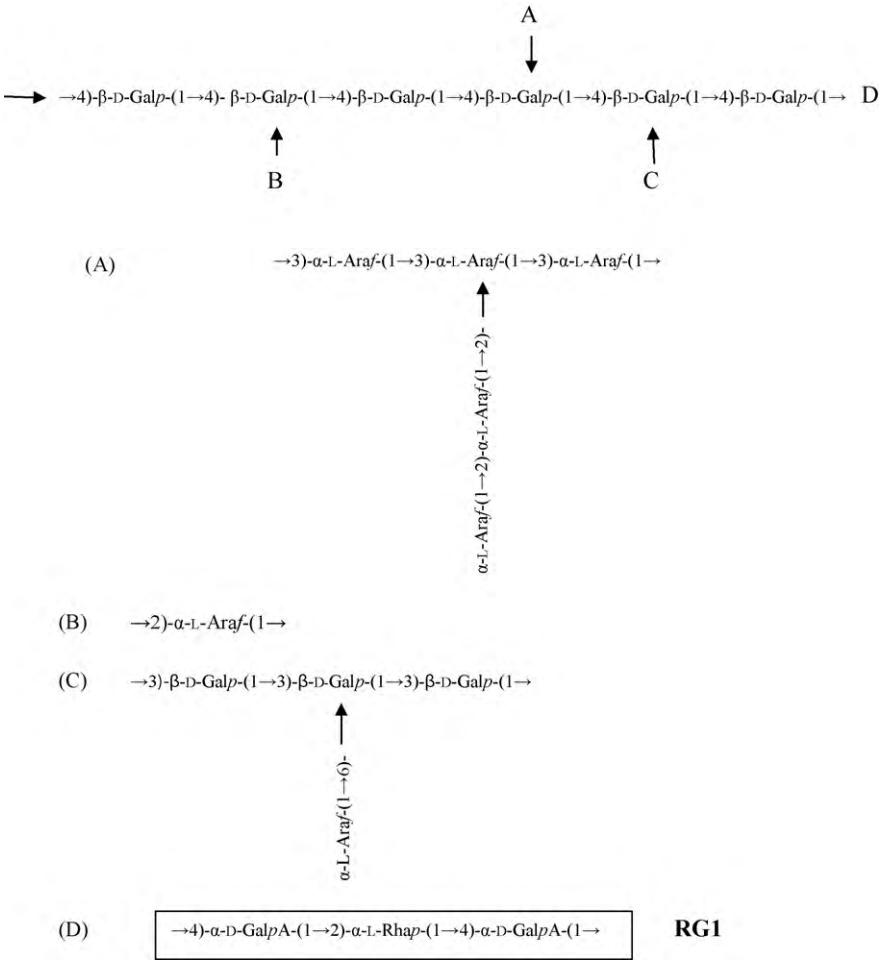

Fig. 6. Structure of purified polysaccharide (CPP) from *C. peruvianus*.

alditol acetates of 2,3,5-Me_3-Ara (7%) and 2,3,4,6-Me_4-Gal (12%), respectively (Table 1). The arabinofuranosyl units were substituted at O-2, O-3 and O-2,3, in accord with formation of alditol acetates of 3,5-Me_2-Ara (2%), 2,5-Me_2-Ara (4%) and 5-Me-Ara (2%), respectively.

The galactopyranosyl units were 3-*O*-, 4-*O*-, 2,4-di-*O*-, 3,4-di-*O*-, 3,6-di-*O*- and 4,6-di-*O*-substituted, since alditol acetates of 2,4,6-Me_3-Gal (7%), 2,3,6-Me_3-Gal (32%), 3,6-Me_2-Gal (5%), 2,6-Me_2-Gal (4%), 2,4-Me_2-Gal (2%), and 2,3-Me_2-Gal (4%) were formed. The rhamnosyl units were 2,4-di-*O*-substituted and 4-*O*-substituted, as demonstrated by the presence of alditol acetates of 3-Me-Rha (10%) and 2,3-Me_2-Rha (3%). Methylation analysis on carboxy-reduced CPP (CPP-CR) showed an increase in the 2,3,6-Me_3-Gal derivative, indicating that 4-*O*-substituted galacturonic acid residues were present. These, plus 4-*O*- and 2,4-di-*O*-substituted Rha*p* residues, strongly suggest a type I rhamnogalacturonan (RG1) structure. These are formed by a repeating (1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p* group, often having O-4 of the rhamnosyl units substituted by an arabinogalactan sequence (Carpita & Gibeaut, 1993).

Table 1
Profile of partially *O*-methylated alditol acetates and glycosidic linkages, obtained by methylation analysis of CPP[a].

O-Me-alditol acetate from CPP	Linkage	Mol %[b]
2,3,5-Me_3-Ara	Terminal	7
5-Me-Ara	→)-2,3-	2
3,5-Me_2-Ara	→)-2-	2
2,3-Me_2-Rha	→)-4-	3
2,5-Me_2-Ara	→)-3-	4
2,3,4,6-Me_4-Gal	Terminal	12
3-Me-Rha	→)-2,4-	10
2,4,6-Me_3-Gal	→)-3-	7
2,3,6-Me_3-Gal	→)-4-	32
2,6-Me_2-Gal	→)-3,4-	4
3,6-Me_2-Gal	→)-2,4-	5
2,3-Me_2-Gal	→)-4,6-	4
2,4-Me_2-Gal	→)-3,6-	2

[a] The uronic acid content was 6%.
[b] The percentage total is 94% because we consider the uronic acid content.

To confirm the presence of the RG1, CPP-HR was partially hydrolyzed to give CPP-HR2, and its offline ESI-MS spectra (negative ion mode), were performed (Fig. 4). The MS^1 spectrum showed all oligosaccharide molecules as the deprotonated $[M-H]^-$ ions. The predominant ions in the mass spectrum suggest the presence of α-D-Gal*p*A-(1 → 2)-L-Rha*p* and α-L-Rhap-(1 → 4)-D-Gal*p*A at *m*/*z* 339 $[M-H]^-$, α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A at *m*/*z* 515 $[M-H]^-$, $[\alpha\text{-D-Gal}pA\text{-}(1\rightarrow2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow4)]_2$ at *m*/*z* 661 $[M-H]^-$, and $[\alpha\text{-D-Gal}pA\text{-}(1\rightarrow2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow4)]_3$ at *m*/*z* 983 $[M-H]^-$. This result indicates the presence of branches of RG1 arising from arabinogalactan (CPP).

Negative ESI-MS^2 provided a sensitive means for structural analysis of oligosaccharides (Fig. 5). The fragment ions observed in the MS^2 spectra were named following an adaptation of the nomenclature by Domon and Costello (1988). The fragmentation of oligosaccharides in the negative-ion ESI-MS^2 condition involves cleavage of the glycosidic linkage between two monosaccharides, and the fragmentation across the glycosidic bond leading to mainly

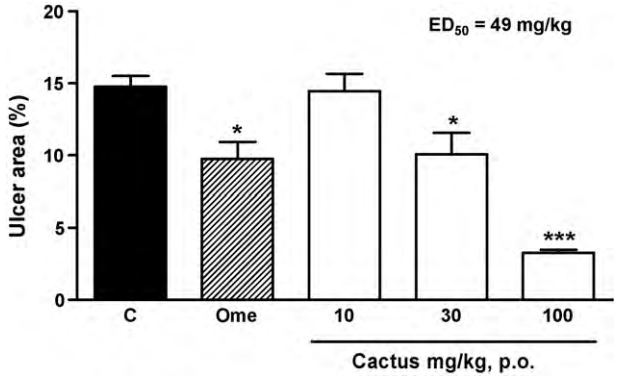

Fig. 7. Protective effect of purified polysaccharide (CPP) from *C. peruvianus*, tested with doses of 10, 30, and 100 mg kg^{-1}, p.o., against ethanol-induced gastric lesions (C: control, water 0.1 mL 100 g^{-1}, p.o. and Ome: omeprazole 40 mg kg^{-1}, p.o.). The results are expressed as mean ± SEM ($n = 6$). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni test. *$p < 0.05$ and *** $p < 0.001$, when compared to control group.

Y and Z-type ions starting from the reducing end, and B and C-type ions counting from the non-reducing end (Körner, Limberg, Christensen, Mikkelsen, & Roepstorff, 1999; Quéméner, Desire, Lahaye, Debrauwer, & Negroni, 2003). The fragmentation processes began with dehydration of precursor ions, resulting in the loss of 18 mass units. For example, that at *m*/*z* 643 corresponded to the loss of 18 mass units form the *m*/*z* ion with 661 (Fig. 5B; Ducasse, Williams, Meudec, Cheynier, & Doco, 2010).

Based on previous results (Souza et al., 2008, 2009), we find that fragmentation on our ESI-MS leads to B and Y-type ions, the B fragment having a double bond between C-1 and C-2. The ion at *m*/*z* 339 gave rise to many fragments, probably due to ring cleavage leading to formation of those of A-type (Fig. 5A). Thus, the MS^2 fragmentations allow us to postulate additional details on oligosaccharide structures, indicating that the Rha unit appears as a reducing end. This could be observed by formation of a fragment at *m*/*z* 235, consistent with the $^{0,2}A_2$-type, and the ion at *m*/*z* 175 (B_1), consistent with the Gal*p*A residue (Fig. 5A). A similar behavior was also observed with the precursor ion at *m*/*z* 661, which provided a fragment ions at *m*/*z* 557 ($^{0,2}A_4$) and *m*/*z* 497 (B_3), as shown in Fig. 5B. The latter had a neutral loss (NL) of 164 a.m.u., consistent with removal of a Rha unit (Figs. 4 and 5B), confirming that the ion at *m*/*z* 661 was from α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-L-Rha*p* (2A, Fig. 4). The absence of an ion at *m*/*z* 467 (B_3), which would be formed by removal of Gal*p*A (NL) from the reducing end, indicates that the sequence α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A was not present (2B, Fig. 4). The ion from hexasaccharide (*m*/*z* 983) was also fragmented, but gave rise to poor daughter ions.

CPP is therefore a type I arabinogalactan (AG1) containing a (1 → 4)-linked β-Gal*p* main-chain, with substituents of arabinosyl units mainly at O-3, O-2 and O-6, as described by Aspinall, Begbie, Hamilton, and Whyte (1967). Moreover, (1 → 3)-linked β-Gal*p* sequences seem to be present in our structure. The arabinosyl units are in turn substituted at O-2, O-3 and O-2,3. This arabinogalactan is probably linked to an RG1 through O-4 of some of the rhamnosyl units. A structure proposed for CPP is shown in Fig. 6.

The present investigation was on the structural characterization of a polysaccharide belonging to a class of polymers whose anti-ulcer activity has been suggested. This activity was determined for the CPP arabinogalactan, oral treatment with 10, 30, and 100 mg kg^{-1} being tested using an *in vivo* model. It significantly reduced the area of gastric lesions induced by EtOH with ED_{50} at 49.0 mg kg^{-1}. Omeprazole (40 mg kg^{-1}, p.o.), the positive control for the test, inhibited the gastric lesions by 35 ± 8% (Fig. 7).

The inhibition of the gastric lesion by CPP suggests its potential use or of its crude plant extract as a phytotherapic medicine in a similar manner to that of another Cactaceae plant *Opuntia ficus-indica*. This has been employed as an Italian folk medicine as an anti-ulcer remedy, having shown a gastroprotector effect (Galati, Mendello, Giuffrida, & Miceli, 2001).

Acknowledgments

The authors would like to thank the Brazilian funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Araucária PRONEX-Carboidratos for their support and Lígia Moura Burci (MSc student) for their help with animal experiments.

References

Alvarez, M., Costa, S. C., Utumi, H., Huber, A., Beck, R., & Fontana, J. D. (1992). The anionic glycan from the cactus *Cereus peruvianus*—structural features and potential uses. *Applied Biochemistry and Biotechnology*, *34*, 283–295.

Alvarez, M., Costa, S. C., Huber, A., Baron, M., & Fontana, J. D. (1995). The cuticle of the cactus *Cereus peruvianus* as a source of a homo-D-galacturonan. *Applied Biochemistry and Biotechnology*, *51/52*, 367–377.

Aspinall, G. O. (1982). *The polysaccharides* New York: Academic Press., 503 pp.

Aspinall, G. O., Begbie, R., Hamilton, A., & Whyte, J. N. C. (1967). Polysaccharides of soy-beans. Part III. Extraction and fractionation of polysaccharides from cotyledon meal. *Journal of the Chemical Society*, *5*, 1065–1070.

Barros, M. J., & Nozaki, J. (2002). Pollutants abatement from effluents of paper and pulp industries by flocculation/coagulation and photochemical degradation. *Química Nova*, *25*, 736–740.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, *3*, 1–30.

Cipriani, T. R., Mellinger, C. G., Gorin, P. A. J., & Iacomini, M. (2004). An arabinogalactan isolated from the medicinal plant *Maytenus ilicifolia*. *Journal of Natural Products*, *67*, 703–706.

Cipriani, T. R., Mellinger, C. G., Bertolini, M. L. C., Baggio, C. H., Freitas, C. S., Marques, M. C. A., et al. (2009). Gastroprotective effect of a type I arabinogalactan from soybean meal. *Food Chemistry*, *115*, 687–690.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Cui, W. W., Eskin, M. N. A., Biliaderis, C. G., & Marat, K. (1996). NMR characterization of a 4-O-methyl-β-D-glucuronic acid-containing rhamnogalacturonan from yellow mustard (*Sinapis alba* L.) mucilage. *Carbohydrate Research*, *292*, 173–183.

Delgobo, C. L., Gorin, P. A. J., Jones, C., & Iacomini, M. (1998). Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry*, *47*, 1207–1214.

Delgobo, C. L., Gorin, P. A. J., Tischer, C. A., & Iacomini, M. (1999). *Carbohydrate Research*, *99*, 41–50.

Domon, B., & Costello, C. E. (1988). A systematic nomenclature for carbohydrate fragmentation in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate Journal*, *5*, 397–409.

Dong, Q., & Fang, J. (2001). Structural elucidation of a new arabinogalactan from the leaves of *Nerium indicum*. *Carbohydrate Research*, *332*, 109–114.

Duan, J., Wang, X., Dong, Q., Fang, J., & Li, X. (2003). Structural features of a pectic arabinogalactan with immunological activity from the leaves of *Diospyros kaki*. *Carbohydrate Research*, *338*, 1291–1297.

Ducasse, M. A., Williams, P., Meudec, E., Cheynier, V., & Doco, T. (2010). Isolation of Carignan and Merlot red wine oligosaccharides and their characterization by ESI-MS. *Carbohydrate Polymers*, *79*, 747–754.

Fincher, G. B., Stone, B. A., & Clarke, A. E. (1983). Arabinogalactan-proteins: Structure, biosynthesis, and function. *Annual Review of Plant Physiology*, *34*, 47–70.

Galati, E. M., Mendello, M. R., Giuffrida, D., & Miceli, N. (2001). Anticulcer activity of *Opuntia ficus-indica* (L.) Mill. (Cactaceae): Ultra structural study. *Journal of Ethnopharmacology*, *76*, 1–9.

Gorin, P. A. J., & Mazurek, M. (1975). Further studies on the assignment of signals in C-13 magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry*, *53*, 1212–1222.

Habibi, Y., Mahrouz, M., & Vignon, M. R. (2005). Isolation and structural characterization of protopectin from the skin of *Opuntia ficus-indica* prickly pear fruits. *Carbohydrate Polymers*, *60*, 205–213.

Körner, R., Limberg, G., Christensen, T. M. I. E., Mikkelsen, J. D., & Roepstorff, P. (1999). Sequencing of partially methyl-esterified oligogalacturonates by tandem mass spectrometry and its use to determine pectinase specificities. *Analytical Chemistry*, *71*, 1421–1427.

Mellinger, C. G., Carbonero, E. R., Noleto, G. R., Cipriani, T. R., Oliveira, M. B. M., Gorin, P. A. J., et al. (2005). Xylans from the medicinal herb *Phyllanthus niruri*. *Journal of Natural Products*, *68*, 1479–1483.

Menestrina, J. M., Iacomini, M., Jones, C., & Gorin, P. A. J. (1998). Similarity of monosaccharide, oligosaccharide and polysaccharide structures in gum exudate of *Anacardium occidentale*. *Phytochemistry*, *47*, 715–721.

Nergard, C. S., Diallo, D., Inngjerdingen, K., Michaelsen, T. E., Matsumoto, T., & Kiyohara, H. (2005). Medicinal use of *Cochlospermum tinctorium* in Mali: Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, *96*, 255–269.

Nozaki, J, Messerschmidt, I., & Rodrigues, D. G. (1993). Tannery waters cleaning with natural polyelectrolytes: Chemical speciation studies of chromium. *Arquivos de Biologia e Tecnologia*, *36*, 761–770.

Quéméner, B., Desire, C., Lahaye, M., Debrauwer, L., & Negroni, L. (2003). Structural characterization by both positive- and negative-ion electrospray mass spectrometry of partially methyl-esterified oligogalacturonides purified by semi-preparative high-performance anion-exchange chromatography. *European Journal of Mass Spectrometry*, *9*, 45–60.

Renard, C. M. G. C., Lahaye, M., Mutter, M., Voragen, F. G. J., & Thibault, J. F. (1997). Isolation and characterization of rhamnogalactan oligomers generated by controlled acid hydrolysis of sugar-beet pulp. *Carbohydrate Research*, *305*, 271–280.

Robert, A., Nezamis, J. E., Lancaster, C., & Hauchar, A. J. (1979). Cytoprotection by prostaglandins in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. *Gastroenterology*, *77*, 433–443.

Sassaki, G. L., Gorin, P. A. J., Souza, L. M., Czelusniak, P. A., & Iacomini, M. (2005). Rapid synthesis of partially O-methylated alditol acetate standards for GC–MS: some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research*, *340*, 731–739.

Sassaki, G. L., Souza, L. M., Cipriani, T. R., & Iacomini, M. (2008). TLC of carbohydrates. In M. Waksmundzka-Hajnos, J. Sherma, & T. Kowalska (Eds.), *Thin layer chromatography in phytochemistry* (pp. 255–276). Boca Raton: CRC Press.

Souza, L. M., Cipriani, T. R., Serrato, R. V., Costa, D. E., Iacomini, M., Gorin, P. A. J., et al. (2008). Analysis of flavonol glycoside isomers from leaves of *Maytenus ilicifolia* by offline and online high performance liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A*, *1207*, 101–109.

Souza, L. M., Cipriani, T. R., Sant'Ana, C. F., Iacomini, M., Gorin, P. A. J., & Sassaki, G. L. (2009). Heart-cutting two-dimensional (size exclusion × reversed phase) liquid chromatography–mass spectrometry analysis of flavonol glycosides from leaves of *Maytenus ilicifolia*. *Journal of Chromatography A*, *1216*, 99–105.

Varner, J. E., & Lin, L. S. (1989). Plant cell wall architecture. *Cell*, *56*, 231–239.

Wagner, H., & Jordan, E. (1988). An immunologically active arabinogalactan from *Viscum album* 'berries'. *Phytochemistry*, *27*, 2511–2517.

Whistler, R. L. (1970). *The Carbohydrates: Chemistry and Biochemistry*. London: Academic Press., p. 447.