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Glucuronoarabinoxylan from coconut palm gum exudate: Chemical structure and gastroprotective effect

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ABSTRACT

A glucuronoarabinoxylan (CNAL) was extracted with 1% aq. KOH (25 °C) from *Cocos nucifera* gum exudate. It had a homogeneous profile on HPSEC-MALLS-RI (M_w 4.6 × 10^4 g/mol) and was composed of Fuc, Ara, Xyl, Glc*p*A (and 4-*O*-Glc*p*A) in a 7:28:62:3 molar ratio. Methylation data showed a branched structure with 39% of non-reducing end units, 3-*O*-substituted Ara*f* (8%), 3,4-di-*O*- (15%), 2,4-di-*O*- (5%) and 2,3,4-tri-*O*-substituted Xyl*p* units (17%). The anomeric region of CNAL ^{13}C NMR spectrum contained 9 signals, indicating a complex structure. The main chain of CNAL was characterized by analysis of a Smith-degraded polysaccharide. Its ^{13}C NMR spectrum showed 5 main signals at δ 101.6, δ 75.5, δ 73.9, δ 72.5, and δ 63.1 that were attributed to C-1, C-4, C-3, C-2 and C-5 of (1 → 4)-linked β-Xyl*p*-main chain units, respectively. CNAL exhibited gastroprotective effect, by reducing gastric hemorrhagic lesions, when orally administered (1 and 3 mg/kg) to rats prior to ethanol administration.

1. Introduction

Cocos nucifera L. is a large palm belonging to the Arecaceae family. It is believed to have its origin in the Indo-Malayan region, from where it spread throughout the tropics (Bankar et al., 2011). The coconut palm is economically important because it provides food, drink, oil, folk medicine, among others. It can also be used for coastal stabilization as windbreaks and as a subsistence crop in many Pacific islands and other tropical regions (Renjith, Chikku, & Rajamohan, 2013; Rinaldi et al., 2009).

The coconut palm produces gum exudates, like other palms as *Livistona chinensis* (Maurer-Menestrina, Sassaki, Simas, Gorin, & Iacomini, 2003), *Scheelea phalerata* (Simas et al., 2004), and *Syagrus romanzoffiana* (Simas et al., 2006). The exudate process occurs mainly after some physical or microbiological injuries, and is found on the trunk of the palm. The coconut exudate is reddish-brown, clear, and vitreous. It can form an aqueous gel in water, although the gum has poor adhesive properties (Nussinovitch, 2010).

The wide industrial application of gum exudates is due to their water-retaining capacity to produce gels or highly viscous solutions, and for their ability to enhance the stability of emulsions and foams. It is known that these properties depend on the chemical structure of gum exudate polysaccharides and on their conformation in solution (Grein et al., 2013; Rinaudo, 2001; Rincón, Muñoz, Pinto, Alfaro, & Calero, 2009; Whistler, 1993).

Polysaccharides are the main components of gum exudates, having complex structures, consisting of a great variety of monosaccharides and glycosidic linkages, and a high number of branches as well (Aspinall, 1969). The most abundant polysaccharide gum exudates are arabinogalactans, such as arabic gum (from *Acacia senegal*), which is composed of Ara, Gal, Glc*p*A, and Rha as major monosaccharides. This polymer is composed of a main chain of (1 → 3)-linked β-D-Gal*p* residues, substituted at O-6 by complex side-chains composed of α-L-Ara*f*, β-D-Glc*p*A, α-L-Rha*p*, and β-D-Gal*p* (Anderson, Hirst, & Stoddart, 1966a, 1996b; Tischer, Gorin, & Iacomini, 2002). Other polysaccharides, such as glucuronoarabinoxylans (GAXs), were also isolated from gum exudates, although

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less common. These polymers have structure similarities with hemicellulosic glucuronoarabinoxylans from the primary plant cell wall, especially from species of the Poaceae family, such as sorghum (Verbruggen et al., 1998), maize (Allerdings, Ralph, Steinhart, & Bunzel, 2006), and wheat (Hromádková, Paulsen, Polovka, Kost'álová, & Ebringerová, 2013; Sun, Cui, Gu, & Zhang, 2011). Acetyl groups, ferulic acid and coumaric acid have also been found in GAXs from plant cell walls (Ishii, 1997). Glucuronoarabinoxylans from gum exudates, as those from palm species, are notably more highly branched than those of the hemicellulose type (Maurer-Menestrina et al., 2003; Simas et al., 2004, 2006).

Plant polysaccharides have showed a variety of biological activities, such as immunomodulatory (Moretão, Buchi, Gorin, Iacomini, & Oliveira, 2003; Schepetkin & Quinn, 2006; Simas-Tosin et al., 2012), anti-ulcer (Cipriani et al., 2008, 2009), antioxidant (Xie et al., 2012), antitumor (Xie et al., 2013), and as adjuvant in sepsis treatment (Dartora et al., 2013; Scoparo et al., 2013). Plant polysaccharides are good candidates as therapeutic biomacromolecules, considering that they are relatively nontoxic and have no significant side effects (Schepetkin & Quinn, 2006).

Glucuronoarabinoxylans from gum exudates are noteworthy molecules as candidates in industry or for therapeutic purposes, mainly because of its high yield, being around 80% of the gum weight (Maurer-Menestrina et al., 2003; Simas et al., 2006). These polymers may vary their chemical structure and conformation, which may be related to the different biological effects observed *in vitro* and *in vivo* (Moretão et al., 2003; Schepetkin & Quinn, 2006). Besides, the coconut palm is widely cultivated on the tropical regions of the planet, and despite of the great consumption of its fruit, the gum is discarded. Considering that there are no studies on coconut palm gum exudate, it was now chosen to evaluate the chemical and structural properties of its polysaccharides. The gastroprotective effects of the isolated glucuronoarabinoxylan were determined as well, using an *in vivo* model.

2. Materials and methods

2.1. Collection of the gum and isolation of polysaccharides

The coconut palm gum exudates were collected from the trunk of various tree specimens in Águas de Santa Bárbara (State of São Paulo, Brazil). The crude gum (9 g) was submitted to aqueous extraction (1.5%, w/v) at 25 °C (24 h). The remaining debris were removed by filtration and 3 volumes of ethanol (EtOH) were added to filtrate giving a precipitate, which was isolated by centrifugation (12,430 × g/20 min/10 °C). After dialysis (cut-off 12–14 kDa) and freeze-drying, the polysaccharide fraction CN was obtained (9% yield). The remaining gum residue was then submitted to aqueous extraction at 50 °C (24 h). The dispersion was filtered and the resulting soluble extract was added to 3 volumes of EtOH to give a precipitate, which was isolated as described above, giving rise to polysaccharide fraction CNH (12% yield). Finally, the remaining aqueous insoluble gum was treated with $NaBH_4$ in solution (pH 10.0), and then dissolved in 1% (w/v) aq. KOH (at 25 °C). After complete solubilization, the alkaline extract was neutralized with 50% (v/v) aq. acetic acid (HOAc) and was added to 3 volumes of EtOH, giving a polysaccharide fraction CNAL (50% yield), isolated as described above (Fig. 1).

2.2. Carboxy-reduction

Carboxy-reduction of polysaccharide CNAL (200 mg) was carried out using two successive cycles of the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (Simas-Tosin et al., 2013; Taylor & Conrad, 1972), to give a carboxy-reduced polysaccharide fraction (CR-CNAL). $NaBH_4$ being used as reducing agent.

2.3. Sodium periodate oxidation and controlled Smith degradation

In order to show the structure of the main chain of the CNAL it was submitted to controlled Smith degradation. CNAL was dissolved in H_2O (1 g in 100 mL) and 0.1 M $NaIO_4$ (100 mL) was then added. The solution was kept for 72 h in the dark, under magnetic stirring. After this time, 1 ml of oxidized solution was removed for determination of periodate consumption, according to the methodology described by Hay et al. (1965). Ethylene glycol (15 mL) was added to stop the reaction. The solution was dialyzed (cut-off 8 kDa/48 h) against tap water and treated with $NaBH_4$ (pH 10.0 for 16 h), neutralized with HOAc, dialysed (cut-off 8 kDa/48 h) and the volume was reduced to 50 mL. The last step of the procedure was a mild acid hydrolysis with TFA (0.1 M) until obtain pH 2.0, for 40 min

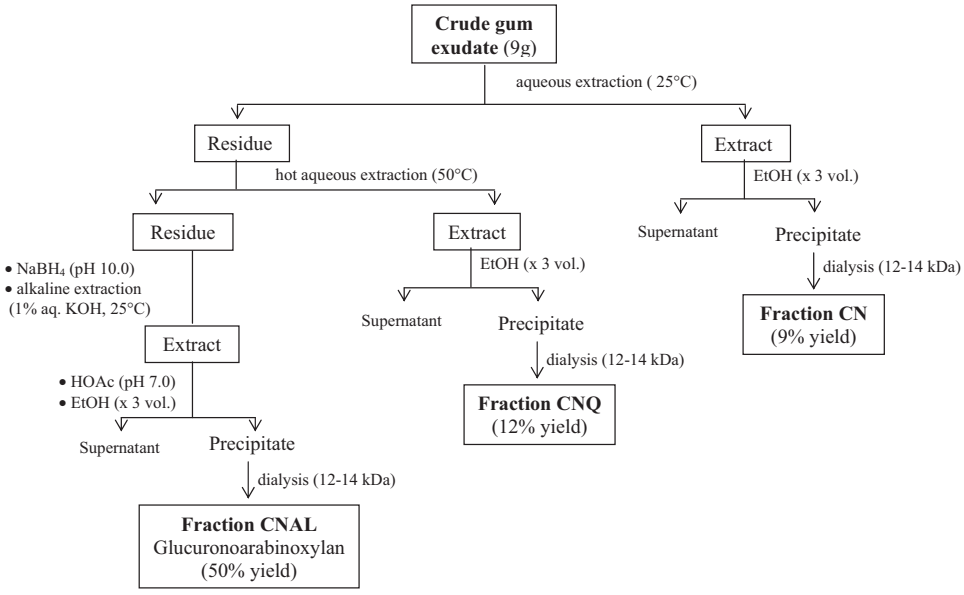

Fig. 1. Flow sheet diagram of isolation and the purification of polysaccharides from coconut gum exudate.

at 100 °C (Goldstein et al., 1965; Gorin et al., 1965; Simas et al., 2004). The degraded polysaccharide solution was raised to pH 5.0 by the addition of 1 M NaOH and excess of ethanol was added (4:1, v/v) to give a precipitate, which was dialyzed (cut-off 2 kDa) for 24 h yielding a Smith degraded polysaccharide fraction S-CNAL.

2.4. Analytical methods

2.4.1. HPSEC-MALLS-RI analysis

HPSEC-MALLS-RI analysis of samples was carried out using a waters high-performance size-exclusion chromatography (HPSEC) apparatus coupled to a differential refractometer (RI) (Waters 2410) and a Wyatt Technology Dawn-F Multi-Angle Laser Light Scattering detector (MALLS). Four columns of Waters Ultrahydrogel (2000, 500, 250, and 120) were connected in series and coupled to a multidetection system. 0.1 M $NaNO_2$ containing NaN_3 (0.5 g/L) was used as eluent. Fractions (1 mg/mL) were dissolved in this solvent and filtered (0.22 μm) before analysis. Data were analyzed using ASTRA 4.70.07 software.

2.4.2. Monosaccharide composition analysis

Each polysaccharide sample (2 mg) was hydrolyzed with 2 M TFA for 8 h at 100 °C, and the product was reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a) and acetylated with a mixture of acetic anhydride (Ac_2O) and pyridine (1:1; v:v) for 18 h at 25 °C (Wolfrom & Thompson, 1963b). The resulting alditol acetates were analyzed by gas chromatography–mass spectrometry (GC–MS) using a Varian Saturn 2000R – 3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer, with He as the carrier gas. A DB-225 capillary column (30 m × 0.25 mm i.d.), which was maintained at 50 °C during injection and then programmed to increase to 220 °C at a rate of 40 °C/min, was used for the quantitative analysis of the alditol acetates. The products were identified by their typical retention times and electron impact profiles. Uronic acid contents were determined by the colorimetric method of Filisetti-Cozzi and Carpita (1991).

2.4.3. Methylation analysis

Polysaccharide fractions CNAL, CR-CNAL, and S-CNAL (5 mg) were methylated according to Ciucanu and Kerek (1984), by dissolution in dimethyl sulfoxide followed by addition of powdered NaOH and CH_3I. Each mixture was vigorously agitated for 30 min and then left, at room temperature, for 18 h. The per-*O*-methylated products were extracted from aqueous solutions using $CHCl_3$, which was evaporated, at 25 °C, to dryness. Each per-*O*-methylated sample was hydrolyzed with 72% (w/w) H_2SO_4 (0.5 mL), at 0 °C, for 1 h, followed by dilution to 8% (Saeman, Moore, Mitchell, & Millet, 1954), being kept at 100 °C, for 16 h. The acid was then neutralized with $BaCO_3$ that was removed by centrifugation (12,000 × *g*, 20 min). After $NaBD_4$ reduction and acetylation with Ac_2O-pyridine, the resulting mixtures of partially *O*-methylated products were examined by GC–MS using a DB-225 capillary column (25 m × 0.25 mm i.d.), held at 50 °C during injection and then programmed to increase to 215 °C, at a rate of 40 °C/min. The partially *O*-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.4.4. Nuclear magnetic resonance spectroscopy

^{13}C NMR, ^{1}H NMR, and ^{1}H (obs.), ^{13}C HSQC spectra were obtained using a 400 MHz Bruker model DRX Avance III spectrometer equipped with a 5 mm broad band. Analyses were performed at 30 °C or 70 °C in D_2O (for fraction CNAL) or Me_2SO-d_6 (for fraction S-CNAL). The chemical shifts are expressed in ppm (δ) relative to external standard of acetone (δ 30.2) or Me_2SO-d_6 (δ 39.51).

2.5. Biological experiments

2.5.1. Animals

Experiments were carried out using female Wistar rats (180–200 g) provided by the Federal University of Parana colony and maintained under standard laboratory conditions (12 h light/dark cycles, temperature 22 ± 2 °C), with food and water provided *ad libitum*. The study was conducted in agreement with the "Principles of Laboratory Animal Care" (NIH Publication 85–23, revised 1985) and approved by the Committee of Animal Experimentation of Federal University of Parana (CEUA/BIO-UFPR; approval number 657).

2.5.2. Induction of acute gastric lesions

Acute gastric lesions were induced in overnight (18 h) fasted rats by oral administration of absolute EtOH as previously described by Robert, Nezamis, Lancaster, and Hauchar (1979), with minor modifications. Animals were orally pretreated with the vehicle (water, 1 ml/kg, control group), omeprazole (40 mg/kg, positive control group) or CNAL (0.3, 1 and 3 mg/kg), 1 h before the oral administration of EtOH P.A. (0.5 ml/200 g), and then euthanized 1 h after EtOH administration. The extent of gastric lesions was determined by removing the stomachs and measuring the area of lesions (mm^2) by computerized planimetry using the program Image Tool® 3.0.

2.5.3. Statistical analysis of gastric lesion rate

Results were expressed as mean ± standard error of the mean (SEM) with 6–10 animals per group. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test using Graph-Pad software (GraphPad software, San Diego, CA, USA). Differences were considered to be significant when $p < 0.05$.

3. Results and discussion

3.1. Homogeneity, molecular mass, and structural analysis of the native polysaccharide from coconut gum

The coconut gum exudate was submitted to sequential aqueous (25 °C and 50 °C) and alkaline extractions generating three polysaccharide fractions (CN, CNH, and CNAL respectively). Each fraction was composed of Fuc, Ara, Xyl, and uronic acids, in 6:32:59:3 (CN), 4:32:60:4 (CNH), and 7:28:62:3 (CNAL) molar ratios (Table 1). These data suggested the presence of glucuronoarabinoxylan-type structures in all fractions. Furthermore, the ^{13}C NMR spectra of all fractions were much similar (data not shown). Considering that the yield of CNAL was the highest (50%) (Table 1), this fraction was chosen to continue the structural characterization studies. CNAL was

Table 1
Monosaccharide composition of polysaccharide fractions.

Fractions	Yields (%)[c]	Monosaccharides (%)[a]					
		Fuc	Ara	Xyl	4-Me-Glc	Glc	Uronic acid[b]
CN	9	6	32	59	–	–	3
CNH	12	4	32	60	–	–	4
CNAL	50	7	28	62	–	–	3
CR-CNAL	85	9	31	57	1	2	nd
S-CNAL	25	–	13	85	–	–	2

nd, not detected.

[a] Relative percentage of alditol acetates obtained by successive hydrolysis, $NaBH_4$ reduction, and acetylation, followed by GC–MS analysis.

[b] Determined by the colorimetric method of Filisetti-Cozzi and Carpita (1991).

[c] Yields of fractions CN, CNH, and CNAL were calculated based on the crude gum weight and yields of fractions CR-CNAL and S-CNAL were calculated based on the CNAL aliquot.

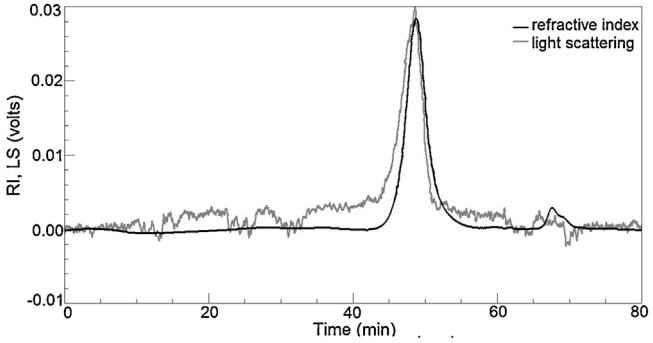

Fig. 2. Elution profiles of CNAL fraction using HPSEC with refractive index (RI) and light scattering (LS) detectors.

analyzed by HPSEC-MALLS, which showed a homogeneous profile (Fig. 2), and a M_w of 4.6 (±0.5) × 10^4 g/mol (*dn/dc* 0.177). Fraction CNAL (200 mg) was carboxy-reduced to characterize the type of uronic acid present. The carboxy-reduced fraction (CR-CNAL; 85% of yield) contained glucose (2%) and 4-Me-glucose (1%) (Table 1), obtained from carboxy-reduction of glucuronic acid units and their 4-*O*-Me-derivatives, respectively.

The methylation analysis (Table 2) of CNAL indicated a highly branched structure, with a great amount of nonreducing end-units of Ara*f* (2,3,5-Me_3-Ara*f*, 16%), Xyl*p* (2,3,4-Me_3-Xyl*p*, 16%), Ara*p* (2,3,4-Me_3-Ara*p*, 2%), and Fuc*p* (2,3,4-Me_3-Fuc*p*, 5%). A lower amount of Ara*f* residues were present as 3-*O*-substituted units (8%). The presence of 2,3,4,6-Me_4-Glc (5%) derivative from CR-CNAL indicated that Glc*p*A (and 4-Me-Glc*p*A) units were present as nonreducing end-units. The majority of Xyl*p* units at CNAL structure were present as 4-*O*- (3%), 2-*O*- (13%), 3,4-di-*O*- (15%), 2,4-di-*O*- (5%), and 2,3,4-tri-*O*-substituted units (17%). The methylation data of CNAL resembled glucuronoarabinoxylans structures from gum exudates of other palms species (Maurer-Menestrina et al., 2003; Simas et al., 2004, 2006) and gums from *Cercidium australe* (Cerezo, Stacey, & Webber, 1969), *Cercidium praecox* (Léon de Pinto, Martínez, & Rivas, 1994), and pineapple gum (Simas-Tosin et al., 2013). These gums contained around 13–27% of totally substituted Xyl*p* units, and 27–43% of non-reducing end units, indicating highly branched structures. The presence of Fuc as non-reducing end units is typical of palm gum exudates, being found in CNAL and other gums from palm species (Maurer-Menestrina et al., 2003; Simas et al., 2004, 2006), suggesting that this monosaccharide could be a potential chemotaxonomic marker for gum exudates.

The ^{13}C NMR spectrum of CNAL confirmed its highly branched structure by the presence of a great number of signals on the anomeric region (Fig. 3A). Signals at δ 108.6–107.1 arose from C-1 of α-L-Ara*f* units (Gorin & Mazurek, 1975). The signal at δ 99.2 could be assigned to C-1 of Glc*p*A in an α-configuration (Cavagna, Deger, & Puls, 1984; Simas et al., 2004). Those signals at δ 103.2, δ 102.7, and δ 101.6 were attributed to C-1 of α-Ara*p*, β-Xyl*p*, and α-Fuc*p* units, respectively (Alquini, Carbonero, Rosado, Cosentino, & Iacomini, 2004; Delgobo, Gorin, Tischer, & Iacomini, 1999; Gast, Atalla, & McKelvey, 1980; Gorin & Mazurek, 1975; Léon de Pinto et al., 1994). The signal at δ 100.5 could be assigned to β-Ara*p* units (Gorin & Mazurek, 1975; Delgobo et al., 1999). Signals at δ 65.1, δ 62.9, and δ 61.3 were assigned to C-5 of nonreducing end-units of β-Xyl*p* (Gorin & Mazurek, 1975), 4-*O*-linked β-Xyl*p* units (Simas et al., 2004), and α-Ara*f* units (Delgobo et al., 1999), respectively. Upfield signals at δ 16.9 and at δ 15.6 arose from–CH_3 group of Fuc*p* units (Alquini et al., 2004). The ^{1}H NMR and ^{1}H (obs.), ^{13}C HSQC spectra of CNAL (Fig. 4) were in agreement with ^{13}C NMR assignments. Anomeric signals at δ 5.260/108.6, δ 5.309/108.1, and δ 5.404/107.8 were typical from α-L-Ara*f* units (Delgobo et al., 1999; Tischer et al., 2002). Signals at δ 4.615/103.2 and δ 5.060/100.5 were attributed to H-1/C-1 of α- and β-L-Ara*p* units, respectively (Agrawal, 1992; Delgobo et al., 1999). H-1/C-1 correlations at δ 4.750/102.7 and δ 5.120/101.6 were from β-Xyl*p* (Gast et al., 1980; Gorin & Mazurek, 1975) and α-Fuc*p* (Alquini et al., 2004) units, respectively. H-1 signals at δ 5.220, δ 5.260, and δ 5.339 which coupled with C-1 signal at δ 99.2 were from α-Glc*p*A units (Cavagna et al., 1984; Simas et al., 2004). Upfield signal at δ 1.289 arose from–CH_3 of Fuc*p* units (Alquini et al., 2004).

3.2. Structural analysis of Smith degraded polysaccharide (S-CNAL): elucidation of main chain of CNAL polysaccharide

The residual Smith degraded polysaccharide (S-CNAL) presented M_w 3.1 (±0.6) × 10^4 g/mol and was composed of Ara, Xyl, and uronic acids in a 13:85:2 molar ratio (Table 1). Methylation analysis of S-CNAL (Table 2) showed mainly Xyl*p* units 4-*O*-substituted (56%), characterizing the main chain of the original polysaccharide (CNAL) as a (1 → 4)-linked xylan, and suggesting that a large proportion of these units were substituted at O-3 and O-2 by periodate sensitive side-chains, which were degraded. Some of the Xyl*p* units of the main chain were 2-*O*-substituted (15%) by side-chains composed of 2-*O*-substituted Xyl*p* (18%) and nonreducing end-units of Ara*f* (11%). Under sodium periodate oxidation, the polysaccharide CNAL consumed 0.80 moles of periodate per monosaccharide unit, which was in agreement with methylation data (Table 2).

The ^{13}C NMR spectrum of S-CNAL (Fig. 3B) contained 5 main signals at δ 101.6, δ 75.5, δ 73.9, δ 72.5, and δ 63.1 that were attributed

Table 2
Partially *O*-methylalditol acetates formed on methylation analysis of polysaccharide fractions.

Partially *O*-methylated alditol acetates[a]	Retention time (min)	Fractions			Linkage type[c]
		CNAL	CR-CNAL[b]	S-CNAL[b]	
2,3,5-Me_3-Ara*f*	6.588	16	20	11	Ara*f*-(1→
2,3,4-Me_3-Fuc*p*	6.655	5	4	–	Fuc*p*-(1→
2,3,4-Me_3-Ara*p*	6.836	2	3	tr.	Ara*p*-(1→
2,3,4-Me_3-Xyl*p*	7.042	16	19	–	Xyl*p*-(1→
2,5-Me_2-Ara*f*	7.900	8	6	–	→3)-Ara*f*-(1→
2,3,4,6-Me_4-Glc*p*	8.174	–	5	–	Glc*p*-(1→
2,3-Me_2-Xyl*p*	8.731	3	3	56	→4)-Xyl*p*-(1→
3,4-Me_2-Xyl*p*	8.731	13	12	18	→2)-Xyl*p*-(1→
2-Me-Xyl*p*	10.943	15	9	–	→3,4)-Xyl*p*-(1→
3-Me-Xyl*p*	10.988	5	5	15	→2,4)-Xyl*p*-(1→
Pentaacetate Xyl*p*	13.885	17	14	–	→2,3,4)-Xyl*p*-(1→

tr.: traces.
[a] Analyzed by GC–MS, after methylation, total acid hydrolysis, reduction with $NaBD_4$ and acetylation.
[b] CNAL after carboxy-reduction and Smith degradation, respectively.
[c] Based on derived *O*-methylalditol acetates.

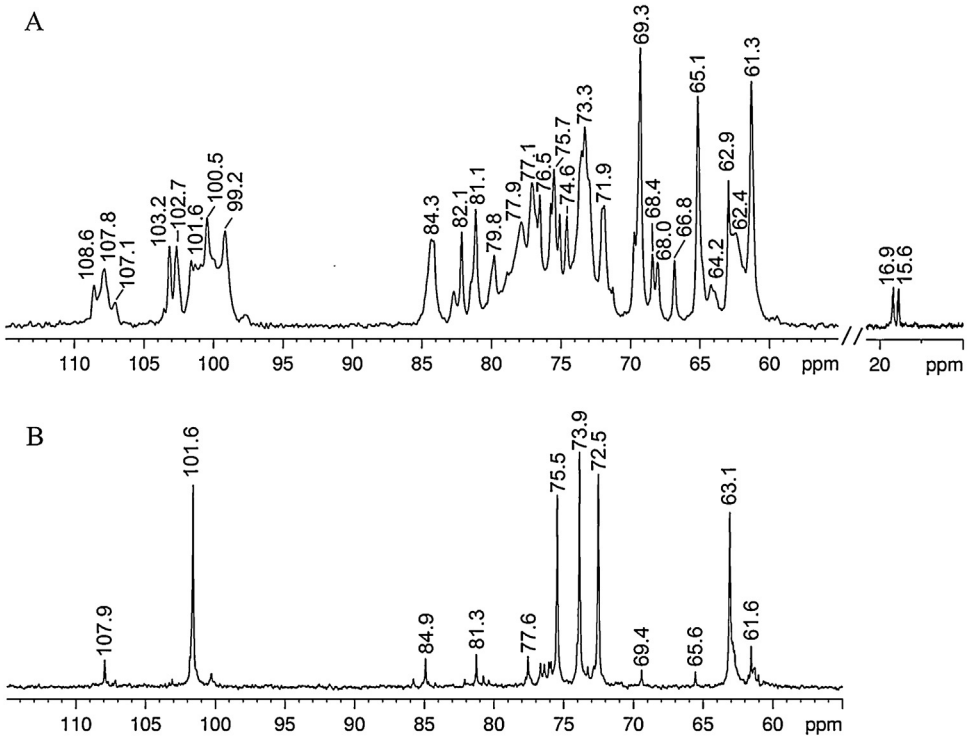

Fig. 3. ^{13}C NMR spectra of native polysaccharide (CNAL) (A) and Smith degraded polysaccharide (S-CNAL) (B). Solvent: D_2O (CNAL) and Me_2SO-d_6 (S-CNAL) at 30 °C with numerical values in δ (ppm).

to C-1, C-4, C-3, C-2 and C-5 of (1 → 4)-linked β-Xyl*p*-main chain units respectively (Gast et al., 1980; Simas et al., 2004; Simas-Tosin et al., 2013). Signals at δ 107.9 and δ 61.6 corresponded to C-1 and C-5 of residual α-L-Ara*f* nonreducing end-units (Gorin & Mazurek, 1975; Simas-Tosin et al., 2013). These data were in accord with other authors that described glucuronoarabinoxylan-type gum exudates, as those of other palm trees (Maurer-Menestrina et al., 2003; Simas et al., 2004, 2006), species of *Cercidium* (Cerezo et al., 1969; Léon de Pinto et al., 1994), and pineapple (Simas-Tosin et al., 2013).

3.3. Gastroprotective effect of polysaccharide CNAL

It has been demonstrated that different polysaccharides isolated from plants have several biological activities, including a gastroprotective effect. Among them, arabinogalactans, rhamnogalacturonans and arabinoxylans were demonstrated to exhibit anti-ulcer activity, by reducing the gastric lesion caused by ethanol (Cipriani et al., 2006, 2008; Mellinger-Silva et al., 2011; Nascimento et al., 2013).

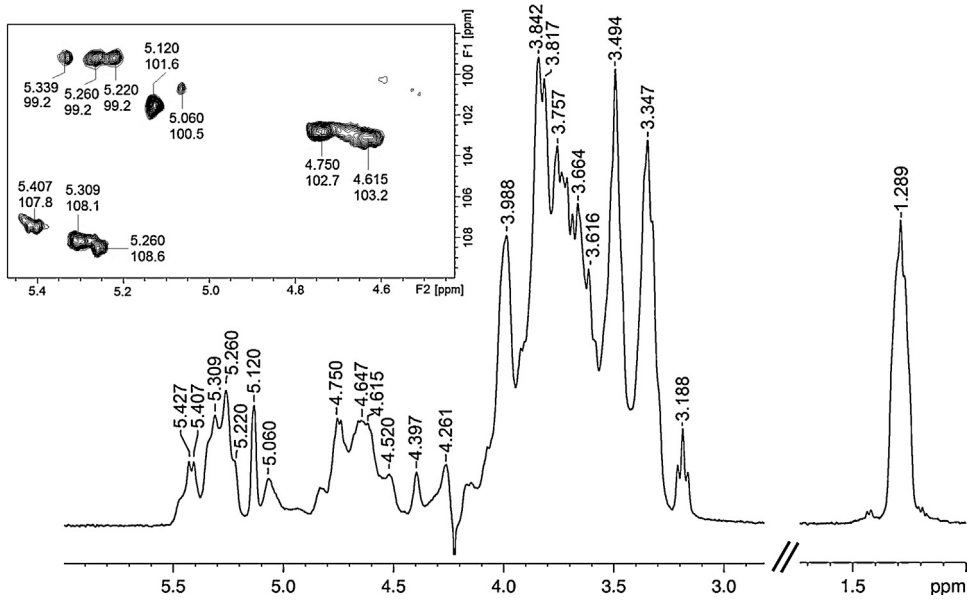

Fig. 4. ^{1}H NMR and anomeric region of ^{1}H (obs.), ^{13}C HSQC (inset) spectra of native polysaccharide (CNAL). Solvent: D_2O at 70 °C with numerical values in δ (ppm).

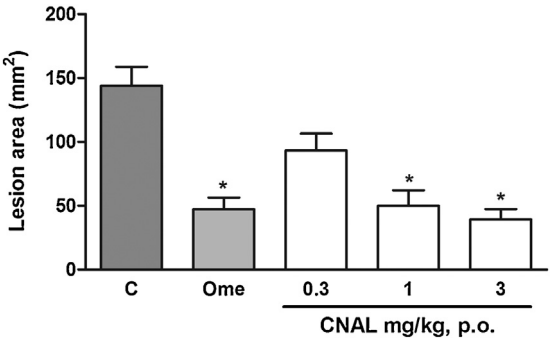

Fig. 5. Gastroprotective effect of CNAL against acute gastric lesions induced by ethanol in rats. The animals were orally treated with vehicle (C: water, 1 ml/kg), omeprazole (Ome: 40 mg/kg) or CNAL (0.3, 1 and 3 mg/kg), 1 h before oral administration of ethanol (0.5 ml/200 g). The results are expressed as mean ± S.E.M. ($n = 6$). Statistical comparison was performed using analysis of variance (ANOVA) followed by *post hoc* Bonferroni's test: $^{*}p < 0.05$ when compared with control group (C).

In order to investigate the potential gastroprotective effect of the glucuronoarabinoxylan now isolated, we performed the model of gastric lesions induced by ethanol. Ethanol is a well known necrotizing agent that rapidly penetrates in the gastric mucosa leading to hemorrhagic erosion and ulcer formation through increasing vascular permeability, membrane damage, and reduction of mucosal protective factors such as mucus barrier and non-proteic sulphydrilic groups (NP-SH) (Repetto & Llesuy, 2002; Siegmund, 2003). The extent of gastric lesions induced by ethanol was determined by removing the stomachs and measuring the area of lesions, showing that the higher doses of CNAL (1 and 3 mg/kg) significantly reduced the hemorrhagic lesions in 65% and 73%, respectively, when compared to control group (C: 143.9 ± 14.8 mm^2) (Fig. 5). In addition, the positive control that was treated with omeprazole (40 mg/kg, p.o.), also inhibited the gastric lesion area by 67%, which was similar to the inhibition observed for the dose of 1 mg/kg of CNAL (Fig. 5). It is important to note that these findings are in accord with data obtained with other heteroxylans. Mellinger-Silva et al. (2011) reported that an arabinoxylan isolated from sugarcane bagasse reduced the area of ethanol-induced lesions in rats by over 50%, although the doses administered were much higher (30, 100, and 300 mg/kg). Acidic heteroxylans obtained from *Maytenus ilicifolia* and *Phyllanthus niruri* also exhibited anti-ulcer activity by reducing the gastric lesions induced by EtOH. The reduction observed reached 65–78% (Cipriani et al., 2008), although the tested doses (30 and 100 mg/kg) were also higher than the doses administered in the present study. Our results demonstrated that the gastroprotective effect of CNAL was not dose-dependent, although the glucuronoarabinoxylan showed a great activity even in lower doses, when compared with other heteroxylans. Considering the efficacy of this polysaccharide in protecting the stomach mucosa, further studies are required to determine the possible mechanism of action involved in its effect.

4. Conclusions

The polysaccharide isolated from coconut gum exudate was characterized as a glucuronoarabinoxylan (CNAL), composed of Xyl, Ara, Fuc, and GlcA (and its 4-*O*-methyl derivative). The main chain of CNAL is composed of (1 → 4)-linked β-Xyl*p* units, which were 2-*O*-, 3-*O*-, and 2,3-di-*O*-substituted by side chains of 3-*O*-substituted Ara*f* units and nonreducing end-units of Ara*f*, Xyl*p*, Fuc*p*, Glc*p*A (and 4-O-Me-Glc*p*A). This structure resembled those of glucuronoarabinoxylans from gum exudates of other palms species. The glucuronoarabinoxylan exhibited gastroprotective effect when orally administered to mice prior to ethanol administration that causes stomach injury. The animals treated with the polysaccharide had a reduction of the hemorrhagic lesions when compared to control group. Our results demonstrated that the gastroprotective effect of CNAL was not dose-dependent, although the glucuronoarabinoxylan had a great activity even in lower doses, when compared with other heteroxylans.

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