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Spondias purpurea Exudate polysaccharide as affinity matrix for the isolation of a galactose-binding-lectin

Daniele M.A. Teixeira [a], Renata C. Braga [a], Ana C.G. Horta [a], Renato A. Moreira [a], Ana C.F. de Brito [b], Jeanny S. Maciel [b], Judith P.A. Feitosa [b], Regina C.M. de Paula [b,*]

[a] *Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal 6020, CEP 60451-980, Brazil*
[b] *Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Caixa Posta 12200, CEP 60455-760, Fortaleza-Ceará, Brazil*

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Abstract

Spondias purpurea L., popularly known as ciriguela, is native and widespread tree from Mexico through Northern Peru and Brazil, particularly in semi-arid zones. This tree exudes a water soluble polysaccharide, constituted of a (1→3) linked galactan backbone substituted at C6 with D-galactose, D-xylose, L-arabinose, L-rhamnose and glucuronic acid units. Brazilian polysaccharide differs from Venezuelan on the amount of acid and arabinose as well as the presence of fucose and glucose as minor sugar. The D-galactose substitution (1→6) confers to the polysaccharide the peculiar capacity of binding α-D-galactose specific lectins after cross-linking with epichlorohydrin. The gel obtained was able to specifically retain D-galactose-binding-lectins, among with those from *Artocarpus incisa*, *Artocarpus integrifolia*, *Erythrina velutina* and *Ricinus communis*. On the other hand, no glucose-binding-lectins were retained.

Keywords: Polysaccharide; Affinity chromatography; Lectins; *Spondias purpurea*

1. Introduction

Spondias purpurea is a tree from *Anacardeaceae* family originally from Central America and widespread in all countries of tropical America. Composition and structural features of *S. purpurea* from Venezuela was investigated by de Pinto et al. (1995, 1996). A Venezuelan polysaccharide sample mainly constitutes of galactose, arabinose, glucuronic acid and 4-*O*-methyl glucuronic acid and xylose, mannose and rhamnose as traces residues (de Pinto et al., 1995). The structure of the polysaccharide was determined for Venezuelan polysaccharide after partial degradation and analysis of the products by ^{13}C NMR. The structure consist of β-D-galactopyranose (1→3) and (1→6) linked and arabinose, rhamnose as well as β-D-glucuronic acid and 4-*O*-methyl α-glucuronic acid as terminal residues (Gutiérrez de Gotera, Martinez, Sanabria, de Pinto, & Igartuburu, 2005).

Lectins are proteins possessing at least one non-catalytic domain, which binds reversibility to a specific monosaccharide through hydrogen bonds, metal coordination, van der Waals and hydrophobic interactions. This sugar-binding property of the lectins also confers to these proteins the capacity to be isolated by affinity chromatography on matrices containing their specific ligand sugar (Luck, Ehwald, Zizka, & Koppitz, 1992).

Affinity separation techniques are based on biospecific molecular interactions. Therefore, they are extremely powerful tools for the isolation of valuable biological macromolecules (Helmholz, Cartellieri, He, Thiesen, & Niemeye, 2003). Three major types of biospecific adsorbents have been described for the purification of lectins by this technique: polysaccharides, either native or modified; matrix-bound glycoproteins and glycopeptides; and matrix-bound mono and disaccharides (Lis & Sharon, 1981). Taking into account that glycoconjugates

* Corresponding author. Tel.: +55 85 33669973; fax: +55 85 33669978.
E-mail address: rpaula@dqoi.ufc.br (R.C.M. de Paula).

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and glycans have been shown to interact with many biological systems both *in vivo* and *in vitro*, a great deal of attention has been focused on the development of polymer or microsphere-bound glicosidic ligands (Caron, Sève, Bladier, & Joubert-Caron, 1998).

Fujita, Oishi, Suzuki, and Imahory (1975) investigated the use of natural Arabic polysaccharides as affinity matrix for the isolation of an Anti-B hemagglutinin produced by *Streptomyces* sp. A large variety of affinity adsorbents for lectin purification has been described. Many of these originated from the laboratory of Professor Jerker Porath. The first two commercial products for lectin separation (Sephadex and Sepharose) were based on polysaccharide crosslinked with epichlorohydrin (Janson, 1987). The same procedure was used by Appukuttan, Surolia, and Bachhawat (1977) for the isolation of *Ricins communis* lectins with guar (*Cyamopsist tetragonolobus*) endospermic polysaccharide (α-D-galactopiranosyl 1→6 (β1→4) mannan). Using similar procedure, affinity matrices were prepared with crosslinked endospermic galactomannan from *Adenanthera pavonina* (Moreira, Castelo-Branco, Monteiro, Tavares, & Beltramini, 1998), *Parkinsonia aculeate* (Garros-Rosa, Reicher, Petkowicz, & Sierakowski, 2006) and *Leucaena leucocephala* (Seshagirirao, Leelavathi, & Sasidhar, 2005). These matrices showed specificity for the isolation of α-D-galactose-binding lectins. The bark exudate polysaccharide from the cashew tree (*Anacardium occidentale* L.) was also studied as a potential material to be used as chromatographic matrix and/or for bioaffinity ligand for proteins (lectins) (Lima, Lima, Salis, & Moreira, 2002). Recently cellulose based matrices were shown to be a promising support for the preparation of biospecific adsorbents for lectin affinity chromatography (Aiulyte, Liesiene, & Niemeyer, 2006).

As some variation in composition were found between species from different geographic locations, the composition and structural features of *S. purpurea* from Northeast of Brazil were investigated in the present work. Matrices from this polysaccharide prepared by crosslinking with epichlorohydrin were investigated as affinity matrices for lectin isolation.

2. Materials and methods

2.1. Materials

Crude polysaccharide sample from *S. purpurea* was collected in October 1996, from native trees at Fortaleza, Ceará, Brazil. The sample was identified by Prisco Bezerra Herbarium where a voucher sample is kept.

Seeds of *Artocarpus incisa* L., *Artocarpus integrifolia* L., *Erythrina velutina* Willd, *Ricinus communis* L., *Canavalia maritima* and *Dioclea altissima Vell* were collected in the State of Ceará, Brazil.

Rabbit red blood cells were obtained by puncture of the ears marginal vein in healthy animals.

2.2. Polysaccharide isolation

The polysaccharide was dissolved in distilled water at room temperature (28 °C) to give a 5% (w/v) solution. Then, the solution was filtered, centrifuged at 10,000*g* during 20 min and precipitated with ethanol. The polysaccharide isolated was re-dissolved and re-precipitated with ethanol.

2.3. Analytical data

The moisture and ash contents of polysaccharide were determined at 105 °C and 600 °C, respectively. The specific rotation of polysaccharide water soluble solutions were measured at 25 °C with a Perkin–Elmer 341 polarimeter at 589 nm.

The viscosity measurements were performed in an Ubbelohde viscometer with capillary diameter of 0.5 mm at 25.0 °C with 0.1 M $NaNO_3$ as solvent.

2.4. Chemical composition of the polysaccharide

Total hydrolysis of *S. purpurea* polysaccharide was performed using trifluoroacetic acid (TFA) (de Paula, Heatley, & Budd, 1998). The sample (50 mg) was dissolved in 4 M TFA (1 mL) and heated at 100 °C in a sealed ampoule for 5 h. These conditions were found to give maximum hydrolysis and minimum degradation of labile monosaccharide. To eliminate the excess of acid, the solution was washed with methanol (5 × 10 mL) and concentrated in a water bath at 40 °C with air flow.

Sodium borohydride solution was added to the dry hydrolysed polysaccharide and the pH adjusted to 9–10 with NaOH. The reduction was carried out for 4 h at 50 °C. To eliminate the residue of borax the solution was passed through an H^+ resin and evaporated to dryness after addition of methanol (3×). The dry reduced polysaccharide was dissolved in 4 mL of acetic anhydride and 3 mL of pyridine and left at room temperature for 48 h. To stop the reaction 6 mL of cold water was added. The acetylated polysaccharide was recovered after phase separation in the organic phase upon addition of 1 mL of saturated $CuSO_4$(aq) and 2 mL of ethyl acetate. The organic phase was evaporated to dryness. The resulting alditol acetates were analyzed by GLC (gas–liquid chromatography) using a model 5890 S II HP Gas Chromatography at 220 °C (FID and injector temperature, 250 °C) with DB-210 capillary column (0.25 mm i.d. × 30 m), film thickness 0.25 μm and nitrogen as carrier gas.

The total content of uronic acid was calculated by conductimetric titration with 0.012 M HCl (de Paula, Santana, & Rodrigues, 2001). Protein content was estimated from the nitrogen content determined by the Kjeldahl method (Baethgen & Alley, 1989), using a nitrogen factor of 6.25.

2.5. Structural characterization

^{13}C nuclear magnetic resonance (NMR) spectra of 10% (w/v) solutions in D_2O at 80 °C were recorded at 125.697 MHz using a Varian Unity 500 spectrometer. Chemical shifts are quoted relative to the CH_3 carbon of internal acetone at 31.07 ppm. A distortion less enhancement through polarization transfer (DEPT) spectrum was recorded in order to determine the multiplicity of carbon peaks. The acquisition and delay times were 1.0 s. DEPT spectrum was obtained with final ^1H pulse flip angle of 135° (DEPT 135). The signals in the ^{13}C NMR spectrum were assigned on the basis of correlated compounds from literature data (Defaye & Wong, 1986; de Paula & Rodrigues, 1995; de Paula, Budd, & Rodrigues, 1997; de Paula et al., 1998; de Pinto, Alvarez, Martinez, Rojas, & Leal, 1993; de Pinto, Martinez, Corredor, Rivas, & Ocando, 1994; de Pinto et al., 2000a; de Pinto et al., 2000b).

2.6. Molar mass

The peak molar mass (M_{pk}) was estimated by gel permeation chromatography (GPC) in a Shimadzu equipment at room temperature using an Ultrahydrogel linear column and 0.1 M $NaNO_3$ as solvent. A differential refractometer and UV at 280 nm were used as detector. The elution volume was corrected to the internal marker of ethylene glycol at 11.25 mL. The GPC was calibrated with pullulan.

2.7. Preparation of the chromatographic matrix

The exudate polysaccharide was treated with epichlorohydrin, in the presence of NaOH, following a factorial design described in Table 1, in comparison with the conditions used by Fujita et al. (1975) (*B*) and by (Garros-Rosa et al., 2006) (A_0, A_1 and A_2) . To the purified polysaccharide, NaOH at 40 °C was added and after homogenization, epiclorohydrin was mixed and vigorously stirred at 40 °C. The reaction was allowed to continue for 24 h, at the same temperature, and then the reaction temperature was raised to 70 °C. After standing at this temperature overnight, the gel was broken into portions, swollen in water and homogenized. It was thoroughly washed with water and fine particles were removed by decantation. The cross-linked gels obtained were stable to acid and alkaline conditions and used as an affinity matrix for chromatographic isolation of lectins.

2.8. Lectin extraction

Seeds from dehulled *Artocarpus incisa* and *A. integrifolia* (previously dried in acetone), *Erythrina velutina*, *Ricinus communis*, *Canavalia maritima* and *Dioclea altissima* were finely ground and extracted with 0.15 M NaCl (10% w/v) for 4 h at room temperature, filtered in nylon tissue and spun at 10,000*g* for 20 min at 7 °C. The clear supernatants were, then, used to isolate the lectins by affinity chromatography.

2.9. Affinity chromatography

Chromatographic columns were prepared with the different gels obtained after treatment with epichlorohydrin. After equilibration with 0.15 M NaCl, seed extracts (from different species) containing lectin were applied (3 mL) to the columns. The elution of the columns was carried out initially with the equilibrium solution (0.15 M NaCl) followed by 0.15 M NaCl containing 0.2 M galactose, 0.2 M

Table 1
Experimental conditions applied to *S. purpurea* gels preparations

Treatment	Volume and concentration used				Final concentration			Ratio	
	NaOH (mL)	NaOH (M)	E[a] (μL)	H_2O (μL)	P (g/mL)	NaOH (M)	E (M)	E/P (mmol/g)	NaOH/E (M/M)
A_0	4.0	3.0	125	0	0.121	2.9	0.4	3.1	7.25
A_1	4.0	3.0	250	0	0.118	2.8	0.7	6.2	1.96
A_2	4.0	3.0	1000	0	0.100	2.4	2.5	24.8	0.96
B	0.6	5.0	115	400	0.435	2.6	1.6	3.7	1.62
*F*1	1.0	3.0	68	432	0.333	2.0	0.6	1.7	3.33
*F*2	1.0	3.0	135	365	0.333	2.0	1.1	3.3	1.82
*F*3	1.0	3.0	270	230	0.333	2.0	2.2	6.7	0.91
*F*4	1.0	3.0	405	95	0.333	2.0	3.3	10.0	0.61
*F*5	1.0	3.0	500	0	0.333	2.0	4.1	12.4	0.49
*F*6	1.0	5.0	135	365	0.333	3.3	1.1	3.3	3.00
*F*7	1.0	5.0	270	230	0.333	3.3	2.2	6.7	1.50
*F*8	1.0	5.0	405	95	0.333	3.3	3.3	10.0	1.00
*F*9	1.0	5.0	500	0	0.333	3.3	4.1	12.4	0.81
*F*10	1.0	2.0	405	95	0.333	1.3	3.3	10.0	0.40
*F*11	1.0	4.0	405	95	0.333	2.7	3.3	10.0	0.82

Polysaccharide was kept constant in 0.5 g.
[a] E, epichlorohydrin.

glucose or 0.1 M β-alanin pH 2.6 buffer solutions. The non-retained and retained fractions had their protein content and hemagglutinating activity determined.

2.10. Haemagglutination assay

The method described by Moreira and Perrone (1977) was used, with 2% suspension of rabbit erythrocytes. The results obtained were expressed as hemagglutination units (H.U/mL), the reciprocal of the highest dilution still giving visible agglutination.

2.11. Polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970), using 15% polyacrylamide gels. Samples were dissolved in 65 mM Tris–HCl, pH 6.8, containing 2% SDS, and heated at 100 °C for 10 min. After electrophoresis, the bands were revealed with silver staining (Blum, Beier, & Gross, 1987). Dalton Mark VII-L Protein standards used are: Serum albumin, Bovine 66,000 Da, Ovalbumin, chiken egg 45,000 Da, Gluceraldehyde-3-phosphate dehydrogenase 36,000 Da, Carbonic anhydrase from bovine erythrocytes 29,000 Da, Trypsinogen 24,000, Trypsin Inhibitor, soybean 20,100 Da, α-lactoalbumin from bovine milk 14.2 Da.

3. Results and discussion

3.1. Composition of the polysaccharide

The approximate compositions (moisture, ash, protein and carbohydrate) of the polysaccharide are shown in Table 1. The level of purity of the polysaccharides, as determined by the protein content, was measured. Some polysaccharides showed a good index of purity (agar, carrageenan and karaya polysaccharide) with low protein content as 0.3–0.4%, while guar, xanthan and pectin had a much higher protein level (4.5–6.4%). These proteins are probably contaminants or associated with polysaccharide chains, via covalent links (proteoglycans), although the existence of these crosslinks in the plant cell walls is still under investigations (Brett & Waldron, 1996). The nitrogen and protein content are similar to those reported for *S. purpurea* (de Pinto et al., 1995) and *S. purpurea* var. *lutea* (de Pinto et al., 2000a) polysaccharides from Venezuela.

Spondias purpurea polysaccharide from Brazil has a negative optical rotation as observed for polysaccharides from *S. purpurea* var. lutea from Venezuela (de Pinto et al., 2000a) and *S. purpurea* (Pérez, Sanchez, Perez, & Vargas, 1995) from Mexico. However it has been reported that the polysaccharide from *S. purpurea* (Venezuelan sample) is dextrorotatory (de Pinto et al., 1995). The dextrorotatory character has been observed in other *Spondias* polysaccharide such as *S. cytherea* (de Pinto et al., 2000a) *and S. mombin* (de Pinto et al., 1995) polysaccharides.

The neutral sugars content was determined by GC/MS after hydrolysis with TFA. Table 2 shows the sugar composition for Brazilian *S. purpurea* polysaccharide in comparison with results from Venezuelan sample. The molar ratio of the two major monosaccharide, galactose/arabinose, is 1.4, which is very low if compared with 6.5 determined for the Venezuelan sample (de Pinto et al., 1995). The amount of acid and arabinose as well as the presence of fucose and glucose as minor sugar are the major differences found in the Brazilian sample.

3.2. ^{13}C NMR of exudate polysaccharide

^{13}C NMR spectrum of Brazilian *Spondias purpurea* polysaccharide in D_2O at 80 °C was complex (Fig. 1). The less intense peaks at 16.6 ppm at higher field can be assigned as methyl carbon from rhamnose and/or fucose residues. At lower field, the two signals (176.7 and 176.0 ppm) are due to carbonyl groups from glucuronic acid residues. In the anomeric region (90–110 ppm) the peaks at 110.0 and at 109.3 ppm may be attributed to α-arabinofuranose residues. These signals are much more intense in Brazilian polysaccharide than in Venezuelan sample (de Pinto et al., 1995; de Pinto et al., 1996), in agreement with the differences found in the sugar analysis. The other anomeric signals were assigned as β-galactopyranose (1→3) (103.7 ppm), β-glucuronic acid (104.6 ppm) and 4-*O*-methyl glucuronic acid (100.6 ppm).

The presence of 4-*O*-methyl glucuronic acid was detected in the Venezuelan sample by paper chromatography analysis, but the peak assigned in the ^{13}C NMR spectrum was overlapped with the primary carbon of galactose residue (de Paula & Rodrigues, 1995). The peak due to methoxyl groups from 4-*O*-methyl glucuronic acid appears very close to those of primary carbons in the ^{13}C NMR of exudate polysaccharide spectrum. In

Table 2
Analytical data of *Spondias* exudate polysaccharides

	S. purpurea	*S. purpurea* var. *lutea*[a]	*S. purpurea*[b]
Moisture (%)	9.3	9.0	8.23
Ash (%)	4.8	4.0	5.17
N (%)	0.3	0.45	0.32
Protein (%)	2.1	2.81	2.00
Specific rotation (°)	−13.3[d]	−37[c]	+50[c]
Composition			
Galactose	43.3	41	59
Arabinose	31.2	12	9
Mannose		4	2
Uronic acid	17.0	31	26
Fucose	3.6	–	–
Xilose	1.3	3	2
Glucose	1.3	–	–
Rhamnose	2.5	9	2

[a] de Pinto et al. (1995).
[b] de Pinto et al. (2000a, 2000b).
[c] Determined at 30 °C.
[d] Determined at 25 °C.

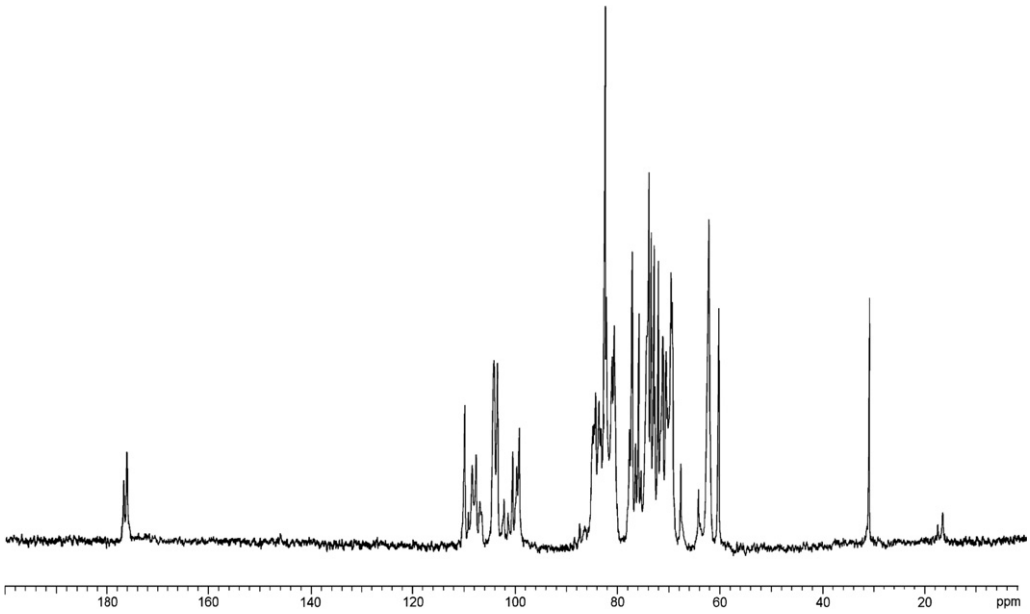

Fig. 1. ^{13}C NMR spectrum of *S. purpurea* polysaccharide in D_2O.

the DEPT-135 experiment, the methylene carbons show opposite amplitude to the methyl carbons. It can be seen in Fig. 2 that the peak at 60.8 ppm is due to methyl carbon of 4-*O*-methyl glucuronic acid. The attributions of main sugar signals based on correlated compounds are shown in Table 3.

3.3. Molar mass distribution

The GPC chromatograms of *S. purpurea polysaccharide* using refractive index and UV detectors are shown in Fig. 3. Broad molar mass distribution was observed with a major peak at 8.04 mL and a less intense one at 9.12 mL. A shoulder was also detected at 6.8 mL. Broad molar mass distributions have been reported for other exudate polysaccharides such as *Acacia senegal* (Vandevelde & Fenyo, 1985), *A. occidentale* (de Paula & Rodrigues, 1995), *A. macrocarpa* (de Paula et al., 1997) and *Albizia lebbeck* (de Paula et al., 2001). In the UV detector at 280 nm the peaks might be due to presence of protein–polysaccharide complex, as found for other exudate polysaccharides, such as *A. occidentale* (de Paula & Rodrigues, 1995) and *A. senegal* (Vandevelde & Fenyo, 1985). The molar mass, determined by pullulan standard curve, for the two main peaks were 6.6×10^5 and 3.9×10^4 g/mol, respectively, for peaks at 8.04 and

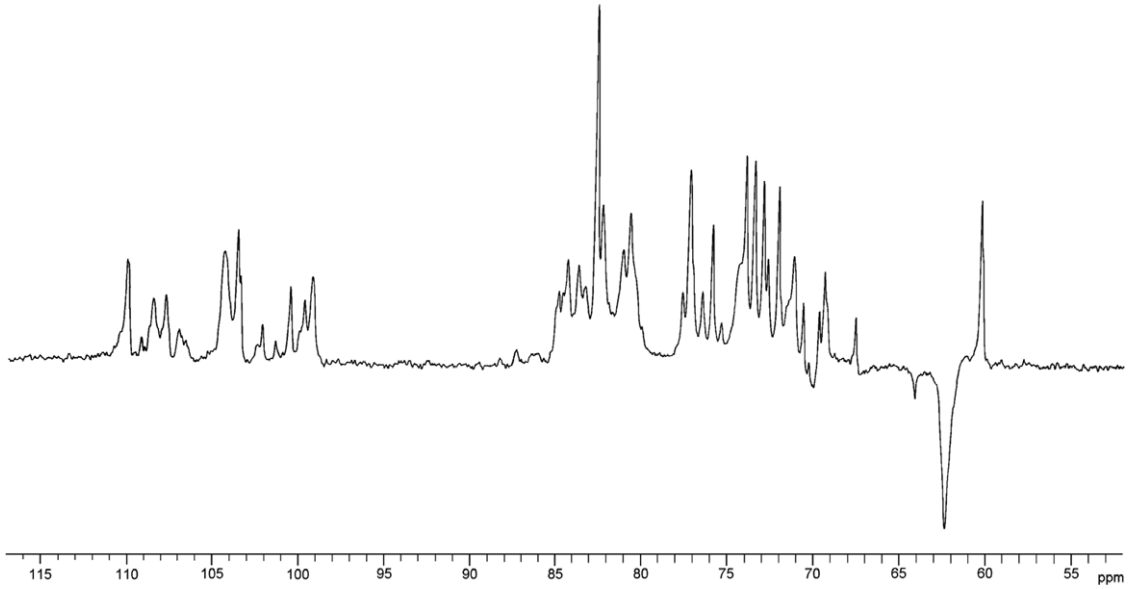

Fig. 2. DEPT-135 spectrum of *S. purpurea* polysaccharide in D_2O.

Table 3
^{13}C NMR chemical shift for *S. purpurea* polysaccharide

Unit	Chemical shift (ppm)							Reference
	C-1	C-2	C-3	C-4	C-5	C-6	O–CH_3	
β-Galactose								
(1→3)linked	103.7	71.3	82.7	69.5	73.7	62.4	–	de Pinto et al. (1995) de Paula et al. (1998) de Pinto et al. (2000a, 2000b) Defaye and Wong (1986)
(1→6)linked	103.7	70.7	72.8	67.7	73.1	69.5	–	de Pinto et al. (1995) de Pinto et al. (2000a, 2000b) de Paula et al. (1998)
α-Arabinose								
(1→3)linked	109.3	80.8	83.4	83.8	62.4	–	–	de Pinto et al. (1995) de Paula et al. (1998)
(1→)linked	110.0	85.0	77.3	84.9	62.4	–	–	de Paula et al. (1997) de Pinto et al. (1995) de Paula et al. (1998)
β-Glucuronic acid	104.6	75.5	76.6	73,7	77.4	176.7	–	de Pinto et al. (1994) de Pinto et al. (1993)
4-*O*-methyl-glucuronic acid	100.6	72.3	74.2	82.4	69.5	176.0	60.8	de Pinto et al. (1994) de Pinto et al. (1993)

9.12 mL. Venezuelan sample is reported to be homogeneous in relation to molar mass with molar mass of 1.6×10^6 g/mol (de Pinto et al., 1996).

3.4. Use of the modified polysaccharide for affinity chromatography

The crosslinked polysaccharides obtained were evaluated with respect to the gel formation, the capacity of binding lectins and the facility of permeation. No gel was obtained from treatments A0, A1 and A2 (Table 4) (the same treatment used for galactomannan from *Parkinsonia aculeate* (Garros-Rosa et al., 2006)). Higher gum concentration was used in treatment *B* (0.435 g/mL) and *F*3–*F*5 and *F*7–*F*11 (0.333 g/mL) result in gel formation. This indicates a clear dependence on the polysaccharide concentration toward gel formation. This behavior can be due to exudate polysaccharide structure that is more branched than galactomannan causing the necessity of a higher gum concentration for crosslinking formation. No or small amount of gels were also formed by treatments *F*1, *F*2 and *F*6 (Table 4), probably due to the low epichlorohydrin(E)/polysaccharide(P) ratio (<5 for this treatments and >5 for other treatments). A gel of low strength was obtained by treatment *F*10 and a very compact gel was formed by treatment *F*3.

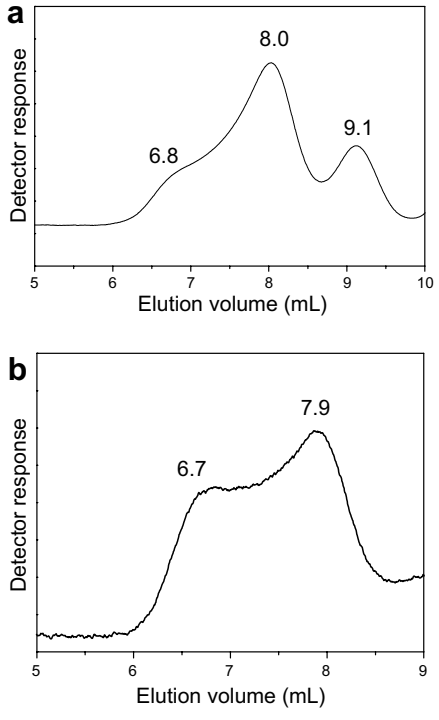

Fig. 3. GPC curves for *S. purpurea* polysaccharide: (a) refractive index detector; (b) UV detector.

Table 4
Mass of the gels after cross-linking

Treatment	Volume after soaked with water (mL)	Mass after cross-linking (g)
A_0	–	–
A_1	–	–
A_2	–	–
B	3.0	0.55
*F*1	–	–
*F*2	–	–
*F*3	3.0	0.49
*F*4	3.7	0.49
*F*5	2.3	0.55
*F*6	–	0.22
*F*7	2.4	0.58
*F*8	3.0	0.55
*F*9	1.7	0.61
*F*10	3.5	0.50
*F*11	2.3	0.49

Jacalin is a tetrameric and bivalent α-D-galactose-specific lectin isolated from jack fruit seeds, first described by Moreira and Ainouz (1981). Its isolation in a pure state can be possible by many methodologies. For example: by affinity chromatography on a crosslinked guar polysaccharide column (Sastry et al., 1986; Swaminathan, Gupta, & Surolia, 2000), on an IgA1-Sepharose affinity column (Hagiwara, Collet-Cassart, Kobayashi, & Vaerman, 1988), by Rivanol (6,9-diamino-2-ethoxyacrilidine lactate) treatment (Ahmed & Chatterjee, 1989), by preparative anion-exchange high-performance liquid chromatography (HPLC) (de Simone, Santos, Araújo, & Pinho, 1994), by affinity chromatography on a D-galactose-agarose matrix (Santos-de-Oliveira, Dias-Baruffi, Thomaz, Beltramini, & Roque-Barreira, 1994), on a galactose-Sepharose 4B (Bourne, Astoul, Zamboni, Peumans, & Menu-Bouaouiche, 2002) and *Adenanthera pavonina* (Moreira et al., 1998) and *Parkinsonia aculeate* (Garros-Rosa et al., 2006) galactomannans. This protein presents two band by SDS–PAGE the non-glycosilated (12 kDa) and glycosilated (15 kDa) subunits and had an absorption value at 280 nm and 1 cm cell $A^{1\%,1\,cm}_{280\,nm}$ of 15.8 (Swaminathan et al., 2000; de Simone et al., 1994; Young, Johnston, Szabo, & Watson, 1989).

Artocarpus integrifolia lectin (jacalin) was applied to *S. purpurea* gel (*B*, *F*4, *F*5, *F*7, *F*8, *F*9 and *F*11), the affinity chromatography for these columns are shown in Fig. 4 and the binding efficiency for this lectin is reported on Table 5. The efficiency of these gels in retaining jacalin was compared. Treatments *F*4 and *F*5 were the most efficient.

Frutalin is a tetrameric and bivalent D-galactose-specific lectin. It has been isolated by affinity chromatography on human stroma (Moreira & Ainouz, 1981) or crosslinked *Adenanthera pavonina* galactomannan (Moreira et al., 1998). This protein shows two bands by SDS–PAGE (15.5 kDa and 12 kDa) subunits and had an absorption value at 280 nm and 1 cm cell $A^{1\%,1\,cm}_{280\,nm}$ of 10.73. Gels used as matrix to retain the jacalin lectin were evaluated to isolate *A. incisa* lectin (frutalin) (Table 5). Although frutalin shows structural homology with jacalin (Campana, Moraes, Monteiro-Moreira, & Beltramini, 2002) a low efficiency on lectin retention by *S. purpurea* gel matrices was observed. The matrix with higher frutalin efficiency was *F*4.

Table 5
Effect of gel preparation conditions on binding efficiency to *Artocarpus* lectin

Columns	Efficiency (mg lectin/g of gel)	
	A. intregrifolia (jacalin)	*A. incisa* (frutalin)
B	19.56	11.02
*F*4	32.55	14.65
*F*5	28.87	8.47
*F*7	2.19	10.84
*F*8	2.18	3.89
*F*9	3.11	4.89
*F*11	3.23	0.94

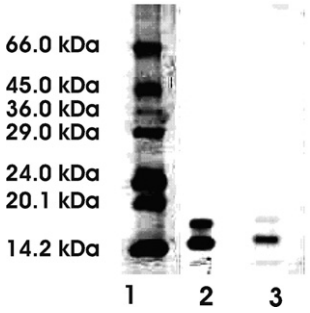

Fig. 5. SDS–polyacrylamide gel eletrophoresis in the presence of the molar mass standards (line 1), *Artocarpus integrifolia* lectin (jacalin) (line 2), *Artocarpus incise* lectin (frutalin) (line 3) isolated from affinity chromatography on *Spondia purpurea F*4 column.

The purity of the jacalin and frutalin obtained by affinity chromatography on the *S. pupurea* matrix (column *F*4) was evaluated by SDS–PAGE. Fig. 5 shows the SDS–PAGE of molar mass standards (line1), jacalin lectin (line 2) and frutalin lectin (line 3) isolated from column *F*4. As can be seen the lectins shows only molar mass bands corresponding to the lectins (15 kDa and 12 kDa for jacalin and 15.5 and 12 kDa for frutalin) and both isolated fractions showed hemagglutinating properties. No other molar mass

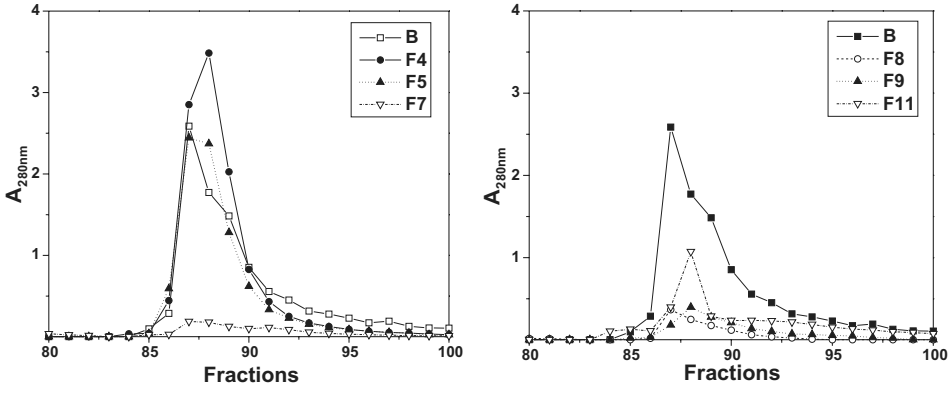

Fig. 4. Affinity chromatography of *Artocarpus integrifolia* in different columns.

Table 6
Effect of lectin type on binding efficiency of *F*4 *S. purpurea* gel

Lectin	Efficiency (mg lectin/g of gel)
Artocarpus intregrifolia (jacalin)	32.55
Artocarpus incisa (frutalin)	14.65
Ricinus communis	5.3
Erythrina velutina	2.4
Dioclea altissima	nd
Canavalia maritime	nd

nd, not detected.

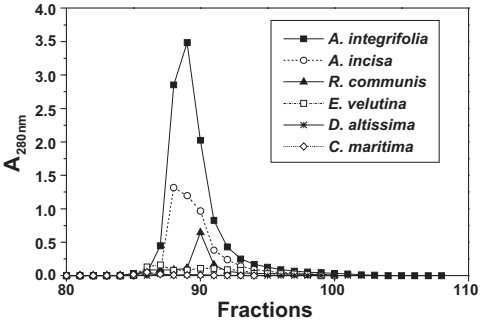

Fig. 6. Affinity chromatography of galactose-binding-lectins *Ricinus communis* and *Erythrina velutina* and D-glucose-binding-lectins from *Dioclea altissima* and *Canavalia maritime* on column *F*4.

bands observed in the crude extract from *A. integrifolia* and *A. incise* seeds (data not shown) were detected at the fraction obtained by *F*4 column.

The matrix from treatment *F*4 was also used to evaluate its capacity to retain other D-galactose-binding lectins from *Ricinus communis* and *Erythrina velutina* and D-glucose-binding-lectins from *Dioclea altissima* and *Canavalia maritime* (Table 6, Fig. 6). No interaction was observed with the D-glucose-binding-lectins from *Dioclea altissima* and *Canavalia maritima* (Fig. 6). The efficiency to bind galactose-biding lectin by *S. purpurea* gels (*F*4) was: *Artocarpus intregrifolia* > *Artocarpus incise* > *Ricinus communis* > *Erythrina velutina*.

4. Conclusion

Spondias purpurea L. exudate polysaccharide is composed of (1→3) linked galactan backbone substituted at C6 with D-galactose, D-xylose, L-arabinose, L-rhamnose and glucuronic acid units. Brazilian polysaccharide differs from Venezuelan on the amount of acid and arabinose as well as the presence of fucose and glucose as minor sugar. The results obtained show that the exudate polysaccharide of *S. purpurea* after crosslinking with epichlrohydrin was able to specifically isolate galactose-binding-lectins. It was also observed that interaction was more effective for the *Artocarpus* lectins than for *Ricinus communis* and *Erythrina velutina* lectins.

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References

Ahmed, H., & Chatterjee, B. P. (1989). Further characterization and immunochemical studies on the carbohydrate specificity of jackfruit (*Artocarpus integrifolia*) lectin. *The Journal of Biological Chemistry, 264*, 9365–9372.

Aiulyte, J., Liesiene, J., & Niemeyer, B. (2006). Evaluation of cellulose-based biospecific adsorbents as a stationary phase for lectin affinity chromatography. *Journal of Chromatography B, 831*, 24–30.

Appukuttan, P. S., Surolia, A., & Bachhawat, B. K. (1977). Isolation of two Galactose-binding Proteins from *Ricinus communis* by affinity chromatography. *Indian Journal Biochemistry and Biophysics, 14*, 382–384.

Baethgen, W. E., & Alley, M. M. (1989). A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. *Communications in Soil Science and Plant Analysis, 20*, 961–969.

Blum, H., Beier, H., & Gross, H. J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis, 8*, 93–99.

Bourne, Y., Astoul, C. H., Zamboni, W. J., Peumans, L., Menu-Bouaouiche, L., et al. (2002). Structural basis for the unusual carbohydrate-binding specificity of jacalin towards galactose and mannose. *Biochemistry Journal, 364*, 173–180.

Brett, C., & Waldron, K. (1996). *Physiology and biochemistry of plant cell walls*. London: Chapman and Hall.

Campana, P. T., Moraes, D. I., Monteiro-Moreira, A. C. O., & Beltramini, L. M. (2002). Unfolding and refolding studies of frutalin, a tetrameric D-galactose binding lectin. *European Journal of Biochemistry, 269*, 753–758.

Caron, M., Sève, A. P., Bladier, D., & Joubert-Caron, R. (1998). Glycoaffinity chromatography and biological recognition. *Journal of Chomatography B, 715*, 153–161.

Defaye, J., & Wong, E. (1986). Structural studies of gum arabic, the exudate polysaccharide from Acacia senegal. *Carbohydrate Research, 150*, 221–231.

de Paula, R. C. M., Budd, P. M., & Rodrigues, J. F. (1997). Characterization of *Anadenanthera macrocarpa* exudate. polysaccharide. *Polymer Internacional, 44*, 55–60.

de Paula, R. C. M., Heatley, F., & Budd, P. M. (1998). Characterization of *Anacardium occidentale* exudate polysaccharide. *Polymer International, 45*, 27–35.

de Paula, R. C. M., & Rodrigues, J. F. (1995). Composition and rheological properties of cashew tree gum, the exudate polysaccharide from *Anacardium occidentale* L.. *Carbohydrate Polymers, 26*, 177–181.

de Paula, R. C. M., Santana, A. S., & Rodrigues, J. F. (2001). Composition and rheological properties of *Albizia lebbeck* gum Exudate. *Carbohydrate Polymers, 44*, 133–139.

de Pinto, G. L., Alvarez, S., Martinez, M., Rojas, A., & Leal, E. (1993). Structural studies of *Melicocca bijuga* gum exudate. *Carbohydrate Research, 239*, 257–265.

de Pinto, G. L., Martinez, M., Beltrán, O., Ricon, F., Igartuburu, J. M., & Luis, F. R. (2000b). Strutural investigation of the polysaccharide of *Spondias mombin* gum. *Carbohydrate Polymers, 43*, 105–112.

de Pinto, G. L., Martinez, M., Corredor, A. L., Rivas, C., & Ocando, E. (1994). Chemical and ^{13}C NMR studies of *Enterolobium cyclocarpum* gum and its degradation products. *Phytochemistry, 37*, 1311–1315.

de Pinto, G. L., Martinez, M., Mendoza, J. A., Avila, D., Ocando, E., & Rivas, C. (1995). Comparison of three Anacardiaceae gum exudates. *Biochemical Systematics and Ecology, 23*, 151–156.

de Pinto, G. L., Martinez, M., Mendoza, J. A., Avila, D., Ocando, E., & Rivas, C. (1996). Structural study of the polysaccharide isolated from

Spondias purpurea gum exudates. *Carbohydrate Research, 290*, 97–103.

de Pinto, G. L., Martinez, M., Sanabria, L., Ricon, F., Vera, A., Beltrán, O., et al. (2000a). The composition of two *spondias* gum exudates. *Food Hydrocolloids, 14*, 259–263.

de Simone, S. G., Santos, R., Araújo, M. F., & Pinho, R. T. (1994). Preparative isolation of the lectin jacalin by anion-exchange high-performance liquid chromatography. *Journal of Chromatography A, 688*, 357–362.

Fujita, Y., Oishi, K., Suzuki, K., & Imahory, K. (1975). Purification and Properties of an Anti-B Hemagglutinin Produced by *Streptomyces* sp.. *Biochemistry, 14*, 4465–4470.

Garros-Rosa, I., Reicher, F., Petkowicz, C. L. O., Sierakowski, M. R., et al. (2006). Characterization of the galactomannans from *Parkinsonia aculeata* seeds and their application on affinity chromatography. *Polímeros Ciência e Tecnologia, 16*, 99–103.

Gutiérrez de Gotera, O., Martinez, M., Sanabria, L., de Pinto, G. L., & Igartuburu, J. M. (2005). 1D and 2D-NMR spectroscopy studies of the polysaccharide gum from *Spondias purpurea* var. Lutea. *Food Hydrocolloids, 19*, 37–43.

Hagiwara, K., Collet-Cassart, D., Kobayashi, K., & Vaerman, J. P. (1988). Jacalin: Isolation, characterization, and influence of various factors on its interaction with human IgA1, as assessed by precipitation and latex agglutination. *Molecular Immunology, 25*, 69–83.

Helmholz, H., Cartellieri, S., He, L., Thiesen, P., & Niemeye, B. (2003). Glicoaffinity chromatography and biological recognition. *Journal of Chromatography A, 1006*, 127–135.

Janson, J. (1987). On the history of the development of sephadex. *Chromatographia, 23*, 361–365.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature, 227*, 680–685.

Lima, R. S. N., Lima, J. R., Salis, C. R., & Moreira, R. A. (2002). Cashew-tree (*Anacardium occidentale* L.)exudates gum: a novel bioligand tool. *Biotechnology and Applied Biochemistry, 35*, 45–53.

Lis, H., & Sharon, N. (1981). Affinity chromatography for the purification of lectins (A review). *Journal of chromatography, 215*, 361–372.

Luck, K., Ehwald, R., Zizka, P., & Koppitz, H. (1992). Affinity sorbent for galactose specific lectins consisting of deproized plant cell wall ghosts. *Plant Science, 82*, 29–35.

Moreira, R. D., & Ainouz, I. L. (1981). Lectins from seeds of jack fruit (*Artocarpus integrifolia* l) - isolation and purification of 2 isolectins from the albumin fraction .1. *Biologia Plantarum, 23*, 186–192.

Moreira, R. A., Castelo-Branco, C. C., Monteiro, A. C. O., Tavares, R. O., & Beltramini, L. M. (1998). Isolation and partial characterization of a lectin from *Artocarpus incisa* L. seeds. *Phytochemistry, 47*, 1183–1188.

Moreira, R. A., & Perrone, J. C. (1977). Purification and Partial Characterization of a Lectin from *Phaseolus vulgaris*. *Plant Physiology, 59*, 783–787.

Pérez, R. M., Sanchez, J., Perez, S., & Vargas, R. (1995). An analytical study of gums from *Leucaena glabrata* and *Spondias purpurea*. *Journal of the Science of Food and Agriculture, 68*, 39–41.

Santos-de-Oliveira, R., Dias-Baruffi, M., Thomaz, S. M. O., Beltramini, L. M., & Roque-Barreira, M. C. (1994). A neutrophil migration-inducing lectin from *Artocarpus integrifoilia*. *Journal of Immunology, 153*, 1799–1805.

Sastry, M. V. K., Banarjee, P. B., Patanjali, S. R., Swamy, M. J., Swarnalatha, G. V., & Surolia, A. (1986). Analysis of saccharide binding to *Artocarpus integrifolia* lectin reveals specific recognition of t-antigen (beta-d-gal(1-]3)d-galnac). *The Journal of Biological Chemistry, 25*, 1726–1733.

Seshagirirao, K., Leelavathi, C., & Sasidhar, V. (2005). Cross-linked Leucaena seed gum matrix: An affinity chromatography tool for galactse-specific lectins. *Journal of Biochemistry and Molecular Biology, 38*, 370–372.

Swaminathan, C. P., Gupta, A., & Surolia, N. S. A. (2000). Plasticity in the primary binding site of Galactose/N-Acetylgalactosamine-specific Lectins. *Journal of Biological Chemistry, 275*, 28483–28487.

Vandevelde, M. C., & Fenyo, J. C. (1985). Macromolecular distribution of Acacia senegal gum (gum arabic) by size-exclusion chromatography. *Carbohydrate Polymers, 5*, 251–273.

Young, N. M., Johnston, R. A. Z., Szabo, A. G., & Watson, D. C. (1989). Homology of the D-Galactose-specific lectins from *Artocarpus integrifoilia*, and *Maclura pomifera* and the role of an unusual small polypeptide subunit. *Archives of Biochemistry and Biophysics, 270*, 596–603.