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Chemical studies on the polysaccharides of *Salicornia brachiata*

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ABSTRACT

A group of 12 polysaccharide extracts were prepared from the tips, stem and roots of an Indian halophyte *Salicornia brachiata* Roxb. obtained by sequential extractions with cold water (CW), hot water (HW), aqueous ammonium oxalate (OX) and aqueous sodium hydroxide (ALK) solutions. Monosaccharide composition analysis revealed that all the polysaccharide extract samples consisted primarily of rhamnose, arabinose, mannose, galactose, glucose, whereas ribose and xylose were present only in some of the extracts. All the extracts exhibited low apparent viscosity (1.47–2.02 cP) and sulphate and contained no prominent toxic metal ions. Fucose was detected only in OX extract of the roots. These polysaccharides were found to be heterogeneous and highly branched (glycoside linkage analysis, size-exclusion chromatography, ^{13}C-NMR, FT-IR, circular dichroism and optical rotation data). Physico-chemical analyses of these polysaccharides including uronic acid, sulphate and protein contents were also carried out. This constitutes the first report on the profiling of *Salicornia* polysaccharides.

1. Introduction

Polysaccharides are relatively complex carbohydrates and the first biopolymer found in nature (Tolstoguzov, 2004). These polymers are made up of either single or many monosaccharides joined together by glycosidic bonds forming large, often branched, macromolecules. They play a number of roles in biological functions like respiration, mechanical strength, source of energy, stress tolerance etc. (Sznaidman, 1999). Polysaccharides occur in the cell wall and in intercellular regions of plants building flexible structures suited to their environment. Polysaccharides can be modified by derivatization involving their hydroxyl groups to impart new properties, giving rise to new functions (Pourjavadi, Barzegar, & Zeidabadi, 2007; Mehta, Kondaveeti, & Siddhanta, 2011; Chhatbar, Prasad, Chejara, & Siddhanta, 2012; Oza, Meena, & Siddhanta, 2012).

Salicornia brachiata Roxb. (belonging to Kingdom-*Plantae*, Division-*Tracheophyta*, Class-*Magnoliopsida*, Order-*Caryophyllales*, Family-*Chenopodiaceae*, Genus-*Salicornia* and Species-*brachiata*) (www.itis.gov) is a marine halophyte, capable of growing in a very high saline soils, salt marshes and mud flats associated with mangrove wetlands of India. *S. brachiata* is erect or decumbent, fleshy jointed branching shrubs or herbs, about 20–45 cm high, which have distinct three parts–roots, stem and the tender tips. *Salicornia* species have been reported to be used in folk medicines for various maladies (Lee et al., 2006; Park & Kim, 2004). Bioassays of the polysaccharides of *Salicornia herbacea* (Lee et al., 2006; Im, Kim, & Lee, 2006) and phytochemical studies of *Salicornai fruticosa* (Radwan, Nazif, & Abou-Setta, 2007) have been reported in the literature. Antitubercular, antibacterial and antioxidant activities were reported from *S. brachiata* of Indian coast (Rathod et al., 2008; Manikandan, Neelakandan, & Usha Rani, 2009; Rathod et al., 2011; Daffodil, Rajalakshmi, & Mohan, 2013). *S. brachiata* is an important halophyte species providing animal fodder, herbal salt and seeds yielding oil that is used by mangrove dependent villagers as a vegetable diet (Ghosh et al., 2002; Eganathan, Subramanian, Latha, & Rao, 2006), while the ash of the whole plant has been reported to be useful in itch treatment (Ravindran, Venkatesan, Balakrishnan, Chellappan, & Balasubramanian, 2005). The MALDI-TOF mass spectral profiling of oligosaccharides of the seedlings of *S. brachiata* was studied and described this plant to be a potential source of dietary supplements, since the mass spectra of the oligomers exhibited xyloglucan building blocks which resembled those of highly nutritious plant soybean (Mishra, Joshi, & Jha, 2013). Cellulose profiling of *S. brachiata* and synthesis of the corresponding fluorogenic cellulose amide derivatives exhibiting metal scavenging properties have been reported (Sanandiya, Prasad, Meena, & Siddhanta, 2010; Sanandiya & Siddhanta, 2013).

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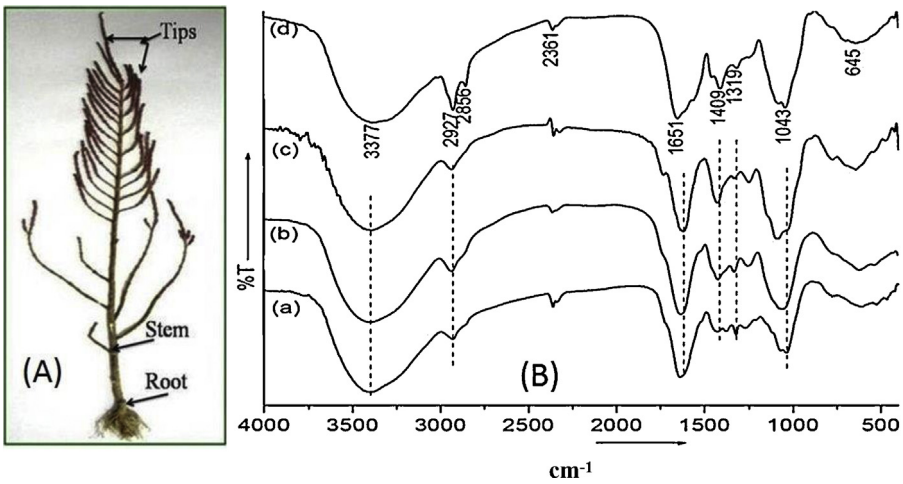

Fig. 1. (A) Image of the herbarium of *Salicornia brachiata*; (B) FT-IR spectra of (a) CWt, (b) HWt, (c) OXt and (d) ALKt.

There exists no report on the detailed chemical studies on the polysaccharides of *Salicornia* sp. Therefore, it was decided to chemically investigate and characterize the polysaccharides of *S. brachiata*.

2. Experimental

2.1. Materials

S. brachiata Roxb. used in this study was collected from Diu (20° 43′56″N, 70° 55′45″E) at the western coast of India. The plant was taxonomically identified and a herbarium of the plant was deposited in the CSIR-CSMCRI Herbarium [No. CSM-AL-II-125-05]. The plants were divided into three parts, namely tips, stems and roots (Fig. 1A). Standard sugars Rha, Fuc, Rib, Ara, Xyl, Man, Gal and Glc (AR) were purchased from M/s Aldrich Chem. Co., USA. Other laboratory grade chemicals were purchased from M/s S.D. Fine Chemicals Ltd, Mumbai, India, and were used without further purification. Demineralizd water (MilliQ) was used for extractions.

2.2. Sequential extraction

Polysaccharides of the tips of *S. brachiata* were extracted to yield cold water extract (CWt), hot water extract (HWt), 0.25 M ammonium oxalate extract (OXt) and 0.75 M sodium hydroxide extract (ALKt) employing the methods reported by Tomshich et al. (1997) and Siddhanta, Goswami, Ramavat, Mody, and Mairh (2001). Dry powdered tips of *S. brachiata* was first de-pigmented and defatted by repeated extractions (3 × 24 h) with methanol in a percolator. For the cold water extract (CWt), de-pigmented dried powder (100 gm) was soaked in 20 volumes (w/v) of demineralized (DM) water overnight at 10 °C. Extract was filtered through muslin cloth followed by vacuum filtration over a Celite bed on a Buchner funnel. The filtrate was clarified by centrifugation at 8000 rpm for 15 min to obtain brown colored clear supernatant, which was concentrated up to 1/3 of its volume on a rotary evaporator under reduced pressure at 50 °C. Concentrated extract was precipitated with isopropyl alcohol (extract:IPA—1:2 v/v) and precipitates were recovered by centrifugation. Precipitated extract were dissolved in minimum volume of distilled water, dialyzed (Sigma dialysis tubing, molecular weight cut off 1200 Dalton) against tap water for 6 h, and then against distilled water until it became chloride free. Salt-free extract was lyophilized on a VirTis Freeze Dryer, USA, to yield crude cold water extracted polysaccharides (CWt). The plant residue obtained after the cold-water extraction was further extracted with hot water at 80 °C for 3 h followed by filtration and was further purified by the method mentioned above to yield the hot water extracted polysaccharides (HWt) (cf. Siddhanta et al., 2001). The plant residue obtained after hot water extraction was submerged in a 0.25 M aqueous solution of ammonium oxalate (1:10 w/w), and the mixture was heated up to 80 °C on a water bath for 2 h. The solution was then separated by filtration and purified it by following the method mentioned above to yield ammonium oxalate soluble polysaccharides (OXt) [cf. Tomshich et al. (1997)]. The plant residue obtained after oxalate extraction was washed with hot water (3 × 1 v) to free it from oxalate, which was then submerged in a 0.75 M solution of sodium hydroxide, and the mixture was heated up to 80 °C on a water bath for 2 h followed by purification method mentioned above to yield alkali soluble polysaccharides (ALKt) [cf. Tomshich et al. (1997)]. The same set of methods of extraction was employed to prepare extracts from the stem and roots of *S. brachiata*.

2.3. Characterization

The weight average molecular weight (Mw) was determined by size exclusion chromatography (SEC) on a Waters Alliance 2695 machine with RI 2414 detector. Columns (Ultra hydragel 120, Ultra hydragel 500) were eluted with 0.1 M $NaNO_3$ at a flow rate of 0.5 mL min^{-1}. Oven and flow cell temperatures were maintained at 45 °C for all measurements. Dextrans of different molecular weights were used as standards for calibration. Area measurements, calculations of molecular mass and polydispersity index were determined using Empower 2 Software, USA. Uronic acid content was estimated by the method of Knutson and Jeanes (1968). Moisture contents were considered as the losses in mass from a sample (1 g) after heating at 100 °C ± 2°C on a Moisture analyzer (SARTORIUS A G GOTIINGEN MA30-000V3, 12201154, GERMANY). Polysaccharide samples were ignited at (600° ± 10 °C for 6 h) and percentage of ash contents was calculated based on the weights of the dried polysaccharide samples. Protein content was calculated from percentage nitrogen (%N with respect to the polysaccharide) determined by Kjeldahl method on a KEL PLUS-KES 201 Digestion unit attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN equipments, Chennai, India) using the factor 6.25 (Marks, Buchsbaum, & Swain, 1985). Apparent viscosity was measured using a Brookfield Viscometer (DV-II +Pro) at 27 °C (1% solution) using SC4-18 spindle at 30 rpm speed.

The monosaccharide composition of polysaccharide samples were determined by reductive hydrolysis method (GC–MS)

Table 1
Yield and physicochemical data of the polysaccharides of the *Salicornia brachiata*.

Samples[a]		Yield[b]	Moisture	Total sugar	Uronic acid	Ash	SO_4^{2-}	Protein	Apparent viscosity[c] (cP)	Molecular weight distribution		
		(% ±SD)[d]								Mw	Mz	PDI
CW	Tips	1.70 ± 0.1	15.0 ± 0.9	54.00 ± 1.8	0.90 ± 0.1	11.80 ± 0.8	3.73 ± 0.1	10.00 ± 0.5	1.47	35,081	73,891	2.76
	Stem	1.00 ± 0.1	9.90 ± 0.5	56.02 ± 1.5	1.34 ± 0.1	12.60 ± 1.0	1.56 ± 0.1	5.50 ± 0.1	1.69	41,373	76,495	2.61
	roots	0.28 ± 0.05	9.90 ± 0.5	45.00 ± 1.1	0.49 ± 0.06	17.30 ± 1.2	2.65 ± 0.1	10.00 ± 0.3	1.68	46,255	106,475	2.49
HW	Tips	3.00 ± 0.2	5.94 ± 0.4	50.93 ± 1.2	1.78 ± 0.1	20.30 ± 1.5	3.30 ± 0.1	13.88 ± 0.5	1.69	52,264	113,575	2.37
	Stem	1.60 ± 0.1	10.89 ± 0.8	44.67 ± 1.0	2.11 ± 0.1	14.00 ± 1.1	2.97 ± 0.1	8.81 ± 0.5	1.86	40,409	80,448	2.33
	Roots	0.60 ± 0.05	6.93 ± 0.4	53.00 ± 1.6	0.86 ± 0.1	21.70 ± 1.5	2.44 ± 0.1	11.63 ± 0.5	1.88	41,058	75,619	2.14
OX	Tips	1.20 ± 0.1	10.89 ± 0.6	48.53 ± 1.6	1.63 ± 0.1	22.00 ± 1.5	2.02 ± 0.05	6.13 ± 0.2	1.42	37,220	66,310	2.11
	Stem	0.54 ± 0.05	10.89 ± 0.5	54.02 ± 1.7	1.85 ± 0.1	14.10 ± 1.2	1.47 ± 0.05	6.13 ± 0.2	1.82	23,879	38,916	1.60
	Roots	0.36 ± 0.05	8.65 ± 0.6	39.00 ± 1.3	1.31 ± 0.05	18.30 ± 1.5	1.32 ± 0.05	7.94 ± 0.3	1.53	22,393	37,694	1.70
ALK	Tips	14.50 ± 0.5	11.54 ± 0.5	52.41 ± 1.5	1.24 ± .05	8.40 ± 1.0	1.25 ± 0.05	2.19 ± 0.1	1.71	92,496	468,751	4.63
	Stem	5.10 ± 0.3	9.00 ± 0.5	58.12 ± 1.5	0.57 ± 0.05	7.10 ± 1.0	0.51 ± 0.05	1.75 ± 0.1	1.51	50,725	99,211	2.11
	Roots	2.60 ± 0.1	14.71 ± 0.8	55.39 ± 1.7	1.03 ± 0.05	12.60 ± 1.0	0.43 ± 0.05	4.56 ± 0.2	2.02	51,097	71,277	1.68

[a] CW = Coldwater extract; HW = hotwater; OX = aqueous ammonium oxalate; ALK = aqueous sodium hydroxide.
[b] Yield was calculated on the basis of dry weight of plant part; other constituents in % weight of the respective polysaccharides.
[c] Apparent viscosity was measured in 1% solution at 27 °C, using spindle SC4-18 at 30 rpm speed.
[d] Data presented are the means of triplicate measurements (±SD).

reported by Stevenson and Furneaux (1991). Glycosyl linkage analyses were carried out by preparing partially methylated alditol acetates (PMAA) of polysaccharides by following the method reported by Ciucanu and Kerek (1984). The sugar composition and glycosyl linkage analyses of the polysaccharide samples were carried out on Shimadzu GCMS-QP2010 machine, using an SGE BP-225 capillary column and helium as a carrier gas. The temperature programs were as follows. For alditol acetates and determination of absolute configuration: 160 °C to 230 °C at 10 °C min^{-1}; for partially methyl alditol acetates (PMAA): 50 °C hold for 2 min to 215 °C at 40 $°C^{-1}$.

Spectral analyses, e.g. FT-IR and inductively coupled plasma-emission spectrophotometry (ICP) were carried out as reported earlier (Meena et al., 2007). The value of % sulphur, obtained from ICP, was multiplied by 3 to get % sulphate (SO_4^{2-}). ^{13}C NMR spectra were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer, Switzerland, at 125 MHz machine. The polysaccharide samples were dissolved in D_2O (60 mg mL^{-1}) and the NMR spectra were recorded at 70 °C, using dimethylsulfoxide (DMSO) as an internal standard (ca. δ 39.4 ppm). Circular dichroism (CD) of polysaccharide fractions (c 0.01% w/v, H_2O) were measured on a Jasco J-815 CD spectrometer, Japan. Circular dichroism (CD) spectra were recorded in the range 200-300 nm at the same concentration. All measurements were performed at room temperature (30 °C) using 1.0 cm quartz cells. Specific rotation $[\alpha]_{589}^{30°C}$ of polysaccharide samples was measured on a DigiPol DP781 (Rudolf Instruments, Inc. USA) machine at 0.01% concentration. The polarimeter was calibrated using sealed standard cells showing specific rotation values +15.59° and −13.79°.

3. Results and discussion

In this investigation extra- and intracellular polysaccharides of the three parts viz. tips, stem and roots (Fig. 1A) of the halophyte *S. brachiata* were extracted and characterized (cf. Tomshich et al., 1997; Siddhanta et al., 2001; Berovič et al., 2003).

3.1. Yields and physico-chemical properties

All the crude polysaccharide fractions of *S. brachiata* were fluffy solid and brown in color. Roots, stem and the tips of *S. brachiata* were separated after collection of the plant, the respective extracts (4 extracts each—coldwater, CW; hotwater, HW; aq. ammonium oxalate, OX and aqueous Sodium hydroxide, ALK) were subjected to chemical investigation. Physicochemical characterization of the polysaccharide were done by estimation of total carbohydrates, uronic acid, sulphate, protein, viscosity, ash and moisture contents and the data are furnished in Table 1. The yields of polysaccharide fractions of *S. brachiata* were calculated based on the dry weight of powdered plant material. The yields of alkali soluble polysaccharide (ALK) of all the three parts were significantly greater (ALKt-14.5%, ALKs-5.10% and ALKr-2.60%), whereas lowest yields were obtained with OXt (1.20%), OXs (0.54%) and CWr (0.28%) in tips, stem and roots, respectively (suffixes—r, s, t stand for respective extract of roots, stem and tips). The CWt, (54.0%) ALKs (58.12%) and ALKr (55.39%) polysaccharide fractions contained greatest total carbohydrate. All the polysaccharide fractions of *S. brachiata* exhibited low apparent viscosity (1.47–2.02 cP), uronic acid content (0.49–2.11 w/w%) and sulphate contents (0.43–3.73 w/w%). Proteins were

Table 2
Carbohydrate profile (GC–MS) of the polysaccharides of *Salicornia brachiata*.

Samples		Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
CW	Tips	2.90	–	1.19	24.58	3.93	10.05	17.69	39.66
	Stem	5.38	–	2.26	34.29	3.68	9.07	27.88	17.45
	Root	6.14	–	–	27.83	1.94	5.11	51.99	6.98
HW	Tips	7.34	–	2.32	35.57	4.70	11.67	16.19	22.21
	Stem	8.31	–	–	42.42	–	7.60	27.26	14.42
	Root	9.59	–	1.88	36.75	6.39	8.91	25.67	10.82
OX	Tips	28.00	–	–	35.94	–	8.91	17.02	10.13
	Stem	12.41	–	2.05	39.82	1.96	5.67	26.24	11.85
	Root	10.07	1.12	1.37	37.16	5.11	8.77	27.34	9.07
ALK	Tips	12.87	–	3.85	53.58	4.62	2.51	17.34	5.23
	Stem	6.40	–	2.93	30.94	36.23	3.44	17.48	2.57
	Root	0.70	–	–	9.77	86.16	0.50	2.65	0.20

Rha = Rhamnose, Fuc = fucose, Rib = ribose, Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose.

Table 3
Glycosyl linkage analysis of the polysaccharides of *Salicornia brachiata*.

Sugar	Deduced linkages	Mol%											
		CWt	CWs	CWr	HWt	HWs	HWr	OXt	OXs	OXr	ALKt	ALKs	ALKr
Rha	→2,4)-D-Rha*p* (1→	–	–	1.95	0.91	–	1.05	19.67	–	–	–	6.40	0.70
	→3,4)-D-Rha*p* (1→	0.64	1.10	1.44	–	2.52	1.42	–	9.59	2.48	12.87	–	–
Rha	→2,3,4)-D-Rha*p* (1→	2.26	4.28	2.75	6.43	5.79	7.12	8.33	2.82	7.58	–	–	–
	→2,3,4)-D-Rib*p* (1→	–	2.26	–	–	–	1.88	–	2.05	1.37	–	2.93	–
Ara	D-Ara*f* (1→	–	–	7.04	–	–	–	–	–	–	–	–	2.49
	→2)-D-Ara*p* (1→	–	–	4.59	–	2.27	–	–	–	–	1.36	5.29	–
	→3)-D-Ara*f* (1→	–	–	2.86	–	–	–	–	1.69	–	–	–	–
	→3)-D-Ara*p* (1→	–	3.82	–	–	–	1.04	–	–	–	–	–	–
	→4)-D-Ara*p* (1→	10.01	2.61	8.29	3.00	11.70	2.16	14.21	–	1.84	6.03	18.42	2.72
	→5)-D-Ara*f* (1→	–	–	–	–	–	3.62	–	2.68	–	–	–	–
	→2,3)-D-Ara*p* (1→	–	–	3.00	–	6.26	–	6.18	–	1.55	–	2.16	3.34
	→2,4)-D-Ara*p* (1→	–	1.22	–	–	–	–	–	–	–	1.97	–	–
	→3,4)-D-Ara*p* (1→	6.15	–	–	5.75	2.53	3.03	1.86	3.75	5.23	15.00	–	–
	→2,3,4)-D-Ara*p* (1→	8.43	26.64	2.04	26.83	19.66	27.94	13.69	31.70	28.54	29.22	5.06	1.23
Xyl	D-Xyl*p* (1→	–	–	–	–	–	–	–	–	–	–	–	2.14
	→3)-D-Xyl*p* (1→	3.93	–	–	–	–	–	–	–	–	–	14.98	76.41
	→2,3,4)-D-Xyl*p* (1→	–	3.68	2.70	4.70	–	5.35	–	1.96	5.11	4.62	21.25	7.61
Man	→4)-D-Man*p* (1→	–	–	1.50	–	–	–	–	–	–	–	–	–
	→6)-D-Man*p* (1→	–	–	–	–	–	–	–	–	–	–	–	0.50
	→3,4)-D-Man*p* (1→	–	–	0.63	–	1.34	–	3.42	–	–	–	–	–
	→3,6)-D-Man*p* (1→	–	–	3.14	–	–	–	–	–	–	–	–	–
	→4,6)-D-Man*p* (1→	–	–	1.34	–	1.37	–	–	–	–	–	–	–
	→2,3,4)D-Man*p* (1→	–	–	–	–	–	–	2.78	–	1.04	–	–	–
	→2,3,6)-D-Man*p* (1→	–	–	–	–	–	–	–	–	–	2.51	–	–
	→3,4,6)-D-Man*p* (1→	–	3.92	–	–	2.17	1.65	–	–	–	–	–	–
	→2,3,4,6)-D-Man*p* (1→	10.05	5.15	–	11.67	2.73	5.76	2.71	5.67	7.73	–	3.44	–
Gal	D-Gal*p* (1→(terminal)	–	–	–	–	0.90	–	1.14	–	–	–	–	–
	→3)-D-Gal*p* (1→	5.46	–	–	–	–	–	6.66	–	–	–	2.64	–
	→4)-D-Gal*p* (1→	–	–	–	–	9.47	–	–	–	–	–	–	–
	→6)-D-Gal*p* (1→	–	–	6.52	1.35	–	–	1.05	–	–	–	–	–
	→3,4)-D-Gal*p* (1→	–	–	3.79	–	–	–	–	–	–	–	–	–
	→4,6)-D-Glc*p* (1→	–	–	3.11	–	1.08	–	–	–	–	–	–	–
	→3,6)-D-Gal*p* (1→	–	–	21.54	–	–	–	–	–	–	–	–	–
	→2,3,4)-D-Gal*p* (1→	–	–	––	1.04	–	–	–	–	–	–	–	2.65
	→2,3,6)-D-Gal*p* (1→	–	–	4.66	–	–	–	–	–	–	–	–	–
	→3,4,6)-D-Gal*p* (1→	–	–	5.40	–	2.27	–	–	–	–	1.55	–	–
	→2,3,4,6)-D-Gal*p* (1→	12.23	27.88	6.97	13.80	14.66	25.67	8.16	26.24	27.34	15.79	14.84	–
Glc	D-Glc*p* (1→(terminal)	–	–	–	–	2.48	–	2.71	–	–	–	–	–
	→3)-D-Glc*p* (1→	7.66	–	–	–	1.94	–	–	–	–	1.93	–	–
	→4)-D-Glc*p* (1→	13.00	–	–	–	2.58	–	–	–	–	–	–	–
	→6)-D-Glc*p* (1→	6.40	–	4.02	1.40	–	–	1.52	–	–	–	–	–
	→2,3)-D-Glc*p* (1→	–	–	–	1.09	–	–	–	–	–	–	–	–
	→2,6)-D-Glc*p* (1→	–	–	–	–	–	–	3.95	–	–	–	–	–
	→3,4)-D-Glc*p* (1→	6.56	–	–	2.20	–	–	–	–	–	–	–	–
	→4,6)-D-Glc*p* (1→	–	–	–	1.33	2.23	–	–	–	–	–	–	–
	→2,3,4)-D-Glc*p* (1→	–	–	–	1.79	–	–	–	–	–	–	–	–
	→2,3,6)-D-Glc*p* (1→	–	–	–	3.51	–	–	–	–	–	–	–	–
	→3,4,6)-D-Glc*p* (1→	–	–	–	5.81	–	–	–	1.10	2.28	–	–	–
	→2,3,4,6)-D-Glc*p* (1→	6.03	17.45	2.96	5.07	4.07	10.82	1.95	10.75	6.79	3.30	2.57	–

estimated to be in the range 1.75–13.88 w/w%. All the polysaccharide samples were low in sulphate content, therefore these were not fractionated further on the basis of their charges. The high polydispersity index values in the molecular weight distribution indicated the highly branched, heterogeneous structure for the polysaccharides unlike synthetic polymers. The results of elemental analyses of the polysaccharide fractions indicated the presence of Al, Ca, K, Mg, Na, P (Table S1). The absence of some prominent toxic metal ions (e.g. As, Cd, Co, Cr, Hg, Mn, Ni and Pb) **in** all the polysaccharide samples suggested that these polysaccharides would be suitable for ingestible applications. High concentration of Ca, Mg, K and Na indicated the presence of inorganic salts due to the marine coastal habitat of the halophytic plant.

3.2. Monosaccharide compositions

The retention times (min) of alditol acetates of standard sugars were: rhamnitol acetate 10.40; fucitol acetate 10.54; ribitol acetate 11.03; arabinitol acetate 11.26; xylitol acetate 12.09; mannitol acetate 15.71; galactitol acetate 16.38; glucitol acetate 16.98 (Fig. S2). The sugar residues in the all polysaccharide samples were identified by comparing the relative retention times with those of the respective standard sugar alditol acetates as well as by comparison of their mass fragmentation patterns. The chromatograms of polysaccharide fractions of tips, stem and roots were depicted in Figs. S3–S5, respectively.

The GC–MS analysis of the alditol acetates of the crude polysaccharide fractions revealed the presence of different carbohydrate moieties in varied proportions (Table 2). The fractions CW and HW of the tips, stem and roots contained predominantly glucose, arabinose and galactose carbohydrate moieties, in a randomly distributed order, whereas OX fractions of all the three parts principally constituted of arabinose, galactose and rhamnose monosaccharides. Alkali extract of stem and roots mostly contained carbohydrates moieties in the order xylose > arabinose > galactose, whereas those in ALKt were arabinose > galactose > rhamnose. All the 12 polysaccharide samples contained at least five carbohydrate moieties i.e. rhamnose, arabinose, mannose, galactose and glucose

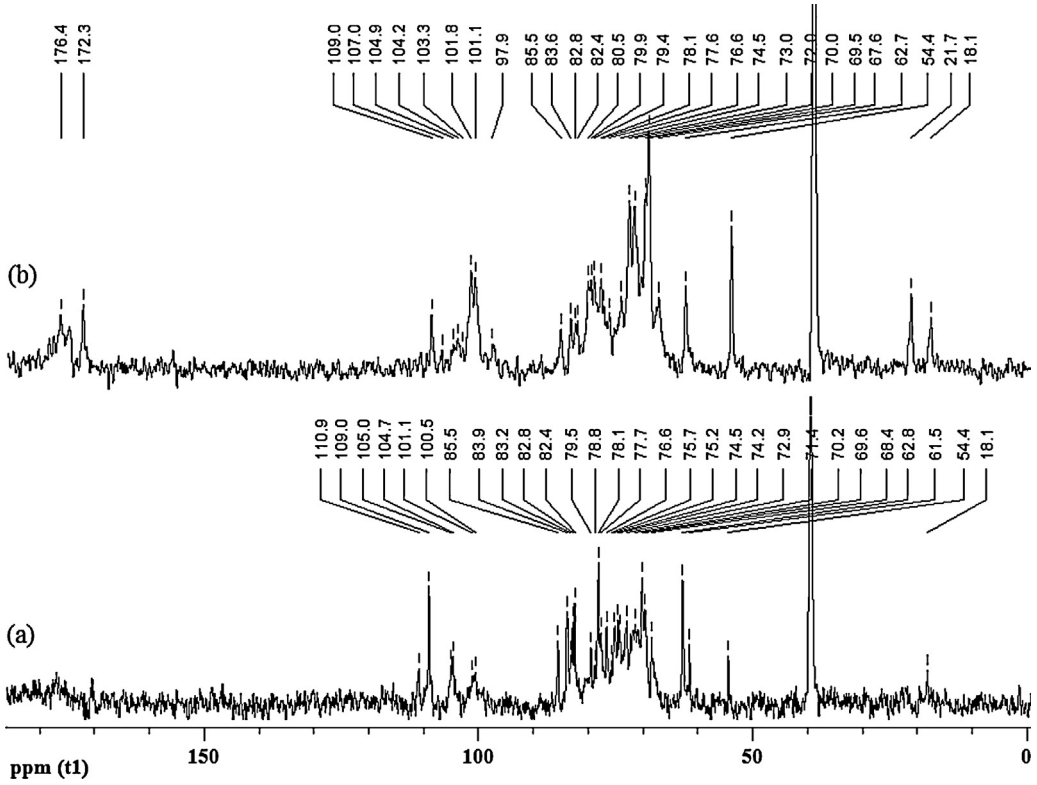

Fig. 2. ^{13}C NMR spectra of (a) CWt and (b) HWt.

in various proportions. Ribose was not found in CWr, HWs, OXt and ALKr fractions where as xylose was not found in HWs and OXt polysaccharide fractions. Fucose was present only in OXr. Uronic acid could not be converted into their alditol acetate as it got separated out during centrifugation of the hydrolyzed sample due to its insolubility in acidic and neutral aqueous pH media.

3.3. Glycosyl linkage analysis

Glycosyl linkage analysis was carried out by GC–MS analysis of partially methylated alditol acetates derivatives (PMAA) of polysaccharide fractions and the results are shown in Table 3. The mass fragmentation patterns of the polysaccharides PMAA samples were compared and validated with those of the respective PMAAs of individual standard sugars provided by CCRC (Complex Carbohydrate Research Centre) data bank (http://www.ccrc.uga.edu./specdb/ms/pmaa/pframe.html) as well as with the ones reported by Sassaki, Gorin, Souza, Czelusniak, and Iacomini (2005). Representative chromatogram and mass fragmentation patterns of PMAA of CWt polysaccharide fraction are depicted in Fig. S6. Those of the remaining other polysaccharide extracts have not been given to avoid repetition. The chromatograms of PMAAs of polysaccharide fractions of tips, stem and roots are depicted in Figs. S7–S9, respectively. The deduced glycosyl linkages obtained are furnished in Table 3. The mole% values of individual sugar moieties were found to be comparable with those obtained in the monosaccharide composition analysis. In general, formation of 1,3,4,5-tetra-*O*-acetyl-6-deoxy-2-*O*-methyl-L-mannitol indicated→3,4)-D-Rha*p* (1→linkage was attributed to pentose sugar, whereas that of 1,2,3,4,5,6-hexa-*O*-acetyl-D-mannitol it is →2,3,4,6)-D-Man*p* (1→linkage was attributed to hexose (Fig. S6). In methylation analysis, the uronic acid residues were not detected since uronic acid content was low (<2%; Table 1). The glycosidic linkage study revealed extremely diverse linkage patterns in all the polysaccharides. For example, the extract CWt contained the following sugars (mol%): Glc (39.66) > Ara (24.58) > Gal (17.69) > Man (10.05) > Xyl (3.93) > Rha (2.90) > Rib (1.19) (Table 2). Each one of these carbohydrate moieties were found to be variously linked (14 types; Table 3) with a polydispersity index value (PDI) of 2.76 (highly branched), which precluded one to decide on a particular backbone structure along with their linkage patterns. In fact the linkage patterns ran from 10 to 23 types in all the polysaccharide samples (Table 3). The PDI data given in Table 1 ranged from 1.60 to 4.63 in all the 12 polysaccharide samples, indicating highly branched structural scenarios. The sample ALKr showed 10 types of linkages (PDI 4.63), whereas CWr exhibited 23 types of linkage patterns (PDI 2.49). Therefore, no specific structure could be assigned to any of the 12 heteropolysaccharide samples with a reasonable degree of certainty.

3.4. FT-IR and ^{13}C NMR spectroscopy

The FT-IR spectra of CWt, HWt, OXt and ALKt were found to be well resolved (Fig. 1B) and the prominent bands appeared in the range, υ_{max} (KBr) (cm^{-1}): 3377–3400 (O—H *str*, *br*, *s*), 2927–2936 (C—H *str*, *w*), 1615–1651 (bound H_2O, *s*), 1409–1426 (C—H bending, *w*), 1249–1252 (>S=O, *w*), 1038–1051 (C—O—C *str*, *s*) [*br* = broad, *s* = strong, *m* = medium, *w* = weak, *str* = stretching]. The characteristic bands of all four fractions were found to be similar to those reported in the literature (cf. Mollet, Rahaoui, & Lamoine, 1998; Sun, Xu, Sun, Xiao, & Sun, 2005). The FT-IR spectra of polysaccharide fractions of stem and roots appeared to have such similarity as mentioned above (Figs. S10 and S11).

The ^{13}C NMR spectra of CWt and HWt showed complex patterns (Fig. 2). The anomeric carbon signals of various sugars were tentatively assigned by comparison with the data reported in the literature (cf. Breitmaier & Hollstein, 1976; Golovchenko, Ovodova, Shashkov, & Ovodov, 2002; Bilan et al., 2002; Coelho, Stasi, & Vilegas, 2003; Gutierrez, Martınez, Sanabria, Pinto, & Igartuburu, 2005; Vieira, Mendes, Gallao, & Brito, 2007; Serrero et al., 2010). The

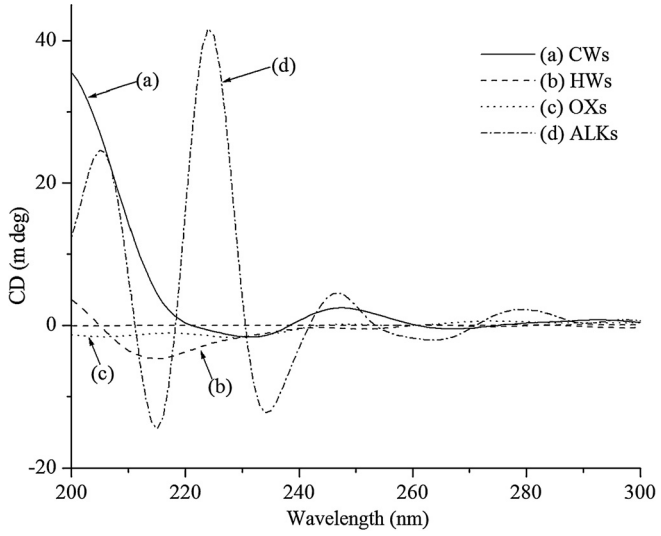

Fig. 3. Circular dichroism spectra of CWs, HWs, OXs and ALKs.

anomeric carbon signals of major sugars in the spectra of CWt were assigned (Fig. 2a): arabinose (110.9 and 109.01 ppm), galactose (105.0 ppm), glucose (104.7 ppm), xylose (101.1 ppm) and mannose (100.5 ppm). On the other hand the ^{13}C NMR spectrum of HWt showed eight anomeric carbon signals which were assigned to arabinose (109.0 and 107.0 ppm), galactose (104.9 and 97.9 ppm), glucose (104.2 ppm), xylose (103.3 ppm), rhamnose (101.8 ppm) and mannose (101.1 ppm) (Fig. 2b). The carbon resonance signals of CWt and HWt were in good agreement with the monomer composition (cf. Table 2). The carbon resonances in CWt and HWt in the range 85.5–54.4 ppm were due to the carbons C2–C6 of various sugar moieties, whereas the one appeared at 18.1 ppm in CWt and two signals at 21.17 and 18.1 were attributed to the methyl carbons of rhamnose. In the ^{13}C NMR of HWt, the downfield carbon resonances were observed at 176.4 & 172.3 ppm, which may be assigned to the carbonyl group of uronic acid residues (Table 2). The anomeric carbon assignments in other polysaccharide fractions were as follows.

In CWs, nine anomeric carbon signals of sugars were assigned to arabinose (110.7, 108.9 and 108.6 ppm), galactose (105.3 and 98.9 ppm), glucose (104.1 ppm), xylose (103.1 ppm) rhamnose (101.6 ppm) and mannose (100.5 ppm) (Fig. S12a). On the other hand the ^{13}C NMR spectrum of HWs showed eight anomeric carbon signals which were assigned to arabinose (108.9 ppm), galactose (104.9 and 97.6 ppm), glucose (104.1 and 103.1 ppm), rhamnose (101.6 ppm) and mannose (100.9 and 100.4 ppm), (Fig. S12b).

In CWr, nine anomeric carbon (C1) signals of sugars in spectra were assigned to arabinose (108.87), galactose (105.15, 98.96 and 97.74 ppm), glucose (104.91 ppm), xylose (103.16 ppm), rhamnose (101.54 ppm) and mannose (100.69 and 100.1 ppm) (Fig. S13a). On the other hand the ^{13}C NMR spectrum of HWr showed seven anomeric carbon signals which were assigned to arabinose (108.99 ppm), galactose (105.19 and 95.56 ppm), glucose (104.41 and 93.67 ppm), xylose (103.22 ppm) and rhamnose (101.65 ppm) (Fig. S13b).

3.5. Circular dichroism and specific rotation

The circular dichroism studies on carbohydrates have also been extensively applied for the determination of the structural characteristics of carbohydrates (McReynolds & Gervay-Hague, 2000; Dentini, Rinaldi, Barbetta, Risica, & Skjak-Bræk, 2006; Siddhanta et al., 2010). The absorption maxima of peak and trough are given in Table 4. The peak/trough ratio was calculated by the equation $(\theta_{peak} - \theta_{trough})/\theta_{trough}$. The pattern of CD spectra of CWs, HWts, OXs and ALKs showed chirality profile of this series of polysaccharides (Fig. 3), which exhibited manifestation of the chiroptical differences as a result of their compositional variations. The CD spectra of polysaccharides of tips and roots are depicted in Figs. S14 and S15, respectively.

All the polysaccharide fractions were chiral having different peak/trough ratios in the CD spectra, peak/trough ratio was the greatest in ALKr (11.94) and so were the specific rotation values of CWs and HWr. The differences in the λ_{max} values in the CD spectra also substantiated their different chiroptical make ups. All the

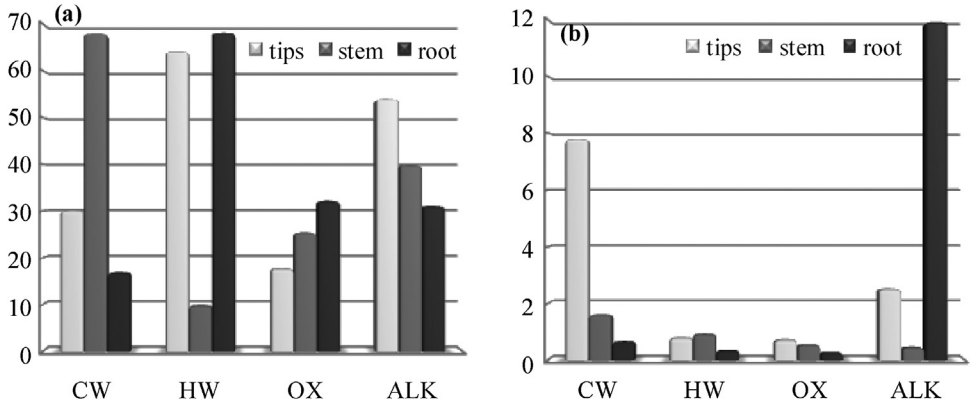

Fig. 4. Comparison of (a) specific optical rotation values and (b) peak/trough rations of CW, HW, OX and ALK of each part of *Salicornia brachiata*.

Table 4
Circular dichroism spectral and optical rotation data of the polysaccharides of *Salicornia brachiata*.

	CWt	CWs	CWr	HWt	HWs	HWr	OXt	OXs	OXr	ALKt	ALKs	ALKr
Peak (λ_{max}, nm)	210	247	213	282	243	228	216	217	250	210	224	217
Trough (λ_{max}, nm)	225	231	220	215	215	213	227	229	225	220	215	236
Peak/trough ratio	7.80	1.59	0.64	0.78	0.91	0.33	0.71	0.53	0.26	2.50	0.44	11.94
Specific rotation[a]	+30.09	+68.14	+16.85	+64.18	+9.74	+68.27	+17.65	+25.36	+32.19	+54.03	+39.87	+31.11

[a] Measured in 0.01% sample in water at 30 °C at 589 nm.

polysaccharide extracts exhibited significant specific rotation values, which showed reversed trends in the quanta in CW/HW and OX/ALK fractions (Fig. 4).

4. Conclusion

The polysaccharides of *S. brachiata* were found to be branched and heterogeneous containing minimum 5 and maximum 8 naturally occurring carbohydrates e.g. ribose, rhamnose, arabinose, xylose, mannose, galactose, glucose and fucose in different compositions and proportions. Fucose was detected only in OXr, the only polysaccharide contained all the eight carbohydrate monomers mentioned above. Xylose was absent in HWs and OXt, whereas ribose was not detected in CWr, HWs, OXt and ALKr. Except fucose all the seven carbohydrate monomers were present in ALKt, ALKs, HWt, HWr, CWt and CWs. Most of all the possible glycosidic linkages of the sugars present in this group of polysaccharides were identified in one or the other extracts, which contained no toxic metal ions and were low in sulphate (≤3.73% w/w). Monosaccharide composition and glycosidic linkage analyses and ^{13}C NMR spectra were coherently explicable to arrive at a particular structural identity. The CD spectra of the polysaccharide samples indicated their varied chiroptical make ups, as one would expect. This chemical study presents an important body of data which would be useful in bioprospecting work on halophytes. Each one of the polysaccharide extract samples was unique in composition and is of biogenetic significance. These polysaccharides are expected to be non-toxic and would contribute to the repertoire of important natural polysaccharide materials. Some of these polysaccharides may be of utility as a platform for generating materials of specific interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.05.072.

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