Original article

Sulfated xylomannans from the red seaweed *Sebdenia polydactyla*: structural features, chemical modification and antiviral activity

*Tuhin Ghosh[1], Carlos Alberto Pujol[2], Elsa Beatriz Damonte[2], Sharmistha Sinha[1] and Bimalendu Ray[1]**

[1]Natural Products Laboratory, Department of Chemistry, University of Burdwan, West Bengal, India
[2]Laboratorio de Virología, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales UBA, Ciudad Universitaria-Pabellón II Piso 4, Buenos Aires, Argentina

*Corresponding author: E-mail: bimalendu_ray@yahoo.co.uk

Background: Many viruses display affinity for cell surface heparan sulfate proteoglycans with biological relevance in virus entry. This raises the possibility of the application of sulfated polysaccharides in antiviral therapy.
Methods: In this study, we analysed polysaccharide fractions isolated from *Sebdenia polydactyla*.
Results: The purified xylomannan sulfate and its further sulfated derivatives showed strong activity against herpes simplex virus type-1 (HSV-1). Their 50% inhibitory concentration values were in the range 0.35–2.8 μg/ml and they lacked cytotoxicity at concentrations up to 1,000 μg/ml. The major polysaccharide, which had 0.6 sulfate groups per monomer unit and an apparent molecular mass of 150 kDa, contained a backbone of α-(1→3)-linked D-mannopyranosyl residues substituted at position 6 with a single stub of β-D-xylopyranosyl residues.
Conclusions: The degree of sulfation seemed to play an important role because desulfation and/or further sulfation of the isolated macromolecules largely influenced their *in vitro* anti-HSV-1 activity.

Introduction

In recent years, many compounds that have potent antiviral activity in cell culture have been detected and some of these compounds are currently undergoing either preclinical or clinical evaluation. Among these antiviral substances, sulfated polysaccharides from marine algae and of synthetic origin are noteworthy. These polysaccharides include mainly agarans, carrageenans, fucans, mannans, rhamnans and ulvans [1–3]. Indeed, some of these macromolecules are in various phases of clinical trials as microbicides [4–9]. Thus, evaluating the potential of sulfated polysaccharides extracted from marine algae as anti-herpes simplex virus type-1 (HSV-1) drug candidates will be of considerable interest.

One important parameter for antiviral activity of a polysaccharide is its degree of sulfation (DS), that is, the number of sulfate groups per monosaccharide residue. In addition, the antiviral potency has been thought to be dependent on molecular mass [3,8,9]. However, the relationships between the chemical structures of sulfated polysaccharides and their antiviral potency remain unclear.

In the course of our screening for anti-HSV-1 drug candidates from seaweed, we analysed the structural characteristics and antiviral properties of diverse types of sulfated polysaccharides [8,9]. Here, we report the structural characteristics of a purified sulfated xylomannan from the red seaweed *Sebdenia polydactyla* and the antiviral potency against HSV-1 of a series of xylomannans with different DS prepared by solvolytic desulfation and further chemical sulfation of the native polymer.

Methods

Chemistry

Plant material and preliminary treatments

Samples of *S. polydactyla* were collected from the Okha coast of Gujarat, India, in August 1995. The seaweeds were washed thoroughly with tap water, dried by forced air circulation and pulverized in a blender (Waring Products, Inc., Torrington, CT, USA). Algal powder (132 g) was depigmented using sequential extraction with petroleum ether and acetone as solvent in Soxhlet apparatus

(Borosil Glass Works, Ltd., Kolkata, India). The unextracted material was placed in a plastic beaker and air dried to yield depigmented algal powder (DAP; 91 g).

Extraction of sulfated xylomannan

Extraction of DAP (10 g) with water (pH 6.0) at a solute to solvent ratio of 1:100 (w/v) was conducted 3× at 25–32°C for 12 h under constant stirring. Separation of the residue from the extract was performed by filtration through a G2 glass filter. The residue was briefly washed with additional distilled water and the wash was collected to maximize polysaccharide recovery. The liquid extract was dialysed extensively against water and lyophilized. The recovered material was dissolved in water; the polysaccharides were precipitated twice with ethanol (4 volumes) and then collected by centrifugation. The final pellet was dissolved in water and lyophilized to yield the water-extracted polysaccharide, named WE (3.4 g). The aqueous 80% ethanol soluble fractions were combined, desalted on Sephadex™ G10 column (2.6×90 cm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) and finally lyophilized to produce the WE-S fraction.

Size exclusion chromatography

The WE fraction was chromatographed on a Sephacryl™ S-300 column (2.6×90 cm; Amersham Biosciences AB, Uppsala, Sweden) using 0.5 M sodium acetate buffer (pH 5.0) as eluent. The flow rate of the column was 0.5 ml/min, and fractions of 10 ml were collected and checked by the phenol–sulfuric acid reaction [10]. The column was calibrated with standard dextrans (500, 70, 40 and 10 kDa).

Chemical analyses

The chemicals used were of an analytical grade or the best available. All determinations were done at least in duplicate. Evaporation was performed under diminished pressure at approximately 45°C (water bath) and small volumes of aqueous solutions were lyophilized. Total sugar and uronic acid were determined by the phenol–sulfuric acid [10] and m-hydroxydiphenyl [11] assays, respectively. For the determination of sugar composition, the monosaccharide residues released by acid hydrolysis were converted into their alditol acetate [12] and analysed by gas–liquid chromatography (GLC; Shimadzu GC-17A; Shimadzu, Kyoto, Japan). Monosaccharides were identified by thin-layer chromatography and GLC–mass spectrometry (GLC–MS; Shimadzu QP 5050 A; Shimadzu) as described previously [13]. Alternatively, tetramethyl silyl (TMS) derivatives of methyl glycosides were analysed by GLC [14].

Sulfate estimation, desulfation and further sulfation

Estimation of sulfate by the modified barium chloride method [15] and infrared (IR) spectrometry [16], and solvolytic desulfation by the method of Falshaw and Furneaux [17] were carried out as described previously [18]. Further sulfation of the purified xylomannan sulfate (F1) was carried out as described previously [19]. Briefly, F1 (30 mg) and SO_3-pyridine (45 mg) was dissolved in 0.5 ml dry *N*, *N*-dimethylformamide by sonication followed by the addition of 50 µl of dry pyridine. The mixture was heated in an oil bath at 90°C under nitrogen atmosphere for 0.5, 1.0, 1.5 and 2.0 h; the solution was subsequently neutralized with NaOH, dialysed and lyophilized to give the sulfated polysaccharides F1S1, F1S2, F1S3 and F1S4, respectively.

Smith degradation

Periodate oxidation of the desulfated polysaccharide (F1D) was carried out as described by Fry [20]. Briefly, a solution of 100 mg of polysaccharide in 50 ml of reagent (50 mM $NaIO_4$ made up in 0.25 M formic acid, pH adjusted to 3.7 with 0.5 M NaOH) was incubated in the dark for 144 h at 4–6°C. Next, the excess of periodate was decomposed with 10 ml ethane-1,2 diol and the solution stirred for a further 1 h period at room temperature. To the vial containing oxopolysaccharide, a solution (50 ml) of 950 mg of $NaBH_4$ in 1 M NaOH was added and the mixture was kept at room temperature for 12 h. The solution was subsequently neutralized with acetic acid, dialysed and lyophilized to give the oxidized and reduced polysaccharide. This preparation was hydrolysed with trifluoroacetic acid (pH 2) for 10 min at 100°C and the resulting hydrolysate was desalted by passing through a column (2.6×90 cm) of Sephadex™ G10. Fractions (5 ml) were collected and analysed for their total sugar contents [10]. Appropriate polymeric fractions were lyophilized to yield a Smith-degraded xylomannan (F1D-Sm; 50 mg).

Linkage analyses

The triethylamine forms [21] of native (F1), desulfated (F1D) and desulfated Smith-degraded (F1D-Sm) xylomannan (5 mg of each) were subjected to three rounds of methylation [22]. Permethylated samples were hydrolysed, converted into their partially methylated alditol acetates and analysed by GLC and GLC–MS as described previously [23].

Spectroscopy

Recording of IR spectra and optical rotation measurements were carried out as described previously [23]. The ^{1}H NMR spectra of the F1, F1D and F1D-Sm were recorded on a Bruker 500 spectrometer (Bruker Biospin AG, Fallanden, Switzerland) operating at 500/600 MHz for ^{1}H. The sulfated xylomannan was converted into sodium salt by passage through a column (7 ml; Bio-Rad, Hercules, CA, USA) of Amberlite IR 120 (H^+), and all samples were deuterium-exchanged

Table 1. Sugar composition of fractions obtained from *Sebdenia polydactyla*

Sugar composition	WE	F1	F2	F1D	F1S1	F1S2	F1S3	F1S4
Total sugar, %*	42	46	39	67	43	42	40	38
Degree of sulfation†	0.5	0.6	0.5	ND	1.0	1.2	1.5	1.6
Xylose, %‡	49	31	28	30	32	31	26	25
Mannose, %‡	51	69	72	70	68	69	74	75

The sugar composition (molecular %) was determined for the following fractions: water-extracted xylomannan (WE), purified major xylomannan sulfate (F1), sulfated derivatives of xylomannan obtained at different time points in the sulfation process (F1S1, F1S2, F1S3 and F1S4 at 0.5, 1.0, 1.5 and 2.0 h, respectively), the minor xylomannan fraction (F2) and desulfated xylomannan (F1D). *Percentage weight of fraction dry weight. †Number of sulfate groups per monosaccharide residue. ‡Molecular percentage of neutral sugars. ND, not determined.

by lyophilization with D_2O and then examined as 1% solutions in 99.8% D_2O.

Virology

Cells and virus

Vero (African green monkey kidney) cells were grown in Eagle's minimum essential medium supplemented with 5% fetal calf serum. For maintenance medium (MM), the serum concentration was reduced to 1.5%. HSV-1 strain F was propagated and titrated by plaque formation in Vero cells.

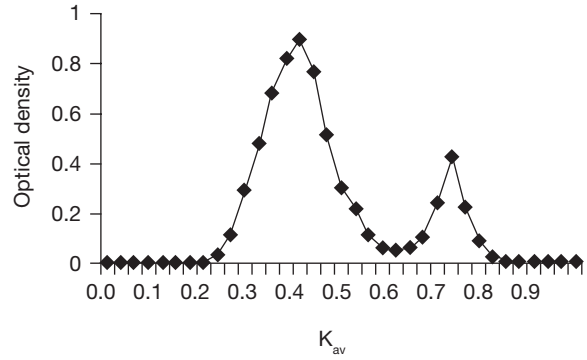
Cytotoxicity assays

Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma–Aldrich, St Louis, MO, USA) method. Confluent cultures in 96-well plates were exposed to different concentrations of the polysaccharides, with three wells for each concentration, using incubation conditions equivalent to those used in the antiviral assays. An aliquot of 10 µl of MM containing MTT (final concentration 0.5 mg/ml) was then added to each well. After 2 h of incubation at 37°C, the supernatant was removed and 200 µl of ethanol was added to each well to solubilize the formazan crystals. After vigorous shaking, the absorbance was measured in a microplate reader at 595 nm. The 50% cytotoxic concentration (CC_{50}) was calculated as the compound concentration required to reduce cell viability by 50%.

Antiviral assays

Antiviral activity was evaluated by a virus plaque reduction assay. Vero cell monolayers grown in 24-well plates were infected with approximately 50 plaque-forming units per well in the absence or presence of various concentrations of the compounds. After 1 h of adsorption at 37°C, residual inoculum was replaced by MM containing 0.7% methylcellulose and the corresponding dose of each compound. Plaques were counted after 2 days of incubation at 37°C. The 50% inhibitory concentration (IC_{50}) was calculated as the compound concentration required to reduce virus plaques by 50%. All determinations were performed twice and each in duplicate.

Figure 1. Elution profile of the water-extracted fraction of the marine red alga *Sebdenia polydactyla* on a Sephacryl™ S-300 column



The crude water-extracted polymeric fraction was isolated as described in the Methods section and eluted through the column with 0.5 M sodium acetate buffer (pH 5.0) at 20 ml/h. The elution of polysaccharide was expressed as a function of the partition coefficient K_{av} ($K_{av}=[V_e-V_0]/[V_t-V_0]$ with V_t and V_0 being the total and void volume of the column determined as the elution volume of glucose and standard dextran [500 kDa], respectively, and V_e is the elution volume of the sample).

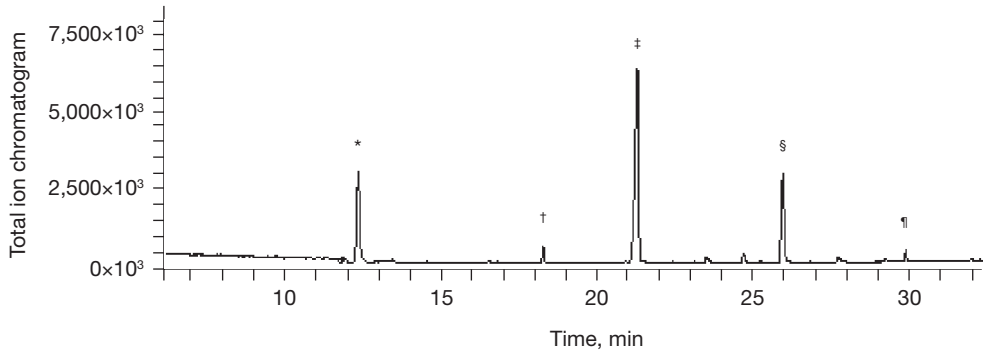
Results

Chemical characterization of sulfated xylomannan from *S. polydactyla*

Isolation, purification and molecular mass

The central goal of this study was to determine the structural features of water-extracted polysaccharides from marine red alga *S. polydactyla*. Therefore, the first step was the determination of sugar composition of the algal powder and, on the basis of the information obtained, to devise a strategy for the extraction of polysaccharides. The DAP contained xylose and mannose as dominant monosaccharides (Table 1) and because xylomannans are usually soluble in aqueous solvents [24–26], DAP was extracted with water. This water-extracted fraction (named WE) amounted to 34% of DAP dry weight and was made up of mannose and xylose residues, and sulfate groups (Table 1).

Figure 2. Total ion chromatogram of partially methylated alditol acetates derived from the desulfated xylomannan of the red alga *Sebdenia polydactyla*



The desulfated xylomannan was completely methylated and then hydrolysed, reduced and the alditol acetates were converted into their partially methylated alditol acetates as described in the *Methods* section. *1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol. †1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylmannitol. ‡1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol. §1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylmannitol. ¶1,2,3,5,6-penta-*O*-acetyl-4-*O*-methylmannitol.

Size exclusion chromatography (SEC) on a Sephacryl™ S-300 column separated the WE into two fractions (Figure 1). The major fraction (F1) amounted to 82% of the total polymers recovered from the column and contained mostly mannose and xylose residues (Table 1). Mannose accounted for >69% of the neutral sugars of F1, which also contained 7% (w/w) of sulfate groups. This purified polysaccharide had a specific rotation $[\alpha]_D^{32}$+51° (*c* 0.19, H_2O) comparable with the xylomannan sulfate in *Nothogenia fastigiata* and *Scinaia hatei* (Liagoraceae), revealing that mannose and xylose residues in F1 belongs to the D-series [25,26].

F1 was subjected to further chemical analyses. First, the apparent molecular mass was determined by SEC. The elution profile of this macromolecule on Sephacryl™ S-300 suggested that the sulfated xylomannan was homogeneous. On the basis of the calibration with standard dextrans, the apparent molecular mass of F1 was 150 kDa.

Chemical modifications

To investigate the effect of the sulfate group on the biological properties of xylomanan sulfate from *S. polydactyla*, we desulfated and further sulfated this macromolecule. Preliminary experiments (data not shown) showed that solvolytic desulfation [17] gave better recovery compared with the methanol–HCl and autodesulfation method [27]. Therefore, F1 was desulfated by solvolysis in dimethyl sulfoxide and the desulfated derivative (F1D) had a recovery yield of 39%. In separate experiments, the purified xylomannan sulfate was further sulfated under various conditions as described in the *Methods* section to yield derivatives F1S1, F1S2, F1S3 and F1S4 with various degrees of sulfation (Table 1).

The Fourier transform IR spectrum of F1 showed an intense absorption band in the region, 1,252 cm^{-1}, which was related to >S=O stretching vibration of the sulfate group [28–30]. In the IR spectrum of the desulfated derivative (F1D), this absorbance became weak; however, in further sulfated derivatives, the intensity of this band became stronger (Supplementary Figure 1), demonstrating that it was associated with the sulfate groups.

To get more information on the structure of xylomannan, periodic acid oxidation of F1D was carried out. The oxidized polymer was reduced with sodium borohydride and subjected to mild acid hydrolysis according to the usual Smith degradation conditions. After separation from the low molecular mass fragments, a Smith-degraded material (F1D-Sm) containing mannose as the only component sugar was obtained in a 50% yield.

Glycosidic linkage analyses

Methylation analyses of the desulfated macromolecule (F1D) gave, *inter alia*, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol residues suggesting that the xylomannan sulfate had a (1→3)-linked backbone (Figure 2). The presence of a large proportion of terminal xylopyranosyl and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylmannitol residues suggested that the polymer was branched at position 6 (Table 2). Linkage analyses of the native xylomannan sulfate F1 yielded a variety of mono-, di- and trimethylated products (Supplementary Figure 2) indicating that the structure of this polymer was highly complex. The increase in the proportions of 2,4,6-tri-*O*-methylmannitol after desulfation, as a consequence of decreased proportions of 4-*O*-methyl- and 2,4-di-*O*-methylmannitol residues, suggested that sulfate esters, when present, were located at position 6

and 2 of the (1→3)-linked mannopyranosyl residues. Small amounts of (1→6)-linked mannopyranosyl residues were also present.

The results obtained from the methylation analyses of the Smith-degraded polymer (F1D-Sm) showed that the degraded polysaccharide was a linear mannan built up of (1→3)-linked mannopyranosyl residues (Table 2). The disappearance of terminal xylitol and 2,4-di-*O*-methyl mannitol residues suggested the presence of a single stub of xylose residues at position 6 of the mannose units of native xylomannan sulfate.

NMR spectroscopy

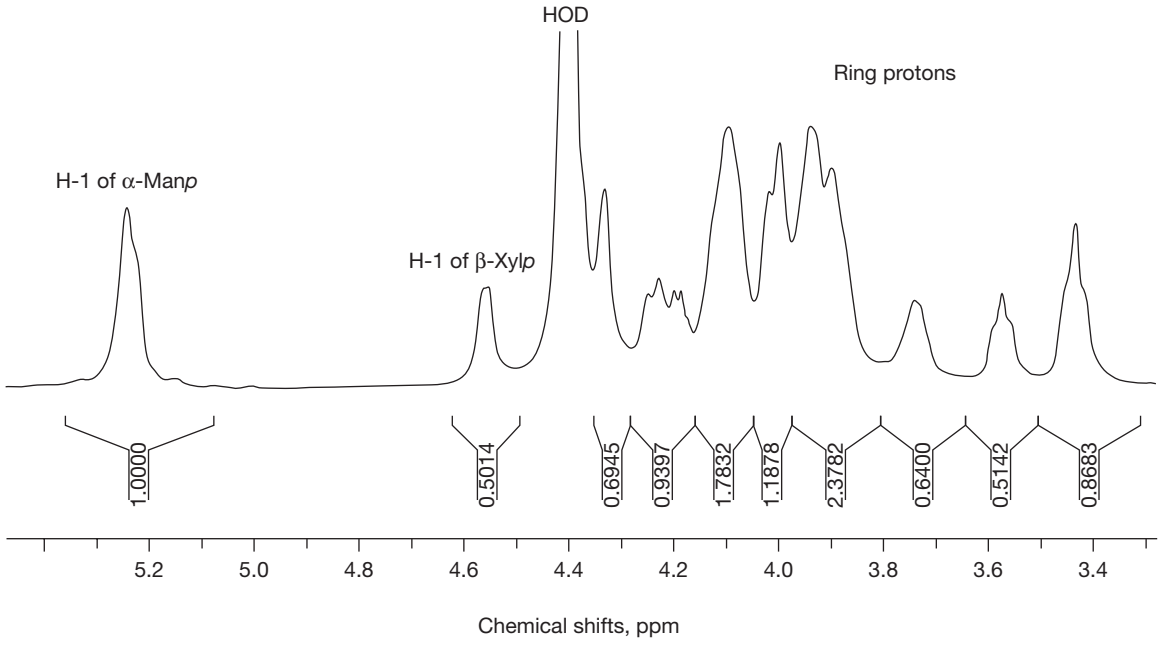
NMR spectroscopy is a convenient method to obtain valuable structural information about polysaccharides. We analysed the sulfated xylomannan by NMR to examine the anomeric configuration and sulfation pattern. The presence of a number of broad signals in the anomeric region of the ^{1}H NMR spectra of the further sulfated xylomannan suggested that their structures were very complex. By contrast, the desulfated xylomannan (F1D) showed only two broad anomeric resonances, one at 5.243 ppm and the other at 4.558 ppm (Figure 3). The anomeric configuration of mannose and xylose residues of a structurally related sulfated xylomannan isolated from other brown algae have been reported to be α and β, respectively [24,25,31]. Therefore, signals at 5.24 and 4.56 ppm were tentatively assigned to anomeric protons of α-(1→3)-linked mannopyranosyl and β-linked terminal xylose residues, respectively. The relative proportion (2:1) of mannose and xylose residues as estimated from the integral of these anomeric signals also fits well with the results obtained from sugar compositional analyses. The disappearance of the latter signal in the NMR spectrum of the Smith-degraded polymer (Supplementary

Table 2. Partially methylated alditol acetates derived from native and desulfated xylomannan of *Sebdenia polydactyla* and the Smith-degraded derivative

	Peak area*		
Methylation products	F1	F1D	F1D-Sm
2,3,4-Xyl†	21	24	ND
2,3,4-Man‡	4	2	ND
2,4,6-Man§	13	47	100
2,4-Man¶	34	26	ND
4-Man#	22	1	ND
Man**	6	ND	ND

Partially methylated alditol acetates derived from native (F1) and desulfated (F1D) xylomannan of *Sebdenia polydactyla* and the Smith-degraded derivative (F1D-Sm). *Percentage of total area of the identified peaks. †1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol. ‡1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylmannitol. §1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol. ¶1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylmannitol. #1,2,3,5,6-penta-*O*-acetyl-4-mono-*O*-methylmannitol. **1,2,3,4,5,6-hexa-*O*-acetylmannitol. ND, not detected.

Figure 3. ^{1}H NMR spectrum at 500 MHz of the sulfated xylomannanan of *Sebdenia polydactyla*



The spectrum was recorded at 80°C for the purified sulfated xylomannanan sample in D_2O solution. The signal for the deuterated water was designated as HOD. H-1 of α-Man*p* and H1 of β-Xyl*p* refer to signals of anomeric protons α-(1→3)-linked mannopyranosyl and β-linked terminal xylopyranosyl, respectively.

Figure 3) confirmed this finding. Similar to the desulfated xylomannan (F1D), the degraded material (F1D-Sm) also included resonance characteristics of polysaccharides, such as signals from ring protons (H-2 to H-6) between 3.44 and 4.33 ppm. Therefore, NMR analyses confirmed the results of the methylation analyses and indicated the presence of α-linked mannopyranosyl and β-linked terminal xylopyranosyl residues.

Antiviral activity of the sulfated xylomannan from *S. polydactyla*

Table 3 summarizes the results of the cytotoxicity and antiviral activity of the native sulfated xylomannan (F1) from *S. polydactyla*, its desulfated fraction (F1D) and the four further sulfated derivatives (F1S1–F1S4) as determined by MTT and plaque reduction assays, respectively. The native polysulfate F1 might be considered a good inhibitor of HSV-1 strain F with an IC_{50} value of 2.8 μg/ml. By contrast, the desulfated derivative F1D was inactive against this virus up to a concentration of 10 μg/ml. The conclusion that can be drawn is that the antiviral activity of this xylomannan is linked to the anionic features of the molecule, given mainly by the presence of sulfate groups. To further assess this feature, the four further sulfated compounds obtained by chemical modification of the purified polysaccharide F1 were also tested against HSV-1. In all cases, the further sulfated derivatives exhibited a more potent antiviral activity in comparison to the native xylomannan F1, with values of IC_{50} ranging from 0.35 to 0.7 μg/ml. The small differences observed in the IC_{50} values of the further sulfated compounds were related to the sulfate content, with higher antiviral activity associated to a greater DS. Moreover, fractions F1S1–F1S4, which presented DS values in the range of 1.0–1.6, showed a significant increase in their antiviral potential in comparison to the native F1, with a DS of 0.6 (Tables 1 and 3). These data confirmed the importance of this parameter in the inhibitory activity of sulfated polysaccharides. Furthermore, the four further sulfated fractions exhibited high selectivity indices (>1,428 to >2,857) because of their lack of toxicity for Vero cells. No effect on cell viability was observed with any of these compounds at concentrations up to 1,000 μg/ml. They also showed a higher inhibitory effect and selectivity when compared with other well known sulfated polysaccharides, such as heparin and dextran sulfate 8000 when assayed as reference substances.

Table 3. Antiviral and cytotoxic activities of sulfated polysaccharides from *Sebdenia polydactyla*

Compound	CC_{50}, μg/ml*	Mean IC_{50}, μg/ml (±SD)†	SI (CC_{50}/IC_{50})
F1	>1,000	2.8 (0.5)	>357
F1S1	>1,000	0.7 (0.1)	>1,428
F1S2	>1,000	0.6 (0.2)	>1,666
F1S3	>1,000	0.47 (0.05)	>2,127
F1S4	>1,000	0.35 (0.03)	>2,857
F1D	>1,000	>10	Inactive
Heparin	>1,000	1.2 (0.3)	>833
Dextran sulfate 8000	>1,000	2.1 (0.4)	>476

The fractions tested were purified xylomannan sulfate (F1), sulfated derivatives of xylomannan obtained at different time points in the sulfation process (F1S1, F1S2, F1S3 and F1S4 at 0.5, 1.0, 1.5 and 2.0 h, respectively) and desulfated xylomannan (F1D). *50% Cytotoxic concentration (CC_{50}), defined as compound concentration required to reduce cell viability by 50%, determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method. †50% Inhibitory concentration (IC_{50}) defined as compound concentration required to reduce virus plaques by 50%. IC_{50} values are the mean (±SD) from duplicate independent tests. Selectivity index (SI) is the ratio between CC_{50} and IC_{50}.

Discussion

In this study, the active anti-HSV-1 principle was isolated from the red seaweed *S. polydactyla* by extraction with water. The major constituent was a sulfated xylomannan (F1), which had a complex structure and a molecular mass of 150 kDa. Recent studies showed that sulfated xylomannan from *S. hatei* and *N. fastigiata* contained a backbone of α-(1→3)-linked D-mannopyranosyl residues substituted at different positions with a single stub of β-D-xylopyranosyl residues and sulfate groups [24,25,31]. The polysaccharide of the present study had a similar backbone, but it differed from *N. fastigiata* xylomannan in the position of xylopyranosyl residues and from *S. hatei* polymer in the location of sulfate group. Moreover, it was highly branched with an average of 25 branching points being present in every hundred mannopyranosyl residues. Therefore, the structure of the polysaccharide described in the present paper is different from these known sulfated xylomannans and it is obvious that its activity against HSV-1 will be different. Furthermore, the native sulfated xylomannan was further sulfated using SO_3-pyridine and desulfated to yield a series of polysaccharides with different sulfate content (DS values varied from approximately 0 to 1.6).

Sulfated polysaccharides are known to affect the growth of animal viruses, including HSV-1 [7,8,32]. Herold and colleagues [33] showed that *N*-sulfation and the presence of carboxyl groups on heparin are key determinants for HSV-1 interactions with host cells because *N*-desulfation and carboxyl reduction abolishes heparin's antiviral activity. The sulfated xylomannan from *S. polydactyla* drastically lost activity when desulfated. Furthermore, this macromolecule (F1) and its further sulfated derivatives (F1S1–F1S4) exhibited a potent inhibitory effect on HSV-1 infection. As can be seen in Tables 1 and 3, the level of antiviral efficacy (determined by IC_{50} and selectivity index values) was directly related to the content of sulfate groups in the molecule. However, the four

further sulfated samples, which varied their DS from 1.0 to 1.6, were highly inhibitory of HSV-1 multiplication. Thus, it appears that a content of one sulfate group per monosaccharide unit (DS of 1.0) is enough to guarantee a good level of selective antiviral effect.

It is believed that the *in vitro* efficacy of sulfated polysaccharides is mainly because of their ability to inhibit the attachment of the virion to the host cell surface [7–9], although in some cases virucidal activity plays a role [9]. However, the native sulfated xylomannan of *S. polydactyla* did not exert any inactivating effect against HSV-1, thus ruling out a direct virucidal action produced by virion inactivation for xylomannans (data not shown).

Sulfated polysaccharides are a potent class of microbicide drug candidate. To date, the performance of microbicide candidates in efficacy trials has been disappointing [34,35], but next-generation concepts currently in or approaching clinical trials offer improved prospects for efficacy. The most plausible approach involves a combination of several drugs, preferentially targeting different steps in the viral infection process [36]. Because sulfated polysaccharides are safe and acceptable [37,38], development of several second-generation combination formulation based on first-generation-led candidates might be more effective [9,39,40]. The polysaccharides of the present study exhibited potent inhibitory action against HSV-1. The extent of inhibition produced by the sulfated xylomannan was similar to that of standard antiherpetic polysulfates, such as heparin and dextran sulfate. In addition, the inhibition of *in vitro* HSV-1 replication was observed at xylomannan concentrations, which did not have any effect on the cell viability. Given the interesting chemical characteristics of the sulfated xylomannans from *S. polydactyla* and the promising *in vitro* antiherpetic properties reported here, these macromolecules represent good candidates for further antiviral research.

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Disclosure statement

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Additional file

The additional file 'Supplementary material' can be accessed at www.intmedpress.com

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