Journal of Scientific & Industrial Research
Vol. 80, December 2021, pp. 1033-1041

Assessment of Anti-microbial and Anti-oxidant Activities of Modified Guar Gum

Manabendra Dhua[1], Sabyasachi Maiti[2]* and Kalyan Kumar Sen[1]
[1]Gupta College of Technological Sciences, Ashram More, G T Road, Asansol 713 301, West Bengal,
[2]Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh 484 887, India

Received 20 December 2020; Revised 29 October 2021; Accepted 02 December 2021

Apart from drug delivery potential of polysaccharides or their chemical derivatives, some chemically modified polysaccharides particularly sulfated polysaccharides showed significant biological property. In this study, guar gum was sulfated by reacting with various ratios of CSA (chlorosulfonic acid) and pyridine to obtain the product with different degree of sulfation (DS). The sulfated guar gum derivatives were examined by FTIR, NMR and degree of sulfation analysed and were further evaluated for their potential antibacterial activity by agar diffusion method. Results obtained from the study indicated that derivatives having higher degree of sulfation exhibited moderate activity against the selected bacterial strains at 200 $\mu g \cdot ml^{-1}$. However, the sulfated guar gum did not show any significant antifungal activity at any concentration studied. The sulfated guar gum demonstrated dose-dependent DPPH and H_2O_2 scavenging activity with maximum activity noted at 2 $mg \cdot ml^{-1}$ concentration. The sulfation of partially oxidized guar gum caused significant improvement of antioxidant activity to that observed for non-oxidized sulfated guar gum derivative. This study revealed that the extent of sulfation and molecular weight had significant impact on antimicrobial and antioxidant activities of guar gum.

Keywords: Antifungal activity, Polysaccharides, Sulfated guar gum

Introduction

Natural polysaccharides are considered superior to synthetic polymers in terms of biocompatibility, biodegradability, chemical modification, bio-adhesive properties, and natural extracellular matrix-mimicking characteristics.[1] Esterification, carboxymethylation, oxidation, amidation and hydroxypropylation are usual mode of structural modification of polysaccharides for obtaining the desired functional properties.[2] Further, the native polysaccharides as well as their chemically modified forms have been tested for their potential applications in the field of dosage form development. Number of studies has revealed that biological activities of polysaccharide are significantly improved after structural modification.[3]

Keeping this in view, several polysaccharide derivatives have been examined for biological activity. The antioxidant activity of oxidized xanthan[4], oxidized curdlan[5], *N*-carboxymethyl chitosan[6], carboxymethyl corn bran polysaccharide[7] and the antiviral activity of curdlan sulfates[8] has been reported. The acetyl and carboxymethyl derivatives of *Ganoderma atrum* polysaccharide has also been investigated for both antioxidant and immune modulating activities.[9]

Recently, the antiviral activity of sulfated polysaccharides have attracted attention of researchers.[10] Besides antiviral activity, the sulfation of polysaccharides may contribute to obtain newer bioactivities. Apart from enhancing water solubility, the sulfation also changes the chain conformation, and consequently alters their biological properties.[11] In contrast to virgin polysaccharides; the sulfation of polysaccharides brought about potential biological activities, such as antiviral, anti-coagulant, antioxidant and antitumor activities.[12]

In most of the cases, the biological activity of sulfated gum seemed to be the function of degree of substitution (DS). The sulfated polysaccharides are sulfate esters where sulfate groups are attached to their hydroxyl group to form a complex structure with change in conformation.[11] Solubility of polysaccharides is also increased as a result of sulfation.

Guar gum belongs to galactomannan class and is isolated from the endosperm of *Cyamopsis tetragonolobus*. The non-toxicity of this gum has permitted its use in the various industries like textile, pharmaceutical, biomedical, cosmetic and food industries.[13] One of the interesting properties of guar

*Author for Correspondence
E-mail: sabya245@rediffmail.com

gum is its viscosity enhancing effect even at very low concentration. The viscous behaviour of guar gum has enabled its use as food stabilizer.[14]

Importance of guar gum in controlled release formulation as well as colon targeted drug delivery system is well established. Chemically, it has a (1→4)-linked β-D-mannopyranose backbone. The α-D-galactose residues are emanated as side chain from 6-positions via 1→6-glycosidic linkages.[15] Sulfonation is a particularly easy and versatile approach for enhancing biological activities of the polysaccharides. The attachment of sulfate group to the hydroxyl group of polysaccharides makes them structurally complex class of polysaccharides. They possess wide bioactivities in contrast to non-sulfated polysaccharides. The negatively charged sulfate groups mainly contribute to their *in vitro* antiviral and anticoagulant activity.[16–19] Further, hypoglycemic[20], antitumor[21,22], and antioxidant[23] and antibacterial[24] activities are also reported for sulfated polysaccharides.

The prevalence of hydroxyl groups in polysaccharides has enabled the researchers to synthesize its sulfated derivatives. A recent study indicated that sulfated guar gum (DS>0.56) possessed significant anticoagulant activity.[25] The sulfated guar gum is also reported to have significant antioxidant properties.[26,27]

These findings indicated that guar gum could be a good basic material for developing new therapeutic molecules. Herein, guar gum was sulfated by controlling chlorosulfonic acid to pyridine ratio. The prepared derivatives were characterized by wet method and FTIR analysis. The sulfated guar gum was evaluated for its potential antimicrobial, antifungal and antioxidant activities. In an earlier report, oxidized xanthan gum showed better antioxidant activity.[4] This observation encouraged us to conduct a preliminary experiment and assess antioxidant activity of sulfated derivative of partially oxidized guar gum.

Materials and Methods

Materials

Guar gum, Nutrient agar media were purchased from HiMedia Lab. Pvt. Ltd., Mumbai, India. Pyridine, chlorosulfonic acid (CSA), hydrogen peroxide (H_2O_2) and dimethylformamide (DMF) were supplied by Merck, Mumbai, India. Amberlite (IR-120), BHA was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Ketoconazole was gifted by Glenmark Pharmaceuticals, Mumbai, India. Other reagents were of AR grade and were employed as collected.

Synthesis of Sulfated Guar Gum

Chlorosulfonic acid was gradually added to 25 ml pyridine at temperature 4–10°C with continuous stirring to prepare sulfating reagent.[12] Guar gum was sulfated by the method described elsewhere.[12] A suspension of guar gum was prepared in 20 ml anhydrous DMF with continuous stirring for 30 min followed by dropwise addition of sulfating reagent. Reaction was continued for 3h at 60°C and then cooled. The pH was maintained between pH 7.0 and pH 8.0 with 2M NaOH solution. The product was then precipitated with 95% ethanol and filtered by muslin cloth. After dissolving the precipitate, it was dialyzed for 72h against water and lyophilized to get the product. This sulfated derivative was synthesized using different chlorosulfonic acid: pyridine ratio viz. 1:1, 1:2, 1:3 and 1:4 and the sulfated samples were designated as SG1, SG2, SG3 and SG4, respectively.

Partial Oxidation of Guar Gum

The guar gum was oxidized following the method reported elsewhere.[4] Briefly, the dispersion of virgin guar gum (1.5%, w/v) in water was heated to 80°C with stirring to obtain a uniform dispersion. The dispersion was adjusted to pH 13.0 with NaOH solution, and 50 ml (30%, w/v) hydrogen peroxide was poured into it. The mixture was kept overnight at room temperature, adjusted to pH 7.0 and then filtered. The partially oxidized guar gum was dialyzed (MWCO: (7–14 kDa) for 3 days against distilled water and freeze-dried. The partially oxidized guar gum was sulfated using chlorosulfonic acid: pyridine ratio of 1:1 following the procedure described earlier and the sample was designated as OSG-1.

Determination of Sulfate Content

This was estimated following the procedure described earlier.[28] In an aqueous solution of sulfated guar gum (2 mg/ml), 30 gm of amberlite (IR-120) resin was added and agitated for an hour. After filtering the solution was titrated with 0.1M NaOH using phenolphthalein indicator. Same was carried out by taking gaur gum for blind determination and sulfur content was determined as follows.

$$S(\%) = \frac{\left(\begin{matrix} ml\ of\ NaOH\ for\ sample - \\ ml\ of\ NaOH\ for\ blind\ sample \end{matrix}\right) \times 320}{mg\ of\ sample}$$

$$DS = \frac{S(\%) \times 162}{[3200 - 102\,S(\%)]}$$

FTIR Analysis

The infrared spectra of native guar gum, oxidized guar gum and sulfated guar gum were recorded in Fourier transform infrared spectrometer (Perkin Elmer) over 4000–500 cm^{-1}.

^{13}C-NMR Study

Solid state ^{13}C-NMR spectrum of guar gum, oxidized guar gum and their sulfated derivatives were recorded in JEOL ECX400 NMR spectrophotometer at 400 MHz.

Antimicrobial Activity

Antibacterial Activity

The activity of sulfated guar gum was evaluated by well diffusion method using nutrient agar medium following the protocol as described earlier.[29] Following incubation at 37°C for 24h, the zone of inhibition was noted and compared to that of penicillin. The activity of sulfated guar gum was evaluated over 50-200 $\mu g \cdot ml^{-1}$ concentration level, against Gram positive bacteria (*S. aureus* [ATCC-29737], *B. subtilis* [ATCC-6633]) and Gram negative strain (*E. coli* [ATCC-8739], *S. typhi* [ATCC-9993]).

Antifungal Activity

Fungal pathogenic strain of *Candia albicans* (ATCC-10231) was used for evaluation of antifungal study. This was assessed by well diffusion technique using Sabouroud Dextrose Agar media as described by Nair *et al*.[30] The zone of inhibition was spotted after incubation of 72 h at 25°C and the same was compared to positive control ketoconazole. The activity of sulfated guar gum was evaluated over concentration level of 50–200 $\mu g \cdot ml^{-1}$.

Antioxidant Activity

1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging[31]

One ml of polysaccharide solution of different concentration (250–2000 $\mu g \cdot ml^{-1}$) was put into to 2 ml of DPPH solution (0.2 $mM \cdot L^{-1}$) and then 2 ml of methanol was mixed. After mixing, the solution was stored at dark for half an hour and UV-absorbance was noted at 530 nm. The scavenging activity was determined as follows.

$$\%\ \text{Scavenging} = \frac{[A_0 - (A - A_b)]}{A_0} \times 100$$

where A_0 = absorbance of DPPH solution, A_b = Sample absorbance in absence of DPPH, A = Sample absorbance with DPPH

Hydrogen Peroxide Radical Scavenging

The derivatives were also assessed for hydrogen peroxide radical scavenging activity following the method reported by Gülçin *et al.*, 2004.[32] A solution of sulfated guar gums was prepared at different concentrations (250 – 2000 $\mu g \cdot ml^{-1}$). Then 2ml 10mM hydrogen peroxide solution in phosphate buffer saline (0.1M pH 7.4) was added to 1ml of sulfated guar gum solution. After proper mixing, incubation at 37°C for 10 min, the UV absorbance was noted against blank at 230 nm. The scavenging activity was obtained as follows:

$$\%\ Scavanging\ of H_2O_2 = \frac{(A_0 - A_1)}{A_0} \times 100$$

where, A_0, and A_1, are absorbance of control, and sample, respectively

Statistical Analysis

The differences in antioxidant activities exerted by native guar gum, SG-1, OSG-1 and BHA samples at 2 mg/ml concentration was analyzed by ANOVA (GraphPad Prism Software, Version 3.00, Trial) and Tukey's test for statistical significance. The difference was regarded significant at 95% confidence level.

Results and Discussion

From the previous studies to prepare sulfated polysaccharides, it was established that high degree of substitution (DS) can be achieved by varying sulfating reagent amount instead of varying temperature of reaction.[11] In this study, guar gum was sulfated by CSA-Pyridine method. It was reported that this method is easier to obtain sulfated derivatives with acceptable yield and degree of substitution.[33,34]

Here, we synthesized sulfated guar gum by using CSA/pyridine sulfating reagent at different ratio. Beyond CSA: Pyridine ratio of 1:1, the variation in sulfating reagents caused detrimental effect on the degree of sulfation. DS with the various ratio of sulfating reagent is given in the Table 1.

Infrared spectra of native gum, sulfated gum and sulfated derivative of partially oxidized gum are displayed in Fig. 1. Sulfated guar gum showed some distinct features which confirmed the sulfation of gum.

Table 1 — Degree of sulfation (DS) of guar gum as a function of CSA: Pyridine ratio

Sample No.	CSA: Pyridine	% Product Yield	Degree of sulfation
SG1	1:1	78.30	0.73 ± 0.09
SG2	1:2	69.75	0.54 ± 0.07
SG3	1:3	76.31	0.49 ± 0.13
SG4	1:4	72.19	0.41 ± 0.10

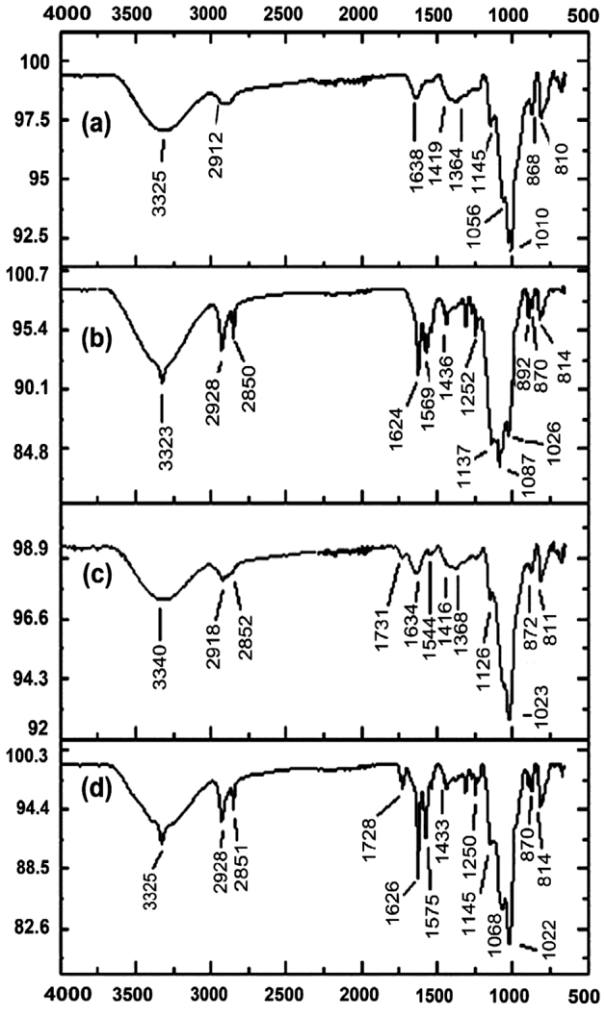

Fig. 1 — FTIR spectra: (a) native guar gum and (b) sulfated guar gum (SG1), (c) oxidized guar gum and (d) sulfated oxidized guar gum

FTIR spectrum of native gum (Fig. 1a) showed a broad spectrum centering at 3325 cm^{-1} was indicative of O-H stretching of primary and secondary hydroxyl groups. C-H stretching was noted at 2912 cm^{-1}. C-O stretching or O-H deformation (coupled) of C-O-H was seen at 1056 and 1010 cm^{-1}. The peaks corresponding to C-O-C glycosidic linkages were visible at 1145 cm^{-1}.

In infrared spectrum of sulfated guar gum (Fig. 1b), C-H stretching appeared at 2928 cm^{-1}. C-O-C stretching of glycosidic linkages and C-O-S vibration of C-O-SO_3 was ascribed to the wave numbers observed at 1137 and 814 cm^{-1}.[35] This suggested that glycosidic linkages remained intact after sulfation. An intense peak at 1087cm^{-1} was still found in the spectrum of sulfated gum. This could be attributed to C-O stretching or O-H deformation (coupled) of C-O-H. A sharp, prominent peak characterizing O-H

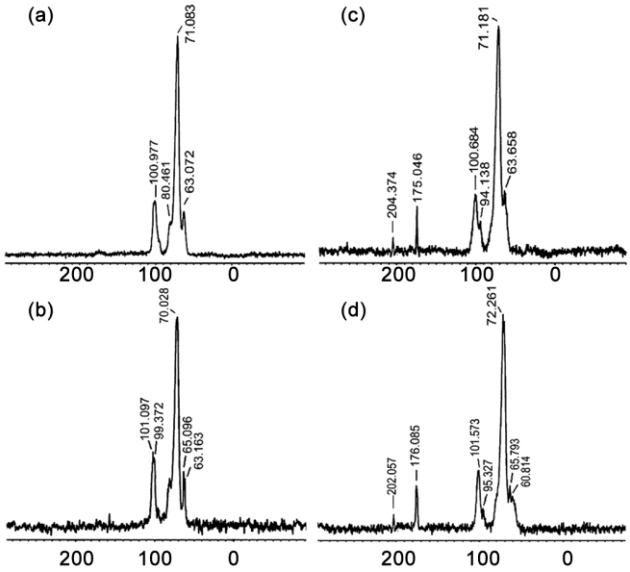

Fig. 2 — ^{13}C-NMR spectrum of (a) guar gum, (b) sulfated guar gum, (c) oxidized guar gum and (d) oxidized sulfated guar gum

stretching was observed at 3323 cm^{-1}. A new band in the spectrum of sulfated guar gum in region 1252 cm^{-1} is due to asymmetrical stretching of S = O group. This peak was not found in the spectrum of native guar gum and thus confirmed the synthesis of sulfated derivative of guar gum.

The liberation of a reactive hydroxyl radical after dissociation is involved in H_2O_2-mediated oxidative degradation. Hydroxyl radicals act as strong oxidizing species and attack the glycosidic bond of polysaccharides and can reduce the molecular weight of polysaccharides.[36] An attempt was made to oxidize guar gum using H_2O_2 and then proceed with the sulfation reaction. The synthesis of sulfated partially oxidized guar gum was established by FTIR spectroscopy (Fig. 1d).

In the infrared spectrum of sulfated oxidized guar gum, O-H stretching vibration appeared at 3325 cm^{-1}, C-H stretching at 2928 cm^{-1}, S=O stretching of –OSO_3 at 1250 cm^{-1}. The peak at 814 cm^{-1} suggested the C-O-S linkage due to sulfation. Further, a new absorption band appeared at 1731 cm^{-1}, which was ascribed stretching vibration of carbonyl group generated after oxidative degradation of O-C-O. This is in agreement to that reported earlier.[37,38] The appearance of peaks at 1022 and 1068 cm^{-1} was indicative of C-O stretching of C-O-H. C-O-C asymmetrical stretching was indicated by the small absorption band 1145 cm^{-1} which suggested presence of glycosidic linkage even after oxidation thus hinted the partial oxidation of guar gum.

Solid state ^{13}C NMR study was performed and the results are depicted in the Fig. 2. A peak at around 100 ppm could be seen in ^{13}C NMR spectrum of guar gum (Fig. 2a) corresponding to C-1 carbon of galactose and mannose unit while the peak at 63 ppm was credited to the C-6 carbon and peaks due to other carbon atom (C-2-5) were found at ~71 ppm.[39] Comparatively more complicated ^{13}C NMR spectrum was obtained after sulfation (Fig. 2b). A downfield shift was observed for the carbon attached directly to the electronegative sulfate group; whereas carbon other than this showed down field shift.[11] The new peak at 65 ppm of the sulfated derivative corresponds to the carbon (O-6 substituted) and thus hinted the prevalence of sulfation reaction at C-6. Moreover, signal at 63 ppm still appeared indicating partial sulfation. Splitting of C-1 signal due to C-2 functionalization is also evident from earlier study. So, splitting of C-1 signal at 95–100 ppm was correlated to sulfation of C-2. C-2 sulfated signal appeared at 99 ppm whereas non-sulfated C-1 appeared at 100 ppm. This result it is evident of nonselective sulfation. On the other hand, ^{13}C-NMR spectrum of oxidized guar gum (Fig. 2c) showed a new peak at 175 ppm and 204 ppm which corresponds to carboxylic acid carbon and carbonyl carbon respectively which confirm that the guar gum was effectively oxidized and at the same time C-1 signal appeared with splitting indicating the partial C-1 oxidation. When oxidized guar gum is further sulfated also showed characteristic new peak at 65 ppm along with peak at about 60 ppm indicating that the sulfation occurred at C-6. In this case C-1 signal was not splitted due to sulfation of C-2 position that might be due to C-1 oxidation leading to steric hindrance.

Because sulfation was done by non-selective method, it is not possible to indicate the exact position of sulfation in the guar gum. Mähner *et al.* (2001) indicated that C-6 hydroxyl group was the most preferential site for sulfation of pullulan, followed by C-3 while C-4 remained mostly non-sulfated and achieved a maximum DS of 2.0.[(28)] The proposed structure of sulfated gum and sulfated oxidized gum is depicted in Fig. 3.

The antimicrobial activity of sulfated gum was assessed against *S. aureus, B. subtilis, E. coli, S. typhi* bacterial strains and *C. albicans* fungal strain by agar diffusion method. The activity was evaluated over concentration level of 50–200 $\mu g \cdot ml^{-1}$ and assessed by measuring zone of inhibition. The data are

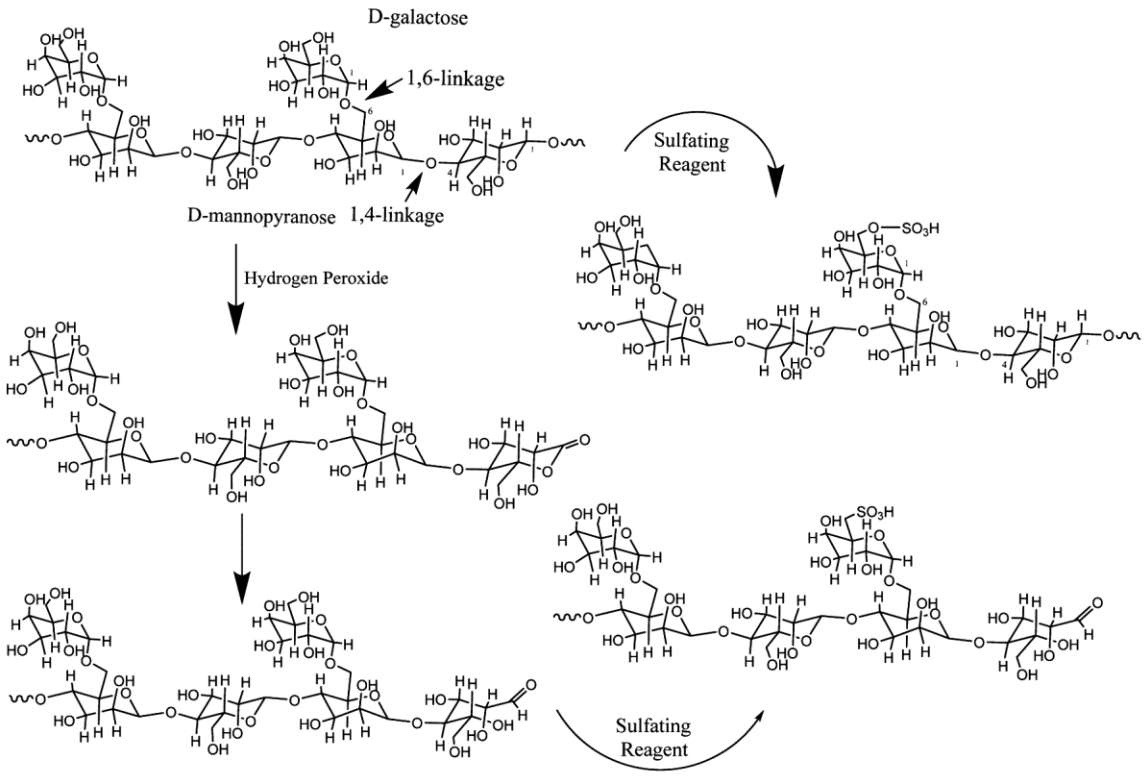

Fig. 3 — Proposed chemical structure of sulfated guar gum and sulfated derivative of partially oxidized guar gum

Table 2 — Antibacterial activity of sulfated guar gum at its varying concentrations and DS

Test organisms	Ampicillin 50 μg·ml^{-1}	Zone of Inhibition in mm											
		SG1 (μg·ml^{-1})			SG2 (μg·ml^{-1})			SG3 (μg·ml^{-1})			SG4 (μg·ml^{-1})		
		50	100	200	50	100	200	50	100	200	50	100	200
S. aureus	++	−	+	+	−	+	+	−	+	+	−	−	−
B. subtilis	++	+	+	+	−	+	+	−	−	+	−	−	−
E. coli	++	+	+	+	−	−	+	−	−	−	−	−	−
S. typhi	++	−	−	+	−	−	+	−	−	−	−	−	−

Note: Zone of Inhibition <7 denoted by −, ≥7mm but ≤10 denoted by + and >10 denoted by ++, Negative (−) sign indicates inactive, No antimicrobial activity of guar gum at 200 μg·ml^{-1}

Table 3 — Antifungal activity of sulfated guar gum at its varying concentrations and DS

Test organisms	Ketoconazole 50 μg·ml^{-1}	Zone of Inhibition in mm											
		SG1 (μg·ml^{-1})			SG2 (μg·ml^{-1})			SG3 (μg·ml^{-1})			SG4 (μg·ml^{-1})		
		50	100	200	50	100	200	50	100	200	50	100	200
C. albicans	++	−	−	−	−	−	−	−	−	−	−	−	−

Note: Zone of Inhibition <7 denoted by -, ≥7mm but ≤10 denoted by + and >10 denoted by ++, − sign indicate inactive

Table 4 — Antioxidant activity of sulfated guar gum by DPPH scavenging method using BHA (butylated hydroxyanisole) as standard

Conc. μg·ml^{-1}	% DPPH Scavenging (Mean ± SD, *n* = 3)						
	Guar gum	SG1	SG2	SG3	SG4	OSG1	BHA
250	18.65 ± 0.42	28.21 ± 0.65	15.70 ± 0.48	19.34 ± 0.79	18.20 ± 0.57	32.60 ± 0.76	35.50 ± 0.80
500	23.23 ± 0.43	31.77 ± 0.32	24.40 ± 0.52	22.70 ± 0.49	19.50 ± 0.52	39.41 ± 0.76	41.07 ± 0.81
1000	34.21 ± 0.65	41.27 ± 0.61	28.57 ± 0.75	32.16 ± 0.68	33.60 ± 0.78	47.35 ± 0.57	60.23 ± 0.97
1500	36.38 ± 0.47	46.15 ± 0.92	35.10 ± 0.40	34.45 ± 0.57	33.14 ± 0.62	51.76 ± 0.87	66.01 ± 0.77
2000	40.22 ± 0.52	52.52 ± 0.58	44.50 ± 1.20	38.34 ± 0.79	36.72 ± 0.65	59.26 ± 0.64	73.89 ± 0.45

presented in Table 2. As is evident, the sulfated guar gum had moderate activity against both types of bacterial strain. More precisely, guar gum with higher DS (SG1) showed comparatively high activity. As pure guar gum did not show antibacterial activity, it was inferred that minimum degree of sulfation is required for antimicrobial activity. However, the antibacterial activity was very less as compared to standard drug ampicillin. The SG1 and SG2 derivatives showed better activity than other derivatives against all bacterial strains at 200 μg·ml^{-1}. Both SG1 and SG2 were found to be effective against bacterial strains under investigation at strength of 200 μg·ml^{-1}. The zone of inhibition of standard drug ampicillin was found to be 21, 20, 15, 17 mm respectively for *S. aureus, B. subtilis, E. coli, S. typhi*. Vijayabaskar *et al.* (2012) reported that sulfation of algal polysaccharide could inhibit the growth of bacterial strains at 1mg/ml.[40]

The result was also in concurrence with the previous findings reported by Seedevi *et al.*, 2017.[41] The authors reported that the sulfated polysaccharides from seaweed *Gracilaria corticata* exhibited antibacterial activities at 25 μg·ml^{-1}.

While considering antifungal activity, the zone of inhibition of ketoconazole was noted as 22 mm. Compared to the standard drug ketoconazole, the zone of inhibition was mostly <7 mm at all concentrations against *C. albicans*, (Table 3). The results indicated that sulfated guar gum failed to show significant antifungal activity against *C. albicans* (Table 3).

Irrespective of the DS values and concentration, the antimicrobial activity of sulfated derivatives was significantly less than the standard. Perhaps, the high molecular weight of sulfated polysaccharides hindered their penetration through the microbial cell membrane barriers to exert considerable effects.[42]

Introduction of electron withdrawing groups such as sulfate group to the polysaccharide structure could help to improve antioxidant activity. Thus, the sulfated guar gum was tested for DPPH and hydrogen peroxide scavenging activity. The DPPH method works on the fact that the encounter of DPPH free radical with an antioxidant (proton-donating substance) reduces the stable radicals and the absorbance value at 517 nm is reduced and henceforth, the percentage of scavenging is regarded as a measure of antioxidant activity.

Results of DPPH scavenging activity are presented in Table 4. It was found that the scavenging activity was 52.5% at 2 mg/ml concentration for the sample having the highest DS (SG1). Notwithstanding, this was exceptionally less in contrast to standard BHA. This result indicated that native guar gum also had

Table 5 — Antioxidant activity of sulfated guar gum by H_2O_2 scavenging method using BHA (butylated hydroxyanisole) as standard

Conc. $\mu g \cdot ml^{-1}$	% H_2O_2 Scavenging (Mean ± SD, n = 3)						
	Guar gum	SG1	SG2	SG3	SG4	OSG1	BHA
250	5.61 ± 0.27	9.01 ± 0.39	8.18 ± 0.50	7.59 ± 0.33	7.13 ± 0.24	11.25 ± 0.35	37.49 ± 0.83
500	10.33 ± 0.36	16.26 ± 0.40	12.11±0.48	10.81 ± 0.49	10.54 ± 0.38	18.56 ± 0.70	61.27 ± 0.90
1000	14.41 ± 0.44	25.77 ± 0.40	16.39±0.47	15.64 ± 0.43	14.33 ± 0.68	29.19 ± 0.86	69.85 ± 0.96
1500	19.10 ± 0.39	31.52 ± 0.57	20.56 ± 0.64	22.14 ± 0.51	18.10 ± 0.60	35.78 ± 0.89	75.61 ± 0.62
2000	25.37 ± 0.51	39.40 ± 0.47	27.83 ± 0.36	26.51 ± 0.57	23.71 ± 0.48	47.15 ± 0.91	81.48±1.07

little antioxidant activity; whereas the sulfated guar gum exhibited better antioxidant activity in dose dependent manner. Li and Shah (2014) noted highest DPPH radicals scavenging activity of sulfated *P. eryngii* polysaccharide. The activity became nearly doubled over that of raw polysaccharide at higher degree of sulfate substitution.[43] Wang *et al.* (2014) reported that high degree of sulfation of *Artemisia sphaerocephala* polysaccharide could strengthen the free radical scavenging activities.[44] They reasoned that the -OSO_3H groups activated the hydrogen atom of the anomeric carbon and promoted its hydrogen atom-donating capacity and thereby antioxidant activity of the polysaccharide.

Wang *et al.* (2010) found that guar gum sulfated with CSA: pyridine (2:1) at C-6 position showed the highest DS (1.01) and best DPPH scavenging activity, corresponding to 69.54% at 5 mg/ml concentration.[26] Another report stated that the region selective sulfation at C-3 and C-2 of guar gum and reduction of molecular weight enhanced the DPPH radical scavenging activity further, even 0.02 mg/ml.[27] Thus, the antioxidant property could be the function of molecular weight, DS and position of sulfation.

Scavenging of H_2O_2 is based on the principle that the antioxidant donates electrons to H_2O_2 and neutralizes it to water. The H_2O_2 scavenging activity of sulfated guar gum is given in Table 5. The test sample with the highest DS (SG1) demonstrated 39.4% H_2O_2 scavenging activity at 2 mg/ml and the same was found to be dose dependent. However, the activity was less than the standard BHA.

Gülçin *et al.* (2004) also reported dose-dependent hydrogen peroxide scavenging activity for aqueous extract of *Urtica dioica* L.[32] However, the same was only 23% for this aqueous extract at 250 $\mu g \cdot ml^{-1}$ dose.

The tailoring of polysaccharide is another way of gaining oligosaccharides with improved solubility and bioactivity. It was interesting to note that the double chemical modification i.e., partial oxidation followed by sulfation of guar gum augmented both DPPH scavenging and H_2O_2 scavenging activities appreciably (Table 4, Table 5). Similar findings were reported by Sun *et al.* (2010) for partially oxidized sulfated polysaccharide κ-Carrageenan.[37] They noted relatively stronger antioxidant activities of κ-Carrageenan with low molecular weight. Because the capacity of low molecular weight polysaccharide to establish intramolecular hydrogen bonds is less, the hydroxyl groups became activated, and thus facilitated free radical scavenging process. Moreover, the introduction of carboxyl groups after partial oxidation of the guar gum could improve hydrophilicity of the oxidized derivatives.[45]

Carboxyl groups, being the strong electron withdrawing group decreased the electron density in the main chain and thereby activating the hydroxyl group to react easily with superoxide anion as well as hydroxyl radicals might have significant impact on the antioxidant activity.[37,46]

The sulfated derivative (SG1) of guar gum exhibited maximum DPPH and H_2O_2 scavenging activities at 2 mg/ml concentration. Hence, ANOVA analysis was done (GraphPad Prism Software, Version 3.00, Trial) for the data obtained at 2 mg/ml concentration to find out any statistical significant differences that may exist between native guar gum, SG1, OSG1 and BHA. The analysis suggested that overall differences in DPPH scavenging activity were statistically significant ($p < 0.001$). Further, Tukey's test indicated that DPPH scavenging activity of SG1, OSG1 and BHA activity was statistically different from native guar gum ($p < 0.001$). Compared to BHA standard, the said activity of SG1 and OSG1 was also different statistically ($p < 0.001$). Further, a significant difference the mean DPPH scavenging activity between SG1 and OSG1 was evident ($p < 0.001$). The statistical comparison was also executed for the H_2O_2 scavenging data obtained for native guar gum, SG1, OSG1 and BHA at 2 mg/ml. However, the trend in overall statistical differences as well as between samples was similar to that observed for DPPH scavenging activity ($p < 0.001$).

Conclusions

This study encompassed the synthesis of sulfated guar gum as a function of CSA: Pyridine ratio. A maximum DS value of 0.73 was obtained with the use of equal amount of CSA and Pyridine. The antimicrobial study revealed that minimum degree of sulfation is required for exhibiting antimicrobial activity and the same was found to be dose-dependent. However, no significant activity against fungal strain *C. albicans* was evident. Further, the sulfated guar gum had DPPH and H_2O_2 radical scavenging activity, though the magnitude was less than the standard antioxidant BHA. One major finding was that the partial oxidation followed by sulfation greatly potentiated the antioxidant activity of guar gum. The findings of this study could be beneficial in food preservation and other pharmaceutical applications.

References

1 Gopinath V, Saravanan S, Al-Maleki AR, Ramesh M & Vadivelu J, A review of natural polysaccharides for drug delivery applications: Special focus on cellulose, starch and glycogen, *Biomed Pharmacother*, **107** (2018) 96–108.

2 Parvathy K S, Susheelamma N S, Tharanathan R N & Gaonka A K, A simple non-aqueous method for carboxymethylation of galactomannans, *Carbohydr Polym*, **62(2)** (2005) 137–141.

3 Xing R, Liu S, Yu H, Guo Z, Li Z & Li P, Preparation of high-molecular weight and high-sulfate content chitosans and their potential antioxidant activity in vitro, *Carbohydr Polym*, **61(2)** (2005) 148–154.

4 Xiong X, Li M, Xie J, Jin Q, Xue B & Sun T, Antioxidant activity of xanthan oligosaccharides prepared by different degradation methods, *Carbohydr Polym*, **92(2)** (2013) 1166–1171.

5 Yan J K, Ma H L, Cai P F, Zhang H N, Zhang Q, Hu N Z, Feng X B & Wu J Y, Structural characteristics and antioxidant activities of different families of 4-acetamido-TEMPO-oxidised curdlan, *Food Chem*, **143** (2014) 530–535.

6 Sun T, Yao Q, Zhou D & Mao F, Antioxidant activity of *N*-carboxymethyl chitosan oligosaccharides, *Bioorganic & Med Chem Lett*, **18(21)** (2008) 5774–5776.

7 Li J, Shang W, Si X, Bu D, Starppe P, Xhou Z & Blanchard C, Carboxymethylation of corn bran polysaccharide and its bioactive property, *Int J Food Sci Technol*, **52(5)** (2017) 1176–1184.

8 Yamamoto I, Takayama K, Gonda T, Matsuzaki K, Hatanaka KI, Yoshida T, Uryu T, Yoshida O, Nakashima H, Yamamoto N, Kaneko Y & Mimura T, Synthesis, structure and antiviral activity of sulfates of curdlan and its branched derivatives, *Br Polym J*, **23(3)** (1990) 245–250.

9 Chen Y, Zhang H, Wang Y, Nie S, Li C & Xie M, Acetylation and carboxymethylation of the polysaccharide from *Ganoderma atrum* and their antioxidant and immunomodulating activities, *Food Chem*, **156** (2014) 279–288.

10 Jyotsna V P, Dhas T S, Mani R & Raguraman V, Antiviral activity of sulfated polysaccharides from *Sargassum ilicifolium* against fish Betanodavirus infection, *Aquacult Int*, **29** (2021) 1049–1067.

11 Liu Y, Liu C, Tan H, Zhao T, Cao J & Wang F, Sulfation of a polysaccharide obtained from Phellinus ribis and potential biological activities of the sulfated derivatives, *Carbohydr Polym*, **77(2)** (2009) 370–375.

12 Wang L, Li X X & Chen Z, Sulfated modification of the polysaccharides obtained from defatted rice bran and their antitumor activities, *Int J Biol Macromol*, **44(2)** (2009) 211–214.

13 Srivastava M & Kapoor V P, Seed galactomannans: An overview, *Chem Biodivers*, **2(3)** (2005) 295–317.

14 Prabaharan M, Prospective of guar gum and its derivatives as controlled drug delivery systems, *Int J Biol Macromol*, **49(2)** (2011) 117–124.

15 Crescenzi V, Dentini M, Risica D, Spadoni S, Skjåk-Braek G, Capitani D, Mannina L & Viel S, C(6)-oxidation followed by C(5)-epimerization of guar gum studied by high field NMR, *Biomacromolecules*, **5(2)** (2004) 537–546.

16 Herold B C, Gerber S I, Polonsky T, Belval B J, Shaklee P N & Holme K, Identification of structural features of heparin required for inhibition of herpes simplex virus type 1 binding, *Virol*, **206(2)** (1995) 1108–1116.

17 Yoshida T, Synthesis of polysaccharides having specific biological activities, *Prog Polym Sci*, **26(3)** (2001) 379–441.

18 Ono L, Wollinger W, Rocco I M, Coimbra T L M, Gorin P A J & Sierakowski M R, In vitro and in vivo antiviral properties of sulphated galactomannans against yellow fever virus (BeH111 strain) and dengue 1 virus (Hawaii strain), *Antivir Res*, **60(3)** (2003) 201–208.

19 Muschin T, Budragchaa D, Kanamoto T, Nakashima H, Ichiyama K, Yamamoto N, Shuqin H & Yoshida T, Chemically sulfated natural galactomannans with specific antiviral and anticoagulant activities, *Int J Biol Macromol*, **89** (2016) 415–420.

20 Wang Y, Peng Y, Wei X, Yang Z, Xiao J & Jin Z, Sulfation of tea polysaccharides: synthesis, characterization and hypoglycemic activity, *Int J Biol Macromol*, **46(2)** (2010) 270–274.

21 Chen T, Li B, Li Y, Zhao C, Shen J & Zhang H, Catalytic synthesis and antitumor activities of sulfated polysaccharide from *Gynostemma pentaphyllum* Makino, *Carbohydr Polym*, **83(2)** (2011) 554–560.

22 Wang X & Zhang L, Physicochemical properties and antitumor activities for sulfated derivatives of lentinan, *Carbohydr Res*, **344(16)** (2009) 2209–2216.

23 Souza B W S, Cerqueira M A, Bourbon A I, Pinheiro A C, Martins J T, Teixeira J A, Coimbra M A & Vicente A A, Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*, *Food Hydrocoll*, **27(2)** (2012) 287–292.

24 Palanisamy S, Vinosha M, Marudhupandi T, Rajasekar P & Prabhu N M, In vitro antioxidant and antibacterial activity of sulfated polysaccharides isolated from *Spatoglossum asperum*, *Carbohydr Polym*, **170** (2017) 296–304.

25 Li Q, He D, Chen W & Ni L, Preparation, characterization and anticoagulant activity of guar gum sulphate, *J Macromol Sci Pure Appl Chem*, **42(8)** (2005) 1085–1094.

26 Wang X, Wang J, Zhang J, Zhao B, Yao J & Wang Y, Structure–antioxidant relationships of sulfated galactomannan from guar gum, *Int J Biol Macromol*, **46(1)** (2010) 59–66.

27 Wang J, Niu S, Zhao B, Wang X, Yao J, Zhang J, Zhao W & Zhao Y, Regioselective synthesis of sulfated guar gum: Comparative studies of structure and antioxidant activities, *Int J Biol Macromol*, **62** (2013) 734–740.

28 Mähner C, Lechner M D & Nordmeier E, Synthesis and characterisation of dextran and pullulan sulphate, *Carbohydr Res*, **331(2)** (2001) 203–208.

29 Krichen F, Karoud W, Sila A, Abdelmalek B E, Ghorbel R, Ellouz-Chaabouni S & Bougatef A, Extraction, characterization and antimicrobial activity of sulphated polysaccharides from fish skins, *Int J Biol Macromol*, **75** (2015) 283–289.

30 Nair M S, Saxena A & Kaur C, Characterization and antifungal activity of pomegranate peel extractand its use in polysaccharide-based edible coatings to extend the shelf-life of capsicum (*Capsicum annuum* L.), *Food Bioproc Tech*, **11** (2018) 1317–1327.

31 Shimada K, Fujikawa K, Yahara K & Nakamura T, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J Agric Food Chem*, **40(6)** (1992) 945–948.

32 Gülçin I, Küfrevioglu O I, Oktay M & Büyükokuroglu M E, Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.), *J Ethnopharmacol*, **90(2-3)** (2004) 205–215.

33 Nguyen T L, Chen J, Hu Y, Wang D, Fan Y, Wang J, Abula S, Zhang J, Qin T, Chen X, Chen X, Khakame S K & Dang B K, In vitro antiviral activity of sulfated *Auricularia auricula* polysaccharides, *Carbohydr Polym*, **90(3)** (2012) 1254–1258.

34 Wei D, Wei Y, Cheng W, & Zhang L, Sulfated modification, characterization and antitumor activities of Radix hedysari polysaccharide, *Int J Biol Macromol*, **51(4)** (2012) 471–476.

35 Cabassi F, Casu B, & Perlin A S, Infrared absorption and Raman scattering of sulfate groups of heparin and related glycosaminoglycans in aqueous solution, *Carbohydr Res*, **63** (1978) 1–11.

36 Kang B, Dai Y D, Zhang H Q & Chen D, Synergetic degradation of chitosan with gamma radiation and hydrogen peroxide, *Polym Degrad Stabil*, **92(3)** (2007) 359–362.

37 Sun T, Huina T, Jing X, Shuo Z & Xin X, Degradation and antioxidant activity of κ-Carrageenans, *J Appl Polym Sci*, **117** (2010) 194–199.

38 Lu Y, Zhao X & Fang S, Characterization, antimicrobial properties and coatings application of gellan gum oxidized with hydrogen peroxide, *Foods*, **8(1)** (2019) 31–42.

39 Chandrika P K S V, Singh A, Rathore A & Kumar A, Novel crosslinked guar gum-g-poly (acrylate) porous superabsorbent hydro gels: Characterization and swelling behaviour in different environments, *Carbohydr Polym*, **149** (2016) 175–185.

40 Vijayabaskar P, Noormohamed V & Ganapathy T, Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae Sargassum swartzii, *Chinese J Nat Med*, **10(6)** (2012) 421–428.

41 Seedevi P, Moovendhan M, Viramani S & Shanmugam A, Bioactive potential and structural characterization of sulfated polysaccharide from seaweed (*Gracilaria corticata*), *Carbohydr Polym*, **155** (2017) 516–524.

42 Alban S & Franz G, Characterization of the anticoagulant actions of a semisynthetic curdlan sulfate, *Thromb Res*, **99(4)** (2000) 377–388.

43 Li S Q & Shah N P, Antioxidant and antibacterial activities of sulphated polysaccharides from *Pleurotus eryngii* and *Streptococcus thermophilus* ASCC 1275, *Food Chem*, **165** (2014) 262–270.

44 Wang J L, Niu S F, Zhao B T, Luo T, Liu D & Zhang J, Catalytic synthesis of sulfated polysaccharides II: comparative studies of solution conformation and antioxidant activities, *Carbohydr Polym*, **107** (2014) 221–231.

45 Bae I Y, Kim H Y, Lee S & Lee H G, Effect of the degree of oxidation on the physicochemical and biological properties of *Grifola frondosa* polysaccharides, *Carbohydr Polym*, **83(3)** (2011) 1298–1302.

46 Xu J, Liu W, Yao W B, Pang X B, Yin D K & Gao X D, Carboxymethylation of a polysaccharide extracted from *Ganoderma lucidum* enhances its antioxidant activities in vitro, *Carbohydr Polym*, **78(2)** (2009) 227–234.