Sulfated modification and anti-HIV activity evaluation of *Lycium barbarum* polysaccharides

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Abstract

BACKGROUND: *Lycium barbarum* polysaccharides (LBPs) belong to a very important class of biological macromolecules from *Lycium barbarum* berries in nature, and have received much attention due to their various biological activities. Since sulfated polysaccharides have antiviral activity in vitro, there is no published research on the sulfated modification of LBPs.

RESULTS: The objective of this study was to investigate the feasibility of sulfated modification of LBPs and their potential application in inhibiting HIV-1. The LBPs with different molecular weight were prepared by fractional precipitation from crude LBP through aqueous extraction, ethanol precipitation and deproteinization. The purified LBPs (G1, G2 and G3) were sulfated by chlorosulfonic acid pyridine method to give rise to the sulfated LBPs (G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4) with different degrees of substitution. The anti-HIV-1activities were evaluated by TD_{50} of the cytotoxicity and IC_{50} of inhibitory activity using the CCK-8 and Magi test with Azidothymidine (AZT) as positive control. The results showed that LBPs and sulfated LBPs showed non-toxicity with the TD_{50} >100 μg/mL. Compared with the LBPs, the inhibition of anti-HIV-1 activity of sulfated LBPs was increased significantly with the IC_{50} value from 0.0924-0.1206 μg/mL to 0.0206-0.0722 μg/mL. The G1S4 (Mw=2.13×10^4 Da and DS=1.12) showed excellent anti-HIV-1 activity with the IC_{50} value near to that of AZT (0.0200μg/mL), which would make it possible to become a candidate compound with anti-HIV-1 activity.

CONCLUSION: The outcome of the study indicated that sulfated modification of LBPs was feasible and sulfated LBPs had good potential as an anti-HIV drug.

Introduction

Lycium barbarum, normally called Goji in China or wolfberry in western countries, is a well-known traditional Chinese medicine all over the world because of its great health benefits and as a food supplements[1,2]. Polysaccharides are the main component in *Lycium barbarum* and considered as the most important functional components in *Lycium barbarum* berries[3]. The polysaccharides of *Lycium barbarum* (LBPs) are a group of water-soluble glycoconjugates and contain 6 monosaccharides (arabinose, rhamnose, xylose, mannose, galactose and glucose) and 18 amino acids[4,5]. They have been identified with varieties of biological and pharmacological activities, such as antioxidants[6], anti-inflammatory[7], and immunodeficiency[8]. Although the various biological functions of LBPs have received extensive attention, little information is available in the literature on the sulfated modification. As sulfated modification can improve the biological activities of polysaccharides effectively or sometime produce new functional properties through partial substitution of hydroxyl group by sulfate group[9,10,11], it has become one of research hotspots in the field of chemical polysaccharides modification. By the way, the sulfated polysaccharides obtained by sulfation of both natural and synthetic polysaccharides have shown excellent anti-HIV activity[12,13]. The anti-HIV mechanism of sulfated polysaccharides could be estimated as the electrostatic interaction between negatively charged sulfate groups in sulfated

polysaccharides and positively charged amino acids in the HIV surface glycoprotein gp120 (HIV gp120), with the positively charged amino acids cumulated region at the C-terminus of the HIVgp120,Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg[14]. The electrostatic interactions between sulfated polysaccharides and HIV oligopeptides were demonstrated by surface plasmonresonance and the degree of sulfation was an essential factor for the potent anti-HIV activity of sulfated polysaccharides[15]. For they were widely available, the LBPs were selected as the research object to investigate the feasibility of sulfated modification and anti-HIV evaluation. In this paper, the sulfated LBPs with different molecular weight (*M*w) were prepared via the ethanol precipitation, deproteinization, and sulfated modification by the method of chlorosulfonic acid pyridine method. The anti-HIV-1 activity of sulfated LBPs was evaluated using MAGI experiments. All these results provide references for the LBPs chemical modification and serve as a promising candidate compounds with anti-HIV-1 activity.

Materials And Methods

Materials *Lycium barbarum* fruits were purchased from Guilin Shili Natural Foods Co., Ltd (Guangxi, China). Acetic acid, n-butanol, Chlorosulfonic acid, and other reagents used were analytical reagent grade and used without further purification. All solutions were prepared with deionized water. Diethylaminoethyl-cellulose (DEAE-52) was purchased from Sinopharm Chemical Reagent Company of Beijing. GPC system (Waters 2696) was purchased from Waters Company of America. The freeze dryer(FD-1C) was purchased from Beijing boyaikang Experimental Instrument Co., Ltd. UV-VIS spectrophotometer(UV-265) was purchased from Shimadzu company of Japan. Inverted microscope (XDS-1B) was purchased from Shanghai Precision Instrument Co., Ltd. FT-IR spectrometer was purchased from Bruker Company of Germany.

Extraction and Purification of polysaccharides from Lycium barbarum **fruits** The dried *Lycium barbarum* fruits were processed into powder. The powder of *Lycium barbarum* fruits was extracted with hot distilled water at a ratio of 1:20 g/mL at 85°C for 1h. After filtration, the residues were extracted 2 times under the same conditions. The filtrates after three times of extraction with Sevag reagent (chloroform/n-butanol 4:1,v/v) were combined and stirred vigorously for 15 minutes by the Sevag method[16]. After centrifugation at 3000 rpm for 25 minutes, the intermediate denatured protein and chloroform layer were discarded and the supernatant was retained. The supernatant was concentrated by rotary evaporator under reduced pressure at 50–55°C. The concentrate was added to 3 times the volume of 95% ethanol and kept at room temperature for 1 hour. After centrifugation at 3000 rpm for 10 minutes, the precipitate was collected, washed with anhydrous ethanol twice and lyophilized to yield the crude LBP.

The crude LBP was dissolved in distilled water (5 mg/mL), in which the refrigerated absolute ethanol was added. After refrigerated at -4℃ for 4h, the mixture was centrifuged, and the precipitate was freeze-dried to obtain the first molecular weight LBP. The filtrated supernatant was added with refrigerated anhydrous ethanol and refrigerated at -4℃ for 4h. After centrifugation, the precipitate was freeze-dried to obtain the second molecular weight LBP. The third molecular weight LBP was obtained by the same operation as the second molecular weight LBP. LBPs with different molecular weight were purified by a diethylaminoethyl

(DEAE) cellulose-52 column (2.5×50 cm). The column was eluted first by filtered (0.45μm membrane) distilled water and then stepwise eluted by 0.1, 0.3, 0.5mol/L NaCl at a flow rate of 1.0mL/min. The eluates were collected and freeze-dried to obtain pure brown LBPs with different average molar weight (*M*w), named as G1, G2 and G3. The polysaccharide content of LBPs was detected by the phenol-sulfuric acid method.

Sulfated modification of LBPs Sulfated modification of LBPs was carried out by the method of chlorosulfonic acid pyridine (Fig. 1)[17,18]. 0.7g of LBPs was dissolved in 15 mL anhydrous DMSO by stirring at room temperature. After the LBPs fully dispersed into the DMSO, the different ratios of LBP to chlorosulfonic acid pyridine complex were 1:1, 1:2, 1:3 and 1:4 g/mL to prepare the sulfated LBPs with different degrees of substitution (DS). The mixture was stirred at reaction temperature of 45°C for 1 h. After reaction, the mixture was cooled to room temperature and poured into ice water, neutralized with ethanol, precipitated, dissolved in distilled water, dialyzed for 72 h, filtered, and the filtrate was lyophilized to obtain sulfated LBPs. G1, G2 and G3 were sulfated respectively, the corresponding sulfated polysaccharides were obtained and named as G1S1-G1S4, G2S1-G2S4, G3S1-G3S4 (as shown in Table 1).

Table 1 Names of sulfated LBPs with different degrees of substitution (DS)

No.	Ratios of LBP to chlorosulfonic acid pyridine complex (g/mL) 2 3 4			
	1:1	1:2	1:3	1:4
G1	G1S1	G1S2	G1S3	G1S4
G2	G2S1	G2S2	G2S3	G2S4
G3	G3S1	G3S2	G3S3	G3S4

Characterization of LBPs

Determination of protein content

The protein content of LBPs was determined by the Lowry method[19,20]. Different bovine serum albumin (BSA) concentrations were prepared as standard and shown in Table 2, in which Folin-phenol reagent was added. At the same time the LBPs solution was ready for detection. Then the absorbance of the standards and sample solution were determined by UV-Vis spectrophotometer at the wavelength of 500 nm.

Table 2 Different bovine serum albumin (BSA) concentrations as standard

No.	1	2	3	4	5	6
Volume of 0.25mg/mL BSA (mL)	0.00	2.00	4.00	6.00	8.00	10.00
Volume of distilled water (mL)	10.00	8.00	6.00	4.00	2.00	0.00
BSA (mg)	0.0	0.50	1.00	1.50	20.00	2.50

According to the absorbance value, the protein content in the LBPs solution was obtained from the standard curve of BSA, and then the mass percentage content of protein in the LBPs was calculated.

Determination of polysaccharide content

The polysaccharide content of LBPs was determined by anthrone-sulfuric acid method[21]. Different glucan concentrations were prepared as standard and shown in Table 3, in which anthrone reagent was added in ice water bath and then transferred into the boiling water bath for 10min and cooled to room temperature. The absorbance of the standard and sample solution was determined by UV-VIS spectrophotometer at the wavelength of 625nm.

Table 3 Different glucan concentrations as standard

No.	1	2	3	4	5	6
Volume of 0.1008 mg/mL glucan (mL)	0.00	5.00	10.00	20.00	30.00	40.00
Volume of distilled water (mL)	50.00	45.00	40.00	30.00	20.00	10.00
Glucan (mg)	0.0	0.5040	1.008	2.016	3.024	4.032

According to the absorbance value the polysaccharide content of LBPs was obtained from the standard curve of glucan, and then the mass percentage content of polysaccharide in the LBPs was calculated.

Degree of substitution (DS)

The DS was used to measure the degree of hydroxyl replaced by sulfated group, which was usually determined by barium chloride-gelatin methods[22,23]. Different K_2SO_4 concentrations were prepared as standard and shown in Table 4, then trichloroacetic acid solution and barium acetate-gelatin solution were added respectively. The absorbance of the mixture was determined by UV-Vis spectrophotometer at the wavelength of 360 nm 15min later.

Table 4 Different K_2SO_4 concentrations as standard

No.	1	2	3	4	5	6
Volume of 100 ug/mL K_2SO_4 (mL)	0.00	0.80	1.20	1.60	2.00	2.40
Volume of distilled water (mL)	3.20	2.40	2.00	1.60	1.20	0.80
SO_4^{2-} (μg)	0.00	80	120	160	200	240

According to the absorbance value, the sulfate content of LBPPs was obtained from the standard curve of K_2SO_4, and then the sulfate content S% in the sample was calculated. The DS was confirmed by

calculation as the following formula[24]:

DS = [1.62×S(%)]/[32-1.02×S(%)]

DS was the degree of LBPs sulfation; S% is sulfur content.

Determination of relative molecular weight (*M*w)

The relative molecular weight of LBPs and sulfated LBPs was determined by HPSEC of the Waters 2696 gel permeation chromatograph (US)[25]. A series of standard dextrans (1.0 mg) with average molecular weight of 1000、5900、11800、22800、47300、112000、212000、404000、788000 Da were dissolved in distilled water with 1.0 mg/mL concentration in turn, which were injected to Ultrahydrogel columns (7.8 cm × 300 cm).The purified water was used as mobile phase at 0.6mL/min flow rate, the injection concentration was 0.30%, and the injection volume was 50 μL with the column temperature at 50°C. Three columns, Ultrahydrogel 100, Ultrahydrogel 250, Ultrahydrogel 1000, were in series with protective column on Empower chromatography workstation.

There was a linear relationship between the retention time and the logarithm of the molecular weight of the series of standard dextrans in the range of 1.0-788 kDa.The formula for calculation was as follows: $\log M = 26.8 - 1.51\ t_R + 0.0363\ t_R^2 - 0.000324\ t_R^3$ ($R^2 = 0.9999$) ($t_R$was the retention time). According to the equation of the calibration curve, the average *M*w of LBPs (G1, G2 and G3) and sulfated LBPs (G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4) were determined respectively.

Fourier transform infrared (FT-IR) spectroscopy analysis

Fourier transform infrared (FTIR) spectra were performed on a Vertex-70 spectrometer (Bruker, Germany) with a scanning range of 4000 – 500 cm^{-1}. The LPBs and sulfated LPBs were ground with dried KBr powder at a mass ratio of 1:100 and pressed into thin slices in a grinding tool for FTIR measurement.

Assay for anti-HIV activity

Cytotoxicity

Cell counting kit-8 (CCK-8) reagent contains WST-8. The dehydrogenase in living cells can reduce WST-8 to water-soluble yellow methyl Zan product, and the amount of methyl Zan generated is directly proportional to the amount of living cells. The number of living cells can be reflected by the absorbance value at 450 nm measured by enzyme labeled instrument. The median toxic dose (TD_{50}) was calculated by regression analysis.

Anti-HIV activity

Human immunodeficiency virus (HIV) infects the host cells by targeting CD4 molecules on the cell surface and the seven transmembrane-spanning G protein-coupled coreceptors, for example, CXCR4 (X4) and CCR5 (R5), via the V3 loop of gp120[26,27]. HIV usingR5 as a coreceptor (R5-HIV) has been isolated

from patients in the all stages. MAGI, ELISA and MTT are frequently used for the evaluation of R5-HIV replication against HIV virus. In this paper, Magi test was used to determine the inhibitory effect of sulfated LBPs on HIV-1 virus.

(1) HeLa-CD4 + β-gal cells (6×10^3 cells/well) were inoculated in flat bottom 96-well microtitre plates, and 40% of them were stratified the next day. (2) DMEM medium containing 20 μg/mL DEAE was prepared, 100μL 2 mg/mL DEAE was added into 9.9 mL DMEM. (3) Diluting solution: The highest non-toxic concentration (e.g. 200 μg/mL) was selected, diluting 5 concentrations with 4 times of ratio. Each concentration solution 100 uL was added into 4 multiple wells. (4) Control group, virus control was added with 100 μL DMEM medium; while cell control was added with 100 μL DMEM medium and 100 μL DMEM medium containing DEAE. (5) Cultured in 5% CO_2 incubator at 37°C for 40 ~ 48h, fixation and staining. (6)100 μL LPBs was added into each well, and the number of blue spots was counted automatically under Olympus inverted microscope. (7) IC_{50} value was calculated by software SPSS Probit.

Results And Discussion

Characterization of LBPs and sulfated LBPs

The characters of LPBs polysaccharides G and their sulfates GS were shown in Table 5. The G and GS were light brown solid and soluble in water. The water solubility of GS was better than that of G and improved by the sulfated modification. The higher water solubility of sulfated LBPs was suitable for their medicinal application[10].

Table 5 Characterizations of the LPBs and sulfated LBPs

Entry	Protein (%)	Polysaccharide (%)	S (%)	DS	Mw×10^4 (Da)
G1	5.65	78.6	-	-	2.52
G1S1	1.98	60.7	0.96	0.05	2.36
G1S2	3.43	63.3	2.20	0.12	2.07
G1S3	3.76	50.8	4.84	0.29	1.98
G1S4	2.86	61.2	12.97	1.12	2.13
G2	7.64	86.3	-	-	8.65
G2S1	2.62	59.2	1.68	0.09	8.26
G2S2	3.47	53.6	5.80	0.36	8.45
G2S3	2.56	82.0	12.63	1.07	7.96
G2S4	1.88	79.4	13.04	1.13	7.68
G3	6.85	80.6	-	-	13.53
G3S1	2.52	59.2	8.71	0.06	13.42
G3S2	2.78	58.4	10.85	0.59	13.51
G3S3	3.35	62.5	11.97	0.98	12.76
G3S4	3.22	60.8	16.45	1.75	12.05

As shown in Table 5, the content of proteins was quantified by the Lowry reaction. Compared with LBPs (G1, G2 and G3), the protein content in all the sulfated LBPs (G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4) was significantly decreased by the sulfated modification in agreement with the reports about the sulfated modification of polysaccharides by Li et al and Xiao et al[28,29]. For example, the protein content of G1 was 5.65%, and that of G1S1 was 1.98%. Also the proteins content of G2 and G3 with 7.64% and 6.85%, and those of G2S1 and G3S1 were 2.62% and 2.52%. The total sugar content was determined by the phenol-sulfuric acid method, the values of G1, G2 and G3 were 78.6%, 86.4% and 80.6%, those of G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4 were about 60%. The relative molecular weight were determined by HPSEC, the *M*w of G1, G2 and G3 were 2.54×10^4 Da, 8.86×10^4 Da and 13.53×10^4 Da, the *M*w of G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4 were decreased a bit by the sulfated modification. The *M*w of G1 was 2.54×10^4 Da, and those of G1S1 and G1S2 were 2.36×10^4 Da and 2.07×10^4 Da. All the sulfated LBPs showed a decrease in *M*w, this may be the degradation of polysaccharides in the process of sulfated modification for the chlorosulfonic acid is a kind of strong acid.

During the sulfated modification, the amount of sulfated esterification reagents was a key factor for the DS value of sulfated LBPs derivatives. The difference in the DS could lead to different biological activities. Thus, four different ratios of LBPs to chlorosulfonic acid pyridine complex were 1:1, 1:2, 1:3, 1:4 g/mL. The determination of sulfur content was carried out by the gelation-barium acetate, the DS values of G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4 were 0.05–1.75 in four grades for each LBPs with different *M*w. The DS values of sulfated LBPs were increased with the higher ratios of LBPs to esterification reagents, the G1S4, G2S4 and G3S4 with the highest DS of 1.12, 1.13 and 1.75 respectively. The DS values of sulfated were results showed that the sulfated modification was successful with the sulfated LBPs. For G1, the DS values of G1S1-G1S4 were 0.05, 0.12, 0.29 and 1.12. For G2, those of G2S1-G2S4 were 0.09, 0.36, 1.07 and 1.13, and for G3, those of G2S1-G2S4 were 0.06, 0.59, 0.98 and 1.75.

Fourier transform infrared (FTIR) spectra was also used to analyzing the difference in the LBPs and their sulfated LBPs. As could be seen from Fig. 2, the overall waveforms, before and after sulfated modification of LBPs, was similar, the changes were mostly concentrated in the 3350 cm^{-1}, 1016 cm^{-1} and 683 cm^{-1} regions. The 3350 cm^{-1} was the OH–stretching vibration peak and narrowed in sulfated LBPs for the hydroxyl groups of LBPs was sulfated. The 1016 cm^{-1} and 683 cm^{-1} were the absorption peaks caused by the S = O and the C-O-S stretching vibrations, the larger absorptions indicated that the sulfated modification of LBPs was successful.

Evaluation of sulfated LBPs on Anti-HIV-1 activity

For testing the toxicity to the cells, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used. By using the CCK-8 assay with Azidothymidine (AZT) approved by FDA as positive control, the median toxic dose (TD_{50}) was calculated by regression analysis. As shown in Table 6, the median toxic dose (TD_{50}) of the G1, G2 and G3 and their sulfates were over 100 μg/mL. They were non-toxic to CCK-8 cells at their maximum soluble concentrations and exhibited inhibitory activities with the

IC_{50} value of 0.02–0.12 μg/mL, which was improved significantly by the sulfated modification. For example, the IC_{50} values of G1, G2 and G3 were 0.1257, 0.0924 and 0.1132 μg/mL, and those of G1S1, G2S1, G3S1 were 0.0365, 0.0524 and 0.0722 μg/mL. The minimum values of IC_{50} was G1S4 with 0.0206 μg/mL and near to that of AZT (IC_{50} = 0.0200 μg/mL).

Table 6 Anti-HIV-1 activities of LBPs and their sulfates

No	DS (%)	*M*w×10^4 (Da)	IC_{50} (μg/mL)	TD_{50} (μg/mL)
G1	-	2.52	0.1206	>100
G1S1	0.05	2.36	0.0365	>100
G1S2	0.12	2.07	0.0289	>100
G1S3	0.29	1.98	0.0363	>100
G1S4	1.12	2.13	0.0206	>100
G2	-	8.65	0.0924	>100
G2S1	0.09	8.26	0.0524	>100
G2S2	0.36	8.45	0.0478	>100
G2S3	1.07	7.96	0.0325	>100
G2S4	1.13	7.68	0.0257	>100
G3	-	13.53	0.1132	>100
G3S1	0.06	13.42	0.0722	>100
G3S2	0.59	13.51	0.0609	>100
G3S3	0.98	12.76	0.0464	>100
G3S4	1.75	12.05	0.0379	>100
AZT		-	0.0200	

The molecular weight of sulfated LBPs had a significant effect on their anti-HIV-1 activity. When the *M*w was about 2×10^4 Da, the activities of G1 and G1S1-G1S4 (IC_{50} values of 0.1206 and 0.0206–0.0365 μg/mL) were better than those of G2 and G2S1-G2S4 (IC_{50} values of 0.0924 and 0.0257–0.0524 μg/mL) with about 8×10^4 Da, and also those of G3 and G3S1-G3S4 (IC_{50} values of 0.1132 and 0.0379–0.0722μg/mL)with about 13×10^4 Da. The reason might be that the sulfated LBPs with lower molecular weight could easily pass through multiple cell membrane barriers and showed better inhibitory activity.

The inhibitory activity of sulfated LBPs on anti-HIV-1 was not only related to molecular weight, but also to the DS values. The molecular weight was similar, the higher the DS value, the higher the inhibitory activity. When the DS was about 1.00, the activities of sulfated LBPs was higher than that of below 1.00 (As shown in Fig. 3). When the *M*w were about 2×10^4, 8×10^4 and 13×10^4 Da, the DS values were about 1.00, the activities of G1S4, G2S4 and G3S4 were higher with the IC_{50} values of 0.0206, 0.0257 and 0.0379 μg/mL. This may be due to the fact that the addition of sulfate group not only provided negative charge which played an important role in antiviral activity, but also changed the binding sites of polysaccharides. Also, it was proved that sulfated modification of LBPs could improve the anti-HIV-1 activity, which reconfirmed the important role of sulfate groups in exhibiting the antiviral activity of sulfated polysaccharides. The higher inhibition is worth emphasizing that is in good agreement with our initial purpose to improve the anti-HIV activity by the sulfated modification of LBPs. The mechanism of relationship between the activity and characters of sulfated LBPs needs further research for the wide applications with anti-HIV-1 activity.

Conclusion

In this paper, the purified LBPs with different molecular weight (G1, G2 and G3) were prepared by fractional precipitation of crude LBPs. Sulfated modification of LBPs was studied by chlorosulfonic acid pyridine method. The four sulfated LBPs with different sulfur content (G1S1-G1S4, G2S1-G2S4 and G3S1-G3S4) were obtained by the 1:1, 1:2, 1:3, 1:4 g/mL ratios of LBPs to chlorosulfonic acid pyridine complex. The characters of LBPs and their sulfates, such as protein content, polysaccharide content, degree of substitution (DS) and relative molecular weight, were determined. The DS values were 0.05–1.75 in four grades for each LBPs with different molecular weight (about 2×10^4 Da of G1, about 8×10^4 Da of G2 and about 13×10^4 Da of G3). The median toxic dose (TD_{50}) of the LBPs and their sulfates were over 100 μg/mL. They were non-toxic to CCK-8 cells at their maximum soluble concentrations and exhibited inhibitory activities with the IC_{50} value of 0.02–0.12 μg/mL, which was improved significantly by the sulfated modification. The minimum values IC_{50} of G1S4 was 0.0206 μg/mL (DS = 1.12 and *M*w = 2.13×10^4Da) and near to that of AZT (IC_{50} = 0.0200 μg/mL). The inhibitory activity LBPs and sulfated LBPs was not only related to molecular weight, but also to DS values. When the molecular weight was about 2×10^4 Da, the activities of G1S1-G1S4 were better than those of G2S1-G2S4 and G3S1-G3S4 with about 8×10^4 Da, and 13×10^4 Da. When the molecular weight was similar and the DS value was about 1, the activities of G1S4, G2S4 and G3S4 were higher than that of G1S1-G1S3, G2S1-G1S3 and G3S1-G1S3 with below 1 of DS. All the results showed that the sulfated modification of LBPs could significantly improve the anti-HIV-1 activity, and the activity was related to the characters of LBPs, especially to the molecular weight and degree of substitution (DS).

Abbreviations

LBP: Lycium barbarum Polysaccharide; AZT: Azidothymidine; *M*w: molecular weight; DEAE: diethylaminoethyl; DMSO: Dimethyl sulfoxide; DS: degree of substitution; TD_{50}: median toxic dose; FT-IR: Fourier transform infrared spectroscopy.

Declarations

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Authors' contributions

Conceptualization, Formal analysis, YZ and NT; Investigation, HW and HY; Writing-original draft, HW; Writing-review and editing, HW and HY. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Jin ML, Huang QS, Zhao K, et al (2013) Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *Int. J. Biol. Macromol.*, 54, 16-23
2. Masci A, Carradori S, Casadei MA, et al (2018) *Lycium barbarum* polysaccharides: Extraction, purification, structural characterisation and evidence about hypoglycaemic and hypolipidaemic effects. A review. *Food Chemistry*, 254, 377-389
3. Cheng J, Zhou ZW, Sheng HP, et al (2015) An evidence-based update on the pharmacological activities and possible molecular targets of *Lycium barbarum* polysaccharides. *Drug Des. dev. Ther.*, 9, 33-78
4. Luo Q, Cai YZ, Yan J, et al (2004) Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sci., 76, 137-149
5. Wang CC, Chang SC, Chen BH (2009) Chromatographic determination of polysaccharides in Lyciumbarbarum Linnaeus. *Food Chemistry*, 116,595-603
6. Liang B, Jin M, Liu H (2011) Water-soluble polysaccharide from dried *Lycium barbarum* fruits: Isolation, structural features and antioxidant activity. *Carbohydr. Polym.*, 83,1947-1951
7. Gan F, Liu Q, Liu Y, et al (2018) *Lycium barbarum* polysaccharides improve CCl4-induced liver fibrosis, inflammatory response and TLRs/NF-kB signaling pathway expression in wistar rats. *Life Sci.*, 192,205-212
8. Zhang XR, Li Yj, Cheng JP, et al (2014) Immune activities comparison of polysaccharide and polysaccharide-protein complex from *Lycium barbarum* L. *Int. J. Biol. Macromol.*, 65,441-445
9. Qian XP, Zha XQ, Xiao JJ, et al (2014) Sulfated modification can enhance antiglycation abilities of polysaccharides from Dendrobium huoshanense. *Carbohydr. Polym.*,101, 982-989
10. Zhang H, Wang JQ, Nie SP, et al (2015) Sulfated modification, characterization and property of a water-insoluble polysaccharide from Ganoderma atrum. *Int. J. Biol. Macromol.*, 79, 248-255

11. Xu Y, Gao YK, Liu F, et al (2018) Sulfated modification of the polysaccharides from blackcurrant and their antioxidant and α-amylase inhibitory activities, *Int. J. Biol. Macromol.* 109, 1344-1354
12. H. Nakashima, O. Yoshida, T. Tochikura, et al (1987) Sulfation of polysaccharides generates potent and selective inhibitors in vitro. *Jpn. J. Cancer Res.*, 78, 1164-1168
13. T. Yoshida (2001) Synthesis of polysaccharides having specific biological activities. *Prog. Polym. Sci.*, 26, 379-441
14. T. Uryu, N. Ikushima, K. Katsuraya, et al (1992) Sulfated alkyl oligosaccharides with potent inhibitory effects on human immunodeficiency virus infection, *Biochem. Pharmacol.*, 43,2385-2392
15. Tungalag Battulga, Oyunjargal Tumurbaatar, Oyundelger Ganzorig et al (2019) Analysis of interaction between sulfated polysaccharides and HIV oligopeptides by surface plasmon resonance. *Int. J. Biol. Macromol.,* 125, 909-914
16. Huang GL, Yang Q, Wang ZB (2010) Extraction and deproteinization of mannan oligosaccharides, *Z. Naturforsch. C.*, 65, 387-390
17. S. Alban, A. Schauerte, G. Franz (2002) Anticoagulant sulfated polysaccharides: Part I. Synthesis and structure-activity relationships of New Pullulan Sulfates. *Carbohydr. Polym.*, 47, 267-276
18. Yang H, Du YM (2003) Chemical modification, characterization and bioactivity of Chinese lacquer polysaccharides from Lac tree rhusvernicifera against leucopenia induced by cyclophosphamide. *Carbohydr. Polym.*, 52,405-410
19. I.D. Nwachukwu, A.T. Girgih, S.A. Malomo, et al (2014) Thermoase-derived flaxseed protein hydrolysates and membrane ultrafiltration peptide fractions have systolic blood pressure-lowering effects in spontaneously hypertensive rats. *Int. J. Biol. Macromol.*, 15, 18131-18147
20. A. Kose, S.S. Oncel (2015) Properties of microalgal enzymatic protein hydrolysates: Biochemical composition, protein distribution and FTIR characteristics. *Biotechnol. Rep.*, 6, 137-143
21. Leng F, Sun S, Jing Y, et al (2016) A rapid and sensitive method for determination of trace amounts of glucose by anthrone-sulfuric acid method. *Bulg. chem. commun.*, 48,109-113
22. Chen S, Chen H, Tian J, et al (2013) Chemical modification, antioxidant and alpha-amylase inhibitory activities of corn silk polysaccharides. *Carbohydr. Polym.,* 98, 428-437
23. Wang ZJ, Xie JH, Shen MY, et al (2018) Sulfated modification of polysaccharides: Synthesis, characterization and bioactivities. *Trends Food Sci. Technol.*, 74, 147-157
24. Keiko Kajiwara, Eiichi Kodama, Masao Matsuoka (2006) A novel colorimetric assay for CXCR4 and CCR5 tropic human immunodeficiency viruses. *Antivir. Chem. Chemoth.*, 17, 215-223
25. Xue S, Yang F, Chen G, et al (2020) Extraction and analysis of polysaccharide from *Momordica charantia*. *Ind. Crops Prod.*, 153, 1-4
26. Wu L, Gerard N.P., Wyatt R., et al (1996) CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature*, 384,179-183
27. Berger E.A., Murphy P.M., Farber J.M (1999) Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.*, 17, 657-700

28. Li H, Wang X, Xiong Q, et al (2020) Sulfated modification, characterization, and potential bioactivities of polysaccharide from the fruiting bodies of *Russula virescens*. *Int. J. Biol. Macromol.*, 154, 1438-1447
29. Xiao H, Fu X, Cao C, et al (2019) Sulfated modification, characterization, antioxidant and hypoglycemic activities of polysaccharides from *Sargassum pallidum*, *Int. J. Biol. Macromol.*, 121, 407-414

Figures

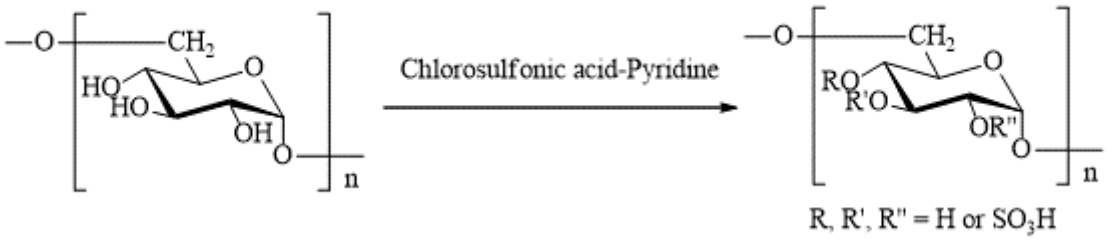

Figure 1

Sulfated modification of LBPs by chlorosulfonic acid-pyridine method

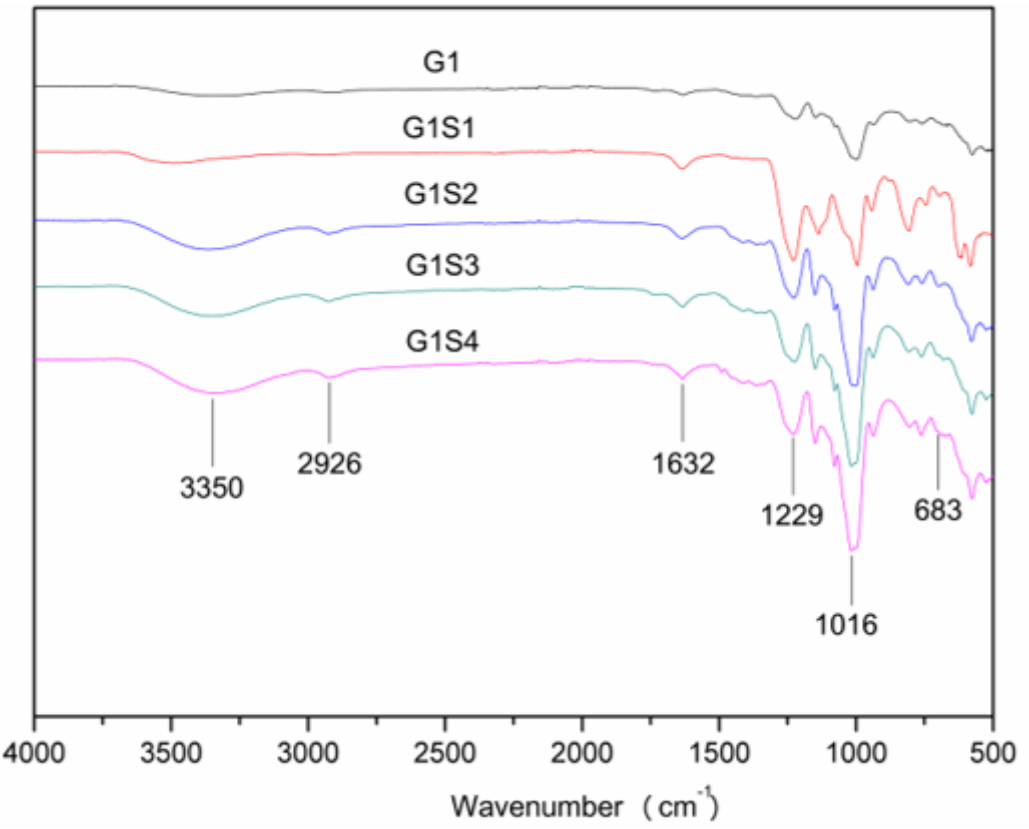

Figure 2

FTIR spectra of G1 and G1S1-G1S4

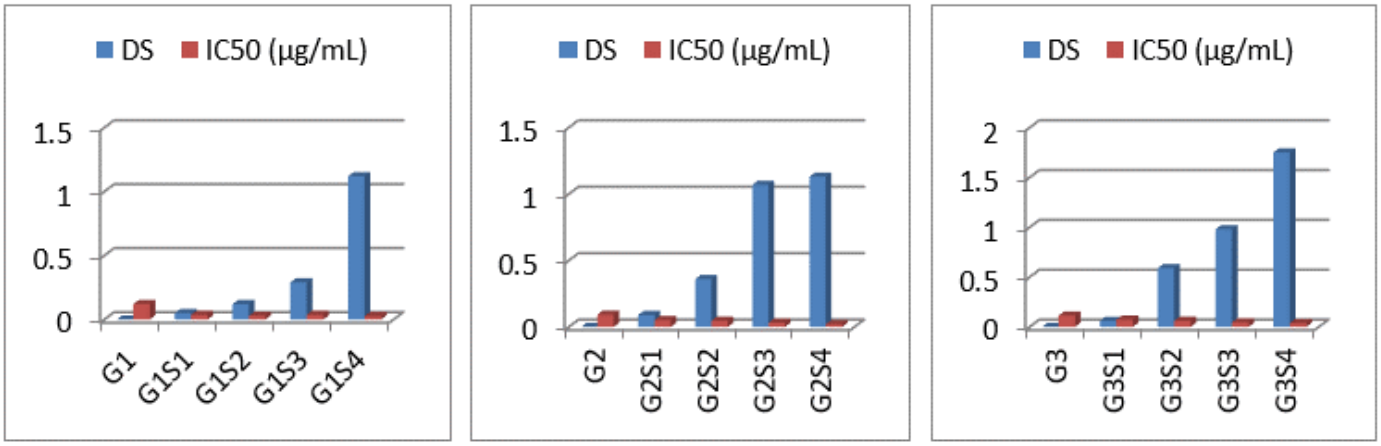

Figure 3

Anti-HIV-1 activities of LBPs and their sulfates