Carbohydrate Polymers 96 (2013) 460–465

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Production, fractionation, characterization of extracellular polysaccharide from a newly isolated *Trametes gibbosa* and its hypoglycemic activity

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ARTICLE INFO

Article history:
Received 5 December 2012
Received in revised form 23 March 2013
Accepted 10 April 2013
Available online 18 April 2013

Keywords:
Optimization
Extracellular polysaccharide
Trametes gibbosa
Fermentation
Molecular weight

ABSTRACT

The submerged fermentation for extracellular polysaccharide (EPS) production from *Trametes gibbosa* was optimized. An optimal medium for EPS production was obtained through central composite design (CCD) as follows: 53.12 g/L maltose and 4.21 g/L polypeptone in distilled water. Furthermore, four groups of EPSs (designated as Fr-I, Fr-II, Fr-III and Fr-IV) were obtained from the culture filtrates by size exclusion chromatography (SEC), and their molecular characteristics were examined by a multiangle laser-light scattering (MALLS) and refractive index (RI) detector system. The weight-average molar mass of Fr-I was determined to be 3.872×10^5 g/mol and its molecular shape was revealed to be a rigid rod in an aqueous solution. Finally, the hypoglycemic effect of the EPS, investigated in streptozotocin induced diabetic mice, decreased plasma glucose, total cholesterol and triacylglycerol concentrations by 17.4%, 14.0% and 12.6%, respectively. The results indicate the potential of this EPS to prevent hyperglycemia in diabetic patients.

1. Introduction

The polysaccharides were a group of high value biopolymers with wide various applications in food, pharmaceutical industries (Poli et al., 2010; Zhong & Tang, 2004). Much interest has been focused on extracellular polysaccharide (EPS) produced by medical fungi due to their biological and pharmacological activities including anti-tumor, antioxidant, hypoglycemic activities, etc. Compared with EPS extraction from cultivating fruiting body of fungus, submerged culture of fungi obviously gave rise to potential advantages of effective EPS production in a compact space and shorter time (Wasser, 2002). Meanwhile, EPS obtained from submerged culture always exhibited the similar biological activity as that of the EPS extraction from fruit body (Kim et al., 2001).

Trametes gibbosa, commonly known as the 'lumpy bracket', is a polypore mushroom that causes a white rot. It grows on beech stumps and the dead wood of other hardwood species. *T. gibbosa* has been reported to possess antitumor activity as a traditional Chinese medicine (Huang, 1998). Ga and Kaviyarasana (2011) investigated the methanolic and water extract of *T. gibbosa* fruit body has antimicrobial and antioxidant activities. Czarnecki and Grzybek (1995) isolated two polysaccharide fractions (registered as TG-1 and TG-2 from the fruit bodies and their antiinflammatory and vasoprotective activities was confirmed. However, to the best of our knowledge, the EPS production by submerged culture of *T. gibbosa*, its molecular characteristics and bioactivity had not been demonstrated.

Though much information on bioactivities of EPS was available, the molecular weight, structure and chain conformation had not been fully investigated due to the small amount of yield and the difficultly in fractionating. Zhang, Zhang, Cheung, and Ooi (2004) reported that the molecular weight was very important to the bioactivity of *Pleurotus* polysaccharide. Hence, data of detailed molecular characterization were critical to elucidate the relationship between physiochemical properties and physiological functions.

Response surface methodology (RSM) was a mathematical/statistical based technique which was useful for analyzing the effects of several independent variables on the response. RSM had been successfully used to produce enzymes, biomass, and metabolites (Kamimura, Medieta, Rodrigues, & Maugeri, 2001; Malinowska, Krzyczkowski, Lapienis, & Herold, 2009). Usually, it applies an experimental design such as central composite design (CCD) to fit a second-order polynomial equation by regression analysis of the experimental data. The equation was used to describe how the test variables affect the response and determine the interrelationship among the variables.

In the present study, a novel *T. gibbosa* was isolated and identified, and submerged culture conditions of *T. gibbosa* were optimized for the production of EPS by RSM. Furthermore, the EPSs fractions

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http://dx.doi.org/10.1016/j.carbpol.2013.04.019

were isolated by gel filtration chromatography and the molecular features were investigated by a SEC/MALLS system. Finally, hypoglycemic effect of *T. gibbosa* EPS was studied in male Kunming mice.

2. Materials and methods

2.1. Isolation and identification of fungus

While searching fungus-pathogenic organisms, a white-rot fungal fruit body was isolated from stumps of beech in our campus. The isolated strain was phylogenetically identified by ITS-5.8S rDNA sequencing analysis. The chromosomal DNA of the strain was isolated from the fresh mycelium using a CTAB method (Reski, Wehe, Hadeler, Marienfeld, & Abel, 1991). The resulting genomic DNA was amplified using 2× Taq MasterMix (Beijing CoWin Biotech Co., Ltd., Beijing City, China) and primers ITS1(5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) on an Applied Biosystems Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA) under the following conditions: 94 °C 5 min (1 cycle); 94 °C 45 s, 55 °C 50 s, 72 °C 60 s (33cycles); 72 °C 5 min (1 cycle). The PCR products were sequenced in both directions by Sangon Biotech Co., Ltd. (Shanghai, China). The obtained nucleotide sequence of the ribosomal sequence was compared with those of GeneBank using the NCBI Blast program, and sequence homology was comparatively analyzed using the Clustal X program (Glass & Donaldson, 1995).

2.2. Microorganism and growth conditions

T. gibbosa was collected in our laboratory and was used throughout this study. Stock cultures were maintained on potato dextrose agar (PDA) slant. Slants were incubated at 26 °C for 8 d and stored at 4 °C, and sub-cultured every one month. The seed culture was grown in a 250 mL flask containing 50 mL of GP medium (0.3% peptone, 3% glucose) at 26 °C on a rotary shaker incubator (150 rpm) for 4 d. Flask culture experiments were performed in 250 mL flasks containing 50 mL media for 10 days after inoculating with 4% (v/v) of the seed culture. *T. gibbosa* was initially grown on PDA medium in a Petri dish, and transferred into the seed medium by punching out 5 mm of the agar plate culture with a self-designed cutter (Park, Kim, Hwang, & Yun, 2001).

2.3. Analytical methods

Samples collected from shake flasks were centrifuged at 9000 × *g* for 15 min, and the resulting supernatant was filtered through a membrane filter (0.45 μm, Millipore). The dry weight of mycelium was measured after repeated washing of the mycelial pellet with distilled water and drying at 70 °C for overnight to a constant weight. The resulting culture filtrate was mixed with four times volume of absolute ethanol, stirred vigorously and kept overnight at 4 °C, thereafter, centrifuged at 10,000 rpm for 15 min to obtain EPS after discarding the supernatant. The precipitate of EPS was lyophilized for further bioactivity analysis, or the precipitate of EPS was redissolved in distilled water and the concentration of the EPS was determined by phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). And each experiment was performed tripartite.

2.4. Purification of EPS

The ethanol precipitates of the crude EPS treated with Sevag reagent (1:4 n-butanol/chloroform, v/v) to remove most of proteins. After removing the proteins and Sevag reagent by centrifugation, the water phase was dialysed against distilled water and lyophilized to yield polysaccharide. The EPS were dissolved in 0.2 M NaCl buffer, and loaded onto a Sepharose CL-6B column (2.4 cm × 100 cm, Sigma Chemical Co., St Louis, MO). The column was eluted with the same buffer at a flow rate of 0.6 mL/min. The eluent was collected by a fraction collector (5 mL eluent/tube). The protein and sugar concentrations of each collector tube were measured. Protein concentration was determined according to Bradford method (Bradford, 1976). The total sugar content in the EPS was determined by phenol-sulfuric acid method using glucose as the standard. The protein moiety in the EPS was monitored by measuring the absorbance at 280 nm, whilst the carbohydrate moiety was monitored at 490 nm (Dubois et al., 1956).

2.5. SEC/MALLS analysis

The molecular weights of the EPS were estimated by SEC coupled with MALLS Dawn DSP detector (Wyatt Technology, Santa Babara, CA) and a refractive index (RI) detector (Optilab rEX, Wyatt Technology, Santa Barbara, CA). The EPS samples were dissolved in a 0.1 M PBS buffer (pH 6.8) containing 0.04% diaminotetraacetic acid–disodium salt (Na_2EDTA) and 0.01% sodium azide and filtered through 0.25 μm filter membranes (Millex HV type, Millipore Co., Bedford, MA) prior to injection into the SEC/MALLS system. The chromatographic system consisted of a degasser (Degasys, DG-1200, uniflow, HPLC Technology, Macclesfield, UK), a SSI 222D pump (Scientific Systems, State College, PA, USA) single-piston isocratic, pulse-dampened HPLC pump (Model 590 Programmable Solvent Delivery Module, Waters Co., Milford, MA), an injection valve (Rheodyne, Inc., Cotati, CA) fitted with a 500 μL loop, and two SEC columns (Shodex OH Pack SB-803 and 805 HQ, JM Science Inc., Buffalo, NY) connected in series. The flow rate was 0.75 mL/min and the injection volume and concentration was 100 μL and 2 mg/mL, respectively. During the calculation of molecular weights of each EPS, the value of d*n*/d*c* (specific refractive index increment) was used from the data in literature (Jumel, Fiebrig, & Stephen, 1996), in which the estimated d*n*/d*c* was 0.14 mL/g. Calculations of molecular weight and root mean square (RMS) radius of gyration for each EPS were performed by the Astra 4.72 software (Wyatt Technology). The RMS radii of each polysaccharide were determined from the slope by extrapolation of the first-order Debye plot (Wyatt, 1993).

2.6. Animal experiments and induction of diabetes

A total 24 5-week-old male Kunming mice was obtained from Henan Experimental Animal Center (Zhengzhou, China) were housed in individual stainless steel cages and acclimatized with free access to food and water for at least 1 week in an air conditioned room (23 ± 2 °C with 55 ± 5% humidity) under a 12:12-h light–dark cycle. The mice were fed with a commercial pellet diet (Henan Experimental Animal Center) throughout the experimental period.

Mouse were adapted for 7 days in the growth room and fasted for 12 h. Diabetes was induced by intramuscular injection of streptozotocin (Sigma Chemical Co., St. Louis, MO) dissolved in 0.1 M sodium citrate buffer (pH 4.5) at a dose of 50 mg/kg body weight (Bolkenta, Yanardağb, Tabakoğlu-Oğuza, & Özsoy-Saçan, 2000; Hwang & Yun, 2010). Two days after injection of diabetogenic agent (streptozotocin), fasting blood glucose was determined before administration of the EPS and the mice with more than blood glucose >300 mg/dL were included in the group of diabetics. All procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" approved by Zhengzhou University of Light Industry.

Table 1
Experimental groups for dose-dependent hypoglycemic activity of *T. gibbosa* EPS.

Group	Oral administration
Normal[a]	None
Control[b]	0.9% NaCl
EPS[b]	200 mg/kg d *T. trogii* EPS

[a] Group of 8 normal mice.
[b] Diabetic mice induced by streptozotocin (50 mg/kg body weight) for 8 mice. The mice were administrated orally with either saline (control) or EPS produced from the submerged mycelial culture of *T. trogii* at 200 mg/kg body weight daily for 3 weeks.

2.7. Animal experimental design and analytical measurements

All the animals were randomly divided into three groups with eight animals in each group (Table 1): normal group, normal mice received 0.9% NaCl solution; control group, STZ-induced diabetic mice treated with 0.9% NaCl solution; diabetic EPS-treated group, diabetic mice high-treated with *T. gibbosa* EPS at the level of 200 mg/kg body weight using an oral zonde daily for three weeks. On the basis of our preliminary test result (doses of 100 and 200 mg/kg) and other experimental designs by several investigators who used natural sources for diabetic therapy (normal dose ranges: 100–300 mg/kg), 200 mg/kg was chosen as a suitable dose for this experiment (Benwahhoud, Jouad, Eddouks, & Lyoussi, 2001; Hwang & Yun, 2010).

The body weight gain and food intake were periodically measured. Blood samples of the experimental animals were collected in heparinized tubes and plasma was separated by centrifugation at 1100 × *g* for 10 min. Each organ was isolated and the weights were measured after washing with 0.9% NaCl. The plasma glucose level was measured using glucose oxidase kit (Biosino Bio-technology and Science Inc., Beijing, China). Total cholesterol, triglyceride levels were measured using a CHOD-PAP method (Biosino Bio-technology and Science Inc., Beijing, China) (Henry, 1974). HDL cholesterol was evaluated by enzymatic test kits (Biosino Bio-technology and Science Inc., Beijing, China) using PEG-modified enzymes (Okada, Matsui, Ito, & Fujiwara, 2001). LDL cholesterol and atherogenic index (AI) were calculated by the following equations:

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \left(\frac{\text{triglyceride}}{5}\right)$$

$$\text{AI} = \frac{\text{total cholesterol} - \text{HDL cholesterol}}{\text{HDL cholesterol}}$$

$$\text{HTR} = \frac{\text{HDL cholesterol}}{\text{total cholesterol}}$$

2.8. Statistical analysis

Data were expressed as mean ± S.D. ($n = 3$). The statistical significance was determined by Student's *t*-test. Experimental results from the experimental design were statistically subjected to analysis of variance (ANOVA) using SPSS (version 11.0, Chicago, IL). Probability values <0.05 and <0.01 were regarded as statistically significant and highly significant, respectively.

3. Results and discussion

3.1. Isolation and identification of the fungi

Based on combined analysis of the fungal morphological characters, the isolated strain was identified as *T. gibbosa* (GeneBank data homology search result >98%). This strain has preserved at the Henan Province Microbiological Culture Collection Center (HPMCC no. 19698).

Table 2
Experimental range and levels of the independent process variables according the 2^2 full factorial central composite design and the responses of the dependent variables on EPS production by *T. gibbosa*.

Run	Maltose (g/L)	Polypeptone (g/L)	EPS (g/L)
1	50(0)	4.59(−1.414)	8.42 ± 0.78
2	40(−1)	7(1)	2.73 ± 0.31
3	50(0)	6(0)	9.57 ± 1.59
4	60(1)	5(−1)	7.07 ± 1.85
5	64.14(1.414)	6(0)	7.93 ± 0.02
6	50(0)	6(0)	10.64 ± 1.45
7	40(−1)	5(−1)	6.99 ± 0.55
8	60(1)	7(1)	9.07 ± 0.10
9	50(0)	6(0)	9.40 ± 0.89
10	50(0)	7.41(1.414)	4.77 ± 0.08
11	−1.414(35.86)	6(0)	5.82 ± 0.70

The arrangements of column x_1 and x_2 were decided by Box–Behnken design for 2 (factor) × 11 (run number); every row of run number represents one experimental replicate, every run was replicated tripartite. The numbers bracketed were the level of variables.

3.2. Optimization of screened medium constituents by response surface methodology

Based on the preliminary results, where the variables (maltose and polypeptone) determined as the optimal carbon and nitrogen sources for EPS production, the central composite design was employed to optimize their individual concentration. For the two factors, this design was up of a full 2^2 factorial design. Table 2 summarizes the central composite experimental plan along with the experimental and the responses for each individual experiment. Regression analysis on the experimental data, the following second order polynomial equation was shown to explain the EPS production:

$$y = 9.87 + 1.18x_1 - 0.93x_2 + 1.57x_1x_2 - 1.56x_1^2 - 1.70x_2^2 \quad (1)$$

where Y represents the response variable. x_1 and x_2 represent the coded values of maltose and polypeptone, respectively. The regression equation was optimized by the DESIGN EXPERT to get the optimum values. The optimal values of the test variables were as follows: maltose = 53.12 g/L and polypeptone = 5.87 g/L. Three dimensional response surface plots (Fig. 1A) and 2D contour plots (Fig. 1B) graphically represent regression equations were generally used to demonstrate the relative effect of maltose and polypeptone. The optimal values obtained from the contour plot were almost equal to the obtained results by optimizing regression equation (Eq. (1)).

For testing the goodness of fit of the regression equation, the coefficient of determination ($R^2 = 93.48\%$), the adjusted coefficient of determination ($R^2_{\text{Adj}} = 0.8696$) and the coefficient of variation (*C.V.* = 11.27%) were shown in Table 3. These values indicated that the accuracy and the general availability of the polynomial model were adequate. The significance of each coefficient was checked using the *F*-test and the *p* value. The *p* value was used as a tool to check the significance of each coefficient, and also indicated the interaction strength between each independent variable (Lim et al., 2005). The ANOVA of the quadratic regression model demonstrated that the model was highly significant, as was evident from the *F*-test with a very low probability value ($p < 0.005$). The Model *F*-value of 14.33 implied that the model was significant, and there was only a less 0.5% chance that a "Model *F*-Value" that was this large occurred because of noise. The "Lack of Fit *F*-value" of 1.95 implied the Lack of Fit was not significant relative to the pure error, and there was a

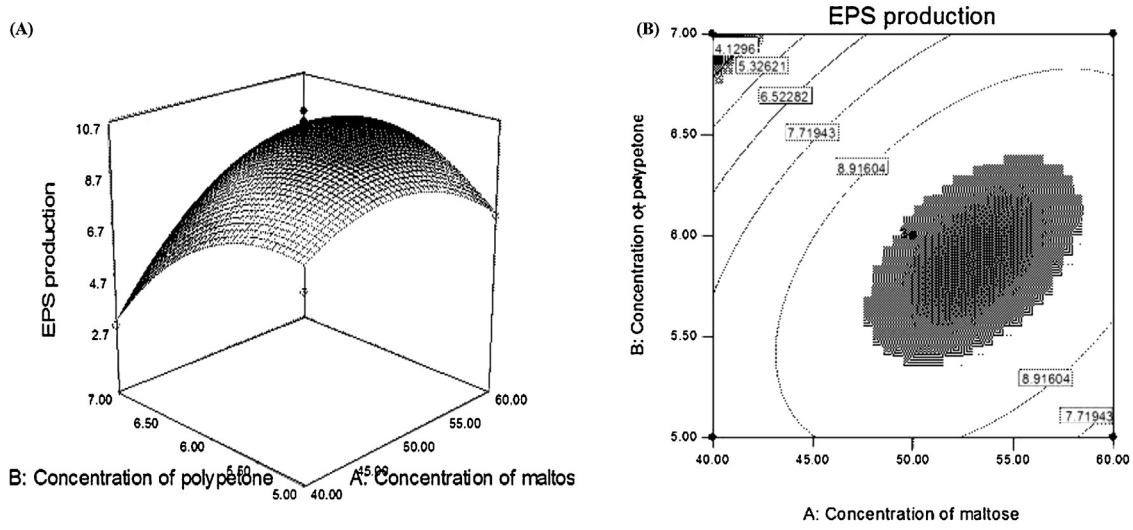

Fig. 1. Response surface plots (A) and 2D contour plots (B) of the model equation fitted to the data of the response surface methodology experiments.

35.66% chance that a "Lack of Fit *F*-value" that was this large could had occurred because of noise. The regression coefficients and the corresponding *p* values were also shown in Table 3. The *p* values of each model, confirmed that the six coefficients ($x_1, x_2, x_1 \times x_2, x_1^2, x_2^2$) were all significant. Therefore, both maltose, x_1 and polypeptone, x_2 were important factors in EPS yield process.

In order to confirm the optimization results, the suggested medium components were confirmed in triplicate. The maximum predicted value of EPS yield obtained was 10.113 g/L where corresponding experimental response was 9.967 g/L. The experimental and predicted values of EPS yields show good agreement with one another.

3.3. Purification and characterization of EPS with SEC/MALLS system

In gel filtration chromatography of the culture filtrate on Sepharose CL-6B, four fractions of EPSs (Fr-I, Fr-II, Fr-III and Fr-IV), which consisted of polysaccharides and proteins, were coeluted as shown in Fig. 2A. It was revealed that Fr-I and Fr-II were glycoproteins. The SEC/MALLS approach could be useful in providing great insight into the characterization of biopolymers without carrying out elaborate fraction procedures prior to analysis. In recent years, SEC instrumentation equipped with both MALLS instrumentation and RI has been used routinely to determine the weight average molecular weight (M_w) of polymers without the use of standards (Cui, Goh, Archer, & Singh, 2007; Li, Weiss, & Roberts, 2009). In this study, the number average molecular weight (M_n), M_w, *z*-average molecular weights (M_z), polydispersity (M_w/M_n), RMS radius, and conformation on polymer samples could be provided by SEC-MALLS-RI system. A typical chromatogram with MALLS and RI detection was shown in Fig. 2B, which was in good agreement with that of gel filtration chromatography on Sepharose CL-6B. Fr-I, Fr-II, Fr-III and Fr-IV peaks appeared between the elution volume of 8.0–11.5, 13.5–14.5, 14.5–15.5 and 15.5–18.0 mL,

Table 3
Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of EPS production by *T. gibbosa*.[a]

Source	Sum of squares	*df*	Mean squares	*F*-value	Probability > *F*
Model	51.13	5	10.23	14.33	0.0055
x_1	11.05	1	11.05	15.49	0.0110
x_2	6.86	1	6.86	9.62	0.0268
$x_1 \times x_2$	9.83	1	9.83	13.77	0.0138
x_1^2	13.84	1	13.83	19.38	0.0070
x_2^2	16.40	1	16.40	22.98	0.0049
Residual	3.57	5	0.71		
Lack of Fit	2.66	3	0.89	1.95	0.3566
Pure error	0.91	2	0.45		
Core total	54.70	10			

[a] Coefficient of determination $R^2 = 0.9348$; adjusted coefficient of determination $R^2_{Adj} = 0.8696$; coefficient of variation, *C.V.* = 0.1127.

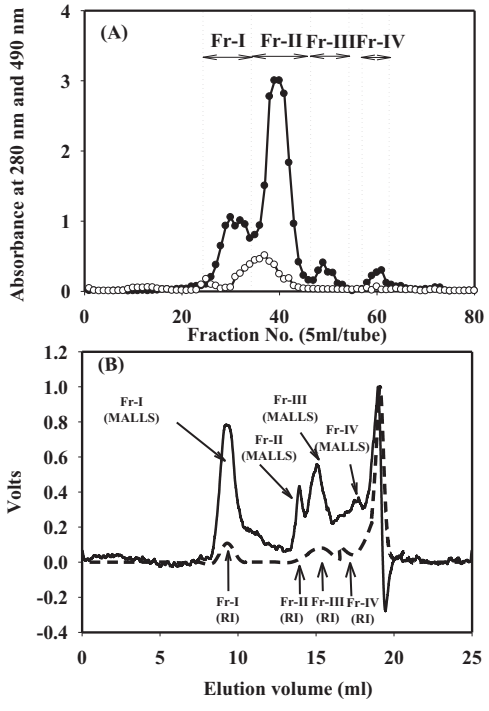

Fig. 2. Elution profiles of the polysaccharides (Fr-I, Fr-II, Fr-III and Fr-IV) in Sepharose CL-6B chromatography (A). Elutes were analyzed by measuring the absorbance at 490 nm for carbohydrate (●) and the absorbance at 280 nm for protein (○). Elution profiles of the polysaccharides (Fr-I, Fr-II, Fr-III and Fr-IV) for the determination of molecular mass in SEC/MALLS system (B). For detailed analysis conditions, see Section 2. (- - -) MALLS detector and (—) refractive index detector. The peaks appearing at elution volume of around 19–22 mL is the baseline noise from the buffer solution.

Table 4
Relevant molecular parameters of EPS (Fr-I, Fr-II, Fr-III and Fr-IV) and the double logarithmic plots of root mean square radius vs. molecular mass for four fractions of EPS produced by submerged mycelial culture of *T. gibbosa* in MALLS analysis.

Parameters[a]	Fr-I (error %)	Fr-II (error %)	Fr-III (error %)	Fr-IV (error %)
M_n (g/mol)	3.531×10^5 (0.3)	2.640×10^4 (0.5)	7.011×10^3 (0.6)	3.671×10^3 (2.3)
M_w (g/mol)	3.872×10^5 (0.3)	2.761×10^4 (0.5)	8.526×10^3 (0.6)	5.659×10^3 (2.7)
M_z (g/mol)	4.304×10^5 (0.6)	2.892×10^4 (1.1)	1.066×10^4 (1.4)	9.102×10^3 (6.3)
M_w/M_n	1.097 (0.4)	1.046 (0.7)	1.216 (0.9)	1.541(3.6)
R_n (nm)	31.4 (1.1)	n/a	n/a	79.5(1.6)
R_w (nm)	33.6 (0.9)	n/a	n/a	103.0(1.1)
R_z (nm)	61.1 (0.8)	n/a	n/a	133.0(0.9)
Double logarithmic plots of root mean square radius vs. molecular mass	0.94	n/a	n/a	n/a

[a] M_n, M_w, and M_z refer number-, weight-, and *z*-average molecular weight, respectively. M_w/M_n means polydispersity ratio. R_n, R_w, and R_z refer number-, weight-, and *z*-average square mean radius of gyration, respectively.

respectively. The molecular mass values for four eluted fractions were calculated for the portions of peaks, which lie within the peak ranges. The relevant molecular parameters of each EPS were summarized in Table 4. The M_w of Fr-I, Fr-II, Fr-III and Fr-IV were determined to be 3.872×10^5, 2.761×10^4, 8.526×10^3 and 5.659×10^3 g/mol, respectively. M_w/M_n was a measure of the width of molecular mass distribution. These can be related to the standard deviation of the distribution of molecular weights in a preparation, whatever form this distribution takes (lognormal, Gaussian, etc.). This information is essential because the functional properties of polysaccharides can be greatly influenced by the molecular weight distribution. The low values of polydispersity ratio for all EPSs mean that these EPS molecules exist much less dispersed in aqueous solution without forming large aggregates (Hwang, Kim, Xu, Choi, & Yun, 2003). The RMS radii for both peaks ranged from 31.4 to 133.0 nm with no clear trends. The slope for Fr-I in the double logarithmic plots of RMS radius vs. molecular mass was shown in Table 4. The study of the dependence of RMS radius of gyration on molecular weight can give the information on the polysaccharide structure. This is, the gross molecular conformation of the EPS can be elucidated from the double logarithmic plot of the RMS radium of gyration vs. the molecular mass according to the following equation:

$$\log\ r_i = k + a \log M_i \quad (2)$$

where r_i is the RMS radius of an EPS molecule, M_i is the molar mass of EPS, k is the intercept at the *Y*-axis (RMS radius of gyration), and a is the critical slope values providing a hint about the conditions of the polymeric chain in aqueous solution for determining the molecular conformation of each EPS (Lim et al., 2005). Slope value of 1/3 would indicate compact globular structure, and 1/2 indicates a flexible random coil polymer. For rigid rods, their corresponding value of slope is unity (Wyatt, 1993). The values of slope of Fr-I indicated 0.94, which implies that the Fr-I molecule exists as a rigid rod form in aqueous solution. It should be mentioned here that the slopes for Fr-II, Fr-III and Fr-IV in the double logarithmic plots of RMS radius vs. molecular mass were not detected because the molecular weights of them were smaller than 5×10^4 g/mol in the aqueous system.

3.4. Hypolipidemic effect of EPS

In this study, the hypoglycemic effects of EPS from *T. gibbosa* in streptozotocin-induced diabetic mice were evaluated with respect to that of the saline administered control group. The organ weights of the experimental animals are showed in Table 5. By the end of the experiment in week 3, the weight of liver decreased significantly by administration of EPS. However, the EPS treatment had no influence on spleen and pancreas weight, compared to the normal and control groups. The effect of EPS on the blood glucose level in streptozotocin-induced diabetic mice over 3-week period is depicted in Table 5. The reduction in blood glucose level was achieved to 17.4% when the EPS was administered. One intracellular mechanism by which the streptozotocin treatment caused degranulation or reduction of insulin secretion by the pancreas is through selective destruction of β-cells in the pancreatic islets (Benwahhoud et al., 2001). Therefore, the blood glucose lowering effect of EPS might be explained by the fact that EPS may act by preservation of a functional portion of pancreatic β-cells that initially survived streptozotocin toxicity or behaving as an insulin-like factor (Hwang et al., 2008).

A maximum reduction of total cholesterol and triacylglycerol of 14.0% and 12.6%, respectively, was obtained at a dose of 200 mg/kg body weight (Table 6). The decrease in total cholesterol and triacylglycerol level in the diabetic mice by EPS reinforces its hypoglycemic potential. In general, the high plasma level of triacylglycerol and total cholesterol observed in diabetic animals may be due to impaired liver function caused by the damage done by streptozotocin (as shown the increased weight of the liver after mice were induced by streptozotocin in Table 6), which acts either directly or indirectly by enhancing the plasma glucose level (Van Horn, 1996).

Table 6 also shows the effect of EPS on the LDL cholesterol, HDL cholesterol and atherogenic index in streptozotocin-induced diabetic mice. At the rate of 200 mg/kg body weight, EPS substantially increased the HDL cholesterol by 36.4% and lowered the LDL cholesterol and atherogenic index by 25.1% and 44.7% as compared to the control group, respectively. This signifies the fact that *T. gibbosa* EPS might behavior as a modulator to correct the liver function by promoting insulin synthesis, thereby reducing the blood glucose level.

Table 5
Effect of *T. gibbosa* EPS on the various organs in streptozotocin-induced diabetic mice for 3 weeks.

Group[a]	Liver (g/100 g BW)	Kidney (g/100 g BW)	Spleen (g/100 g BW)	Pancreas (g/100 g BW)
Normal	4.16 ± 0.05a	1.31 ± 0.15a	0.30 ± 0.08NS	0.35 ± 0.07NS
Control	5.34 ± 0.08c	1.75 ± 0.11b	0.29 ± 0.04	0.31 ± 0.09
EPS	4.73 ± 0.15b	1.50 ± 0.19ab	0.28 ± 0.07	0.40 ± 0.04

Each value is the mean ± SE ($n = 8$). Values with different letters in the same column significantly different among the groups at $p < 0.05$.
[a] See Table 1.

Table 6
Effect of *T. gibbosa* EPS on plasma glucose, total cholesterol, triglyceride levels, plasma HDL cholesterol, LDL cholesterol, and atherogenic index in streptozotocin-induced diabetic mice for 3 weeks.

Group[a]	Normal	Control	EPS
Plasma glucose, total cholesterol, and triglyceride levels			
Glucose (mmol/L)	5.97 ± 1.17a	36.90 ± 0.99c	30.47 ± 2.68b
Total cholesterol (mmol/L)	2.82 ± 0.26a	4.64 ± 0.13c	3.99 ± 0.41b
Triglyceride (mmol/L)	1.23 ± 0.10a	1.67 ± 0.06c	1.46 ± 0.07b
Plasma HDL cholesterol, LDL cholesterol, and atherogenic index			
HDL cholesterol (mmol/L)	1.36 ± 0.06c	0.77 ± 0.08a	1.05 ± 0.10b
LDL cholesterol[b] (mmol/L)	1.39 ± 0.07a	3.54 ± 0.04c	2.65 ± 0.30b
Atherogetic index[c]	1.20 ± 0.10a	5.06 ± 0.50c	2.80 ± 0.03b

Each value is mean ± SE for 8 mice. Values with different letters in the same column significantly different among the groups at $p < 0.05$.
[a] See Table 1.
[b] Total cholesterol − HDL cholesterol − (triglyceride/5).
[c] (Total cholesterol − HDL cholesterol)/HDL cholesterol.

In turn, this reduced the level of triacylglycerol and total cholesterol in blood plasma of diabetic animals.

4. Conclusions

The optimization of submerged culture medium for EPS production was addressed with statistical methodology. Furthermore, four groups of EPS were obtained by gel filtration chromatography and characterized by SEC/MALLS analysis. The SEC/MALLS approach was expected to provide greater insight into the conformation and component of biopolymers with high polydispersity without further isolation and purification. Furthermore, the *T. gibbosa* EPS was proved to have hypoglycemic capacity. Although the exact mechanism of *T. gibbosa* EPS is not fully understood, the above result suggests that a combination of mechanisms might be involved in exhibiting hypoglycemic effect.

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