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Potential antidiabetic activity of extracellular polysaccharides in submerged fermentation culture of *Coriolus versicolor* LH1

John Powen Yang[a,*], Taihao Hsu[b,c], Fangyi Lin[b], Wenkuang Hsu[a], Yucheng Chen[a]

[a] *Natural Products Chemistry Research Laboratory, Department of Bioindustry Technology, Dayeh University, Changhua 51591, Taiwan*
[b] *Microbiology Laboratory, Department of Bioindustry Technology, Dayeh University, Changhua 51591, Taiwan*
[c] *Microbiology Laboratory, Department of Medicinal Botanicals and Healthcare, Da-Yeh University, Changhua 51591, Taiwan*

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ABSTRACT

The separation and purification of extracellular polysaccharides from *Coriolus versicolor* LH1 were investigated along with their α-glucosidase inhibition properties. Three polysaccharide fractions (ePS-F2-1, ePS-F3-1, and ePS-F4-1) were separated from the culture medium of LH1 using a DEAE anion-exchange column and a Sephadex™ G-50 gel filtration column. Their chemical compositions was determined. On the basis of an α-glucosidase inhibition assay, the enzyme inhibition activities of ePS-F2-1, ePS-F3-1, and ePS-F4-1 were investigated. Among these, ePS-F4-1 had the highest enzyme inhibition effects on α-glucosidase. According to the results of the chemical component analysis, ePS-F3-1 and ePS-F4-1 are the polysaccharides which are combined with triterpenoides, and ePS-F2-1 is complexed with proteins and triterpenoides.

1. Introduction

Diabetes mellitus is a common metabolic disease in which the concentration of glucose in the blood is above the standard level. This is due to insulin deficiency or functional disturbance of the receptors, which causes blood glucose to rise and induce disorders in the metabolization of fat and proteins. The number of patients with diabetes mellitus has grown each year. According to the vital statistics from International Diabetes Federation (IDF), 6.0% of the world population had diabetes in 2007. Because the number of patients is increasing every year, the percentage of diabetes prevalence is expected to reach 7.3% by the end of 2025 (Mbanya, Gan, & Allgot, 2006). For this reason, there is an urgent need to create new diabetes medicines.

There are five types of oral antidiabetes medication at present: insulin secretagogues, insulin sensitizers, biguanide, α-glucosidase inhibitors, and dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors). Of these, α-glucosidase inhibitors have been used to reduce blood glucose after digestion. Some enzyme-inhibitory medicines have been used in clinical practice; for example, Acarbose, Voglibose, and Miglitol have been effective and have been used to treat type 2 diabetes mellitus (non-insulin dependent) and accompany insulin treatment for type 1 diabetes mellitus (insulin-dependent).

There are three major ways to acquire α-glucosidase inhibitors: extraction from natural animals, plants and microorganisms, microorganisms' metabolism and synthesis products (Wang & Chang, 2009). Of these, some edible mushrooms can produce α-glucosidase inhibitors by extraction or fermentation. According to a number of studies, various edible mushrooms extracts can help develop immunity from disease, inhibit tumors, provide anti-oxidants, stimulate free-radical scavenging activity, fight viruses, inhibit diabetes, and lower blood lipid levels. These extracts are being used in health foods as well as in the development of natural medicines (Lu, Li, Suo, & Li, 2010). Polysaccharides are present in many edible mushrooms and have the effect of lowering blood sugar. Polysaccharides can be present in fruiting bodies, mycelia, and culture media, and are obtained in different forms, such as polysaccharides, polysaccharopeptides (PSP), and polysaccharide–proteins, through extraction, separation, and purification (Wu, Xiao, Yang, & Zhang, 2009).

Coriolus versicolor (syn. *Trametes versicolor*), is a member of the *Basidiomycota* phyla, *Polyporales* order, and *Polyporaceae* family by taxonomy (Emberger, 2008). It can be used in medicines and health foods to treat disease and to protect body functions. The polysaccharides of *C. versicolor* have notable anti-oxidation effects, can lower blood sugar, and have been used in the treatment and prevention of hepatitis B, tumor diseases such as cancer of the liver, breast, and stomach, and certain immune-deficiency diseases (Li & Xu, 1987; Qian, 1997; Ren, Dai, & Li, 1993).

Recently, a new strain of *C. versicolor* named LH1 was identified. It has been reported that the exopolysaccharopeptides (ePSP)

* Corresponding author. Tel.: +886 4 8511888x2296.
E-mail address: johnyangpw@yahoo.com.tw (J.P. Yang).

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from LH1 cultured with *Lycium barbarum* extract exhibits significant immunomodulatory activity (Lin et al., 2008). However, other bioactivity of extracellular polysaccharides (ePS) from LH1, such as antidiabetic activity, was unknown. Therefore, the objectives of this study were to further probe into the bioactivity of LH1 ePS separated from fermentation cultures and purified. To study new chemical components in the culture medium without damage, use of physical processing was the basis of experiment design. Hence, we conducted our experiments without the Sevag method to denature and remove free proteins in the processing of crude ePS. Saponin is a kind of glucoside in which the aglycon is combined with triterpenoides or spirostanol. Such chemical components are seldom reported in research of the fermentation culture medium of mushrooms (Lu et al., 2008). Therefore, our study investigated the culture medium to determine the activity of α-glucosidase inhibition on polysaccharides that were bound with proteins or triterpenoides. Also, the molecular weights (MW), chemical compositions, FTIR spectra, and enzyme inhibitory activities of purified ePS were evaluated.

2. Materials and methods

2.1. Chemicals

The extracellular polysaccharides (ePS), which were produced from the culture medium of *C. versicolor* LH1, were provided by Dr. Hsu Tai-Hao, Da-Yeh University, Changhua County, Taiwan. Sevag reagent was a mixture of chloroform and n-butyl alcohol in 5:1 (v/v). ρ-Nitrophenyl-α-D-glucopyranoside (ρNPG), α-glucosidase (EC 3.2.1.20, from baker's yeast), 1-phenyl-3-methyl-5-pyrazolone (PMP), α-glucosidase, phosphate buffer, bovine serum albumin, glucose, rhamnose, arabinose, xylose, mannose, and galactose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acarbose was obtained from Bayer Healthcare Company Ltd. (Germany). Krestin (PSK) was obtained from Kureha Co. Ltd. (Japan). DEAE-Sepharose™ CL-6B and Sephadex™ G-50 medium gel were purchased from GE Healthcare (Sweden). All other chemicals were analytical-grade commercial preparations.

The analysis instrument was a spectrophotometer (Spectrophotometer U-3310, Hitachi, Japan). The separation and purification installations were a high pressure pump (L-7100, Hitachi, Japan) and two empty chromatography columns (FG-38-40 and FG-19-100, RAY-E Creative Co., Ltd.).

2.2. Cultivation of C. versicolor

The culture was maintained on potato dextrose agar (PDA) plates at 25 °C. For seeding, the cultures were cultured in normal culture medium (4.0% glucose, 0.15% peptone, 0.15% KH_2PO_4, and 0.15% $MgSO_4 \cdot 7H_2O$) in Erlenmeyer flasks at 25 °C on a rotary shaker at 150 rpm; 5-day-old cultures were used. Batch fermentation of *C. versicolor* LH1 was carried out in a 20 L fermenter (Biotop, Taiwan) in normal culture medium. Fermentations were carried out at 25 °C, pH 4.5–5.0, and 100 rpm for 7 days. After fermentation, the cell mass and the medium were separated by centrifugation at 6000 rpm for 30 min. The medium of *C. versicolor* LH1 was lyophilized until used.

2.3. Quantification and precipitation of ePS

2.3.1. Quantification of carbohydrate and polysaccharides

The contents of polysaccharides in the culture medium were determined from the total sugars using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as the standard and the reducing sugars determined using the DNS method (Miller, 1959) and also used glucose as the standard. The amount of polysaccharides was calculated as (total sugars minus reducing sugars) (Lee, Yang, & Wang Jennifer, 2006).

2.3.2. Quantification of proteins

Proteins were quantified using the Biuret method (Tsuyoshi Ohnishi & Barr, 1978). 4.0 mL of Biuret reagent was added to a tube to which 1.0 mL of the sample proteins had already been added. After 30 min of incubation at 37 °C, the absorbance was read at 540 nm against a blank and the sample color. BSA was used as the standard.

2.3.3. Quantification of triterpenoids

Total triterpenoids were quantified by the colorimetric method using vanillin/glacial acetic acid (Fan & He, 2006). The test samples in tubes were evaporated to dryness in an oven. For each tube, 0.3 mL of 5% vanillin/glacial acetic acid (w/v) and 1.0 mL of perchloric acid solution were added successively. For the reaction, we modified the reaction conditions (e.g., the sample solution was incubated for 10 min at 60 °C and then cooled in the icebox for 3 min). The absorbance of the sample was measured at 548 nm after adding 5.0 mL of glacial acetic acid. Ursolic acid was used as the standard.

2.3.4. Precipitation of crude ePS

The precipitated crude ePS from the medium was concentrated by adding 4 vol of 95% ethanol and allowed to precipitate overnight at 4 °C. However, to arrive at the largest recovery amount of crude ePS, concentrating the amount of total ePS before precipitation was important. Therefore, we studied the precipitation of different concentrations of total ePS in the medium. After precipitation, the supernatant and precipitate were separated by centrifugation at 6500 rpm for 20 min. All of the precipitates were dissolved again in hot water. We then analyzed the polysaccharides and found the optimal recovery content of crude ePS. The recovery percentage of crude ePS and the residual percentage of reducing sugars was calculated form the following equations:-

$$\text{Recovery (\%) of crude ePS} = \frac{\text{Crude ePS}}{\text{Total ePS}} \times 100 \quad \text{(A.1)}$$

$$\text{Residual (\%) of reducing sugars} = \frac{RS_1}{RS_0} \times 100 \quad \text{(A.2)}$$

Total ePS was the amount of polysaccharides in the culture medium, crude ePS was the amount in the precipitated, RS_0 was the amount of reducing sugars in the culture medium, and RS_1 was the amount of reducing sugars in the precipitate. All of the methods of analysis were described above.

2.4. Chromatographic fractionations of ePS

Before chromatography, the precipitate (crude polysaccharides) was dialyzed by membrane (MWCO 12–16 kDa) to remove some smaller compounds for a more accurate chromatogram during ion-exchanger fractionations. Then the precipitate inside of the membrane was filtered through a 0.45 μm nylon membrane. The filtrate (1.0 g, pH 3.86) was injected into a column of DEAE-Sepharose™ CL-6B (38 mm × 90 mm) that was eluted with a stepped gradient NaCl aqueous solution (from 0 to 0.6 M) with an increasing 0.1 M concentration for each fraction and 1.0 M NaCl for the final elution. The eluate was collected in 10 mL amounts for each tube by a fraction collector (Advantec SF-2100W, Japan). The absorbance at wavelength 490 nm for polysaccharides after using the phenol–sulfuric acid method, and the absorbance of ultraviolet at wavelength 280 nm was monitored for the aromatic group of proteins and the aromatic group of triterpenoids. The fractions were further tested to determine the effect of enzyme inhibition.

2.5. Analysis of inhibition of α-glucosidase activity

In vitro, the method used to analyze the inhibition of α-glucosidase activity was that of Gaxiola et al. (2005) using ρNPG as a substrate and modifying the dosage. The assay mixture (1.6 mL) contained 0.20 mL of 0.1 M phosphate buffer (pH 7.0), 0.2 mL of substrate solution (2.5 mM ρNPG in 0.1 M phosphate buffer), 0.2 mL of enzyme solution (0.2 U/mL α-glucosidase in 0.01 M phosphate buffer containing 0.2% BSA), and the indicated concentration of each sample (Gaxiola et al., 2005). The reaction mixture was incubated for 15 min at 37 °C and stopped by adding 0.8 mL of 0.2 M Na_2CO_3. The amount of ρNP released was measured on a Spectrophotometer U-3310 (Hitachi, Japan) at 400 nm.

2.6. Purification by gel permeation chromatography (GPC) and Sevag method

The fractions that showed enzyme inhibition during DEAE Sepharose™ CL-6B column elution were desalted using a dialysis membrane (MWCO 12–16 kDa) and freeze-dried. Before purification, the fraction powders were dissolved in distilled water (the concentration was 1.0 mg/mL), and the samples were filtrated through a 0.45 μm nylon membrane. After that, the filtrate (1.0 mL) was applied to a Sephadex™ G-50 column (19 mm × 700 mm, fractionation range 1.5–30 kDa), which was eluted with a 0.1 M Na_2HPO_4 buffer (pH 9.0) with a 0.4 mL/min flow rate. The eluate was collected in 10 mL amounts for each tube by a fraction collector. The polysaccharides, proteins and triterpenoids were determined described in Section 2.4.

To denature and remove free proteins, 5 vol of Sevag reagent was added to the sample, and the precipitated proteins were separated by centrifugation at 6500 rpm for 10 min. This was repeated four times when Sevag reagent layer was clean. After purification by addition of the Sevag reagent, the samples were dialyzed again by membrane (MWCO 12–16 kDa) to remove smaller compounds, and then the inhibition of α-glucosidase and the chemical composition was determined.

2.7. Determination of molecular weight

The molecular weight of the polysaccharides was determined by high-performance gel-filtration chromatography (HPGFC), which was performed on a Jasco HPLC system with one PolySep-GFC-P 3000 (300 mm × 7.8 mm), a UV detector (Jasco UV-2075 Plus) at 280 nm wavelength, and an RI detector (Jasco RI-2031 Plus). The mobile phase used a 0.1 M Na_2HPO_4 buffer (pH 9.0), and the flow rate was 0.8 mL/min at 30 °C. The samples were dissolved in distilled water. The injection amount of sample (1.0 mg/mL) was 20 μL. The molecular mass was estimated by reference to a calibration curve made from a set of dextran standards of known molecular weight (50, 20, 10, and 5 kDa) and sucrose (0.342 kDa).

2.8. Analysis of monosaccharide composition

The method used to hydrolyze the polysaccharides into monosaccharides was in accordance with the report from Lv et al. (2009); modified doses of 200 mg of polysaccharide samples were dissolved in 20 mL of 3 M trifluoroacetic acid (TFA) in an ampoule (20 mL). The ampoules were kept in an oven at 110 °C for 2 h to hydrolyze the polysaccharides into monosaccharides. After cooling, the hydrolyzed samples of the ePS were carried out with 1-phenyl-3-methyl-5-pyrazolone (PMP) as a derivative. The method of derivation of PMP was also in accordance with the report from Lv et al. (2009). The hydrolyzed samples of the ePS were dried using an evaporator. Then, the samples and the monosaccharide standards were dissolved in 0.3 M NaOH solution (pH 13.3) and prepared at a concentration of 2.4 mg/mL. Then, 10 mL of 0.5 M PMP methanol solution was added to each 15 mL of sample or standard NaOH solution. Each mixture was allowed to react for 30 min at 70 °C, then cooled to room temperature and neutralized (pH 7.0) with 0.3 M HCl. The resulting solutions were dried using an evaporator and then redissolved in 15 mL distilled water. Finally, sample solutions were extracted with 15 mL of chloroform; the process was repeated three times, then the aqueous layer was filtered through a 0.45 μm membrane.

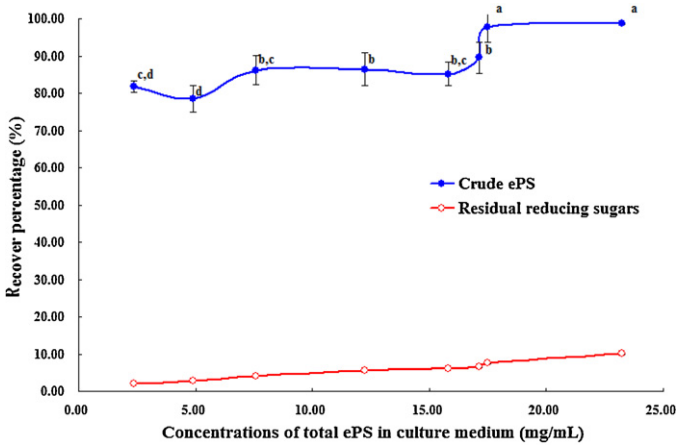

Fig. 1. Recovery percentage at different concentrations of crude ePS, reducing sugar and lost ePS in culture medium. Eq. (A.1): The recovery percentage of crude ePS was calculated as the amount of crude ePS (precipitated) divided by the amount of total ePS (culture medium) multiplied by 100%. Eq. (A.2): The residual percentage of reducing sugars was calculated as the amount of reducing sugars (precipitated) divided by the amount of reducing sugars (culture medium) multiplied by 100%.

The method of analysis used was high performance liquid chromatography (HPLC) with an ODS-C18 column and a UV detector. The following standard sugars were analyzed as references: rhamnose, arabinose, xylose, mannose, glucose and galactose. The HPLC was operated according to Lin, Jia, Huang, and Wang (2006) and performed using the following conditions: mobile phase A:B = 78:22 (A: 0.1 M amino acetic acid buffer, B: acetonitrile); flow rate: 1.0 mL/min; injection amount: 20 μL; UV detector: WL 235 nm; column temperature: 30 °C.

2.9. Infrared spectral analysis

The IR spectrum of the purified fraction was determined using a Fourier transform infrared spectrophotometer (FTIR-8400S, Shimadzu, Japan) equipped with Shimadzu IR solution 1.30 software. The purified fraction was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–400 cm^{-1}.

2.10. Data handling

Results were expressed as means ± standard deviations of four replicated determinations. MINITAB® software (Minitab, Inc., USA) was used for data analysis.

3. Results and discussion

3.1. Influence of precipitate conditions on ePS

Precipitation with 4 vol of 95% ethanol overnight at 4 °C can yield crude ePS, which could be responsible for the higher yields obtained under such conditions. As is shown in Fig. 1, the recovery percentage of crude ePS rose as its concentration in the culture

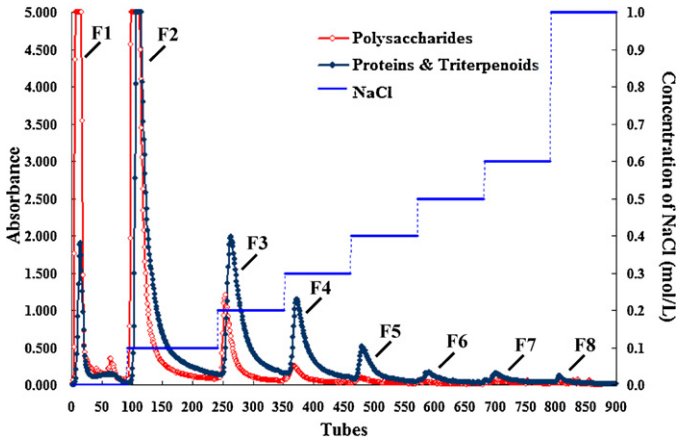

Fig. 2. DEAE-Sepharose™ CL-6B anion-exchange column chromatogram of the crude ePS. The column was eluted with water, NaCl (0.1–0.6 M), and a NaCl (1 M) step gradient at a flow rate of 1 mL/min.

medium increased. For example, if the concentration of total ePS in the culture medium was 23.3 mg/mL, the recovery percentage was 98.7 ± 0.3%. Also, under such conditions the residual ratio of reducing sugars was 10.0 ± 0.1%. The results show that the carbohydrates in the precipitate included polysaccharides and reducing sugars. The residual ratio of reducing sugars increased with the increase of the concentration of the total ePS to be precipitated in ethanol. In this situation it was more difficult to separate out the residual reducing sugars.

3.2. Separation and purification of ePS components

The ePS was fractionated using a DEAE-Sepharose™ CL-6B column Eight fractions ePS-F1, ePS-F2, ePS-F3, ePS-F4, ePS-F5, ePS-F6, ePS-F7, and ePS-F8 were obtained (Fig. 2) (the percentages recovered of each fraction were 26.7, 48.7, 8.9, 9.9, 1.3, 2.2, 1.0, and 1.2, respectively, in relation to the total amount of initial ePS). These were obtained from an aqueous water and NaCl gradient (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 1.0 M) respectively. The results showed that ePS-F8 had the strongest negative charge owing to the elute solvent was the highest concentration of NaCl (1.0 M NaCl), ePS-F2 had the weakest negative charge owing to the elute solvent was the low concentration of NaCl (0.1 M NaCl), and ePS-F1 had a no zero or a positive charge owing to the elute solvent was water. The strongest signals of polysaccharides were ePS-F1 and ePS-F2, the intermediate strength signal was ePS-F3, ePS-F4 and ePS-F5 were weak polysaccharide signals, and ePS-F6, ePS-F7, and ePS-F8 did not have detected polysaccharides signals. ePS-F2, ePS-F3, ePS-F4, and ePS-F5 displayed greater α-glucosidase inhibition than ePS-F1, ePS-F6, ePS-F7, and ePS-F8, as shown in Table 1. These four fractions were then each separately purified using a Sephadex™ G-50 column.

The chromatography results from the Sephadex™ G-50 column are shown in Fig. 3. All of them showed only one symmetrical peak on polysaccharide for numbers ePF-F2-1, ePF-F3-1, ePF-F4-1, and ePF-F5-1. ePS-F2 and ePS-F3 showed two peaks on proteins (or triterpenoids), but ePS-F4 and ePS-F5 had only one signal. As can be seen on the chromatogram in Fig. 3, these four fractions were difficult to purify. Therefore, we used Sevag method to remove the free proteins, which were not bound to polysaccharides. The activity of enzyme inhibition on ePS-F2-1, ePS-F3-1, ePS-F4-1, and ePS-F5-1 is also shown in Table 1.

3.3. Enzyme inhibition activity of different polysaccharide fractions

Some polysaccharides have exhibited strong α-glucosidase inhibition capability. In the investigation, different polysaccharide fractions separated and purified from ePS showed different enzyme inhibition activity (Table 1).

The enzyme inhibition activity of the LH1 culture medium and the crude ePS were lower than that of Krestin (PSK) and Glucobay (Acarbose), but ePS-F5 showed a higher enzyme inhibition than Krestin (PSK) and Glucobay (Acarbose) in this study. Moreover, after removal of proteins with Sevag reagent, two first purified polysaccharides (ePS-F3-1 and ePS-F4-1) had higher enzyme inhibition than Krestin (PSK). However, the presence of some protein (free proteins) without use of Sevag reagent can raise the activity of enzyme inhibition of α-glucosidase. For example, the fraction ePS-F3-1 and ePS-F4-1 showed stronger inhibition after removal of proteins with Sevag reagent, but ePS-F5 had no activity after treatment with Sevag reagent. In other words, the free proteins in ePS-F5 were associated with some low molecular weight materials which raise the inhibition of ePS-F5-1. In addition, due to the free proteins some triterpenoids were also removed by dialysis. This is why the ratios of polysaccharides–triterpenoids were smaller in samples not treated with Sevag reagent. In our research, the ratio of polysaccharides–triterpenoids is related to α-glucosidase inhibition. The smaller ratio of polysaccharides–triterpenoids caused stronger enzyme inhibition. Although the ratios of polysaccharides–triterpenoids were smaller in ePS-F6, ePS-F7, and ePS-F5-1, their amounts were still very small; therefore, the IC_{50} value could not be calculated.

α-Glucosidase, one of the starch hydrolysis enzymes in our intestine, can hydrolyze starch to glucose and then absorb the glucose into intestinal micromodules. In our study, ePS-F3-1 and ePS-F4-1 from the culture medium of *C. versicolor* LH1 showed strong inhibition of α-glucosidase. Therefore, ePS-F3-1 and ePS-F4-1 can potentially provide antidiabetic activity after eating.

3.4. Molecular mass

On the basis of calibration with standard dextrans, the apparent molecular weights of the polysaccharides (ePS-F2-1, ePS-F3-1, and ePS-F4-1) were 1.6 kDa, 3.4 kDa, and 5.0 kDa, respectively. Compared to the polysaccharides PSK-Pro (MW 150 kDa) (Taichi et al., 2011), CVE (the polysaccharide from *C. versicolor* fruiting bodies, MW 500 kDa) (Zhang, Han, & Pan, 2001) and PSP (MW 100 kDa) (Chan & Yeung John, 2006), the enzyme-inhibitory ePS from LH1 had a lower molecular weight. The bioactivity of mushroom polysaccharides can be classified into three groups: (a) antidiabetic activity, when the molecular mass is between 3 and 5 kDa; (b) anti inflammation activity, when the molecular mass is between 10 and 100 kDa; (c) anti-tumor activity, when the molecular mass is over 30 kDa (Wang, Chen, & Hua, 1998). According to this bioactivity classification for mushroom polysaccharides and the effect of α-glucosidase inhibition, it is apparent that ePS-F3-1 and ePS-F4-1 are responsible for the antidiabetic activity.

3.5. Monosaccharide analysis

The content of sugars in the polysaccharides was determined through use of a PMP-derivation method to hydrolyze the polysaccharides. On hydrolysis by 3 M CF_3COOH, three kinds of polysaccharide were derived with PMP and then detected by HPLC analysis. As shown in Table 2, ePS-F2-1 was composed of D-mannose, L-rhamnose, D-glucose, D-galactose, and D-arabinose, and the relative proportions were estimated by HPLC as 1.8:2.5:2.5:1.8:1.0. ePS-F3-1 was composed of D-mannose,

Table 1
Concentration resulting in a 50% α-glucosidase inhibitory effect in different processes.

Processes[b]/sample name	IC_{50} value	Ratio of each component (w/w/w)		
	Solid (mg/mL)[a]	Carbohydrates	Proteins	Triterpenoids
Contrast				
Glucobay (Acarbose)	0.6 ± 0.0			
Culture medium				
LH1 culture medium	16.6 ± 0.2	17.2	12.9	1.0
Precipitation				
Krestin (PSK)	5.9 ± 0.0	25.7	21.5	1.0
ePS	9.8 ± 0.1	34.9	3.5	1.0
DEAE Sepharose™ CL-6B				
ePS-F1[c]	Not active	129.6	15.1	1.0
ePS-F2	19.3 ± 0.0	39.0	36.3	1.0
ePS-F3	2.8 ± 0.0	7.8	13.9	1.0
ePS-F4	1.4 ± 0.0	4.2	9.8	1.0
ePS-F5	0.5 ± 0.0	3.4	1.6	1.0
ePS-F6[d]	Not active	6.1	N.D[e]	1.0
ePS-F7[d]	Not active	4.9	N.D[e]	1.0
ePS-F8[d]	Not active	N.D[e]	N.D[e]	1.0
Sephadex™ G-50				
ePS-F2-1	50.3 ± 0.2	69.2	49.4	1.0
ePS-F3-1	2.1 ± 0.0	13.1	N.D	1.0
ePS-F4-1	1.0 ± 0.0	6.5	N.D	1.0
ePS-F5-1[d]	Not active	12.5	N.D	1.0

[a] IC_{50} is defined as the concentration that resulted in a 50% α-glucosidase inhibition, and the results are means ± standard deviation of four independent replicates ($n = 4$).
[b] Krestin (PSK) and crude polysaccharide (ePS) were precipitated by 4 vol of 95% ethanol; ePS-F2 to ePS-F8 were NaCl-eluted fractions separated by a DEAE-Sepharose™ CL-6B column, but ePS-F1 was a water-eluted fraction; ePS-F2-1 to ePS-F5-1 were purified by the Sevag method.
[c] Had a very weak inhibitory effect and did not achieve a 50% enzyme inhibitory effect.
[d] Small amount; weak inhibitory effect and did not achieve a 50% of enzyme inhibitory effect.
[e] N.D, not detected.
*Effect of α-glucosidase inhibition was calculated as {1 − [(absorbance of sample − absorbance of sample color) ÷ (absorbance of reagent − absorbance of reagent color)]} × 100%. The assay mixture was incubated for 15 min at 37 °C, and the absorbance was measured at wavelength 400 nm. The values of 50% inhibitory concentration (IC_{50}) are expressed as mean ± *SD*.

L-rhamnose, D-glucose, D-galactose, and D-xylose, and the relative proportions were 2.2:1.0:6.9:7.1:2.5. ePS-F4-1 was composed of D-mannose, D-glucose, D-galactose, and D-xylose, and the relative proportions were 2.8:3.9:2.3:1.0. The results of determination of monosaccharides series by HPLC has been reported above, and the sugars series of ePS fractions are consistent with the sugars series reported in the reference from Lin et al. (2008), as shown in Table 2.

3.6. Infrared spectral analysis

The infrared spectra of the purified ePS-F2-1, ePS-F3-1, and ePS-F4-1 fractions were found to be similar as shown in Fig. 4; the spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1}. In IR spectra, the band between 3400 cm^{-1} and 3300 cm^{-1} represented the stretching vibration of O—H in the constituent sugar residues. The small band between 3250 cm^{-1} and 3100 cm^{-1} was associated with stretching vibration of C—H. The characteristic absorption band appeared at 1600 cm^{-1} and was assigned to the stretch of C=O of PSK, ePS-F2-1, ePS-F3-1, and ePS-F4-1 (Sun, Fang, Goodwin, Lawther, & Bolton, 1998), whereas the band between 1440 cm^{-1} and 1395 cm^{-1} was associated with the stretching vibration of C—O (Yang et al., 2008). The band between 1200 cm^{-1} and 1000 cm^{-1} was associated with stretching vibration of C—O from sterol and polysaccharides (Suo, Sun, & Wang, 2010). The prominent band between 1035 cm^{-1} and 1111 cm^{-1} was representative of C—O—C and C—O—H. The small absorption bands at between 935 and 900 cm^{-1} in three FTIR spectra (ePS-F2-1, ePS-F3-1, and ePS-F4-1) could be associated with β-glycosidic linkages between the sugar units (Yang et al., 2008). The absorption bands at 1151 cm^{-1} in four FTIR spectra (PSK, ePS-F2-1, ePS-F3-1, andePS-F4-1) could be the antisymmetric α-(1, 4) glycosidic linkages stretching mode between the sugar units (Sekkal, Dincqb, Legrandb, & Huvenneb, 1995), where might be effected by α-glucosidase. The characterization of three polysaccharides (ePS-F2-1, ePS-F3-1, and ePS-F4-1) by IR analysis above showed the absorption of polysaccharides. Moreover, compared to the spectrum of ursolic acid standard, the stronger absorption bands between 1700 cm^{-1} and 1550 cm^{-1} and the absorption bands between 1550 cm^{-1} and 1450 cm^{-1}, between 1450 cm^{-1} and 1350 cm^{-1}, and between 2350 cm^{-1} and 2250 cm^{-1} are typical of absorption of triterpenoids. The presence bands between 3356 cm^{-1} and 3361 cm^{-1} are the saponins shifted, indicative of

Table 2
Sugar composition of polysaccharide fractions.

Samples	Ratio of each monosaccharide (w/w/w)						Reference
	Man	Rha	Glc	Gal	Ara	Xyl	
ePS-F2-1	1.8	2.5	2.5	1.8	1.0	N.D	In this study
ePS-F3-1	2.2	1.0	6.9	7.1	N.D	2.5	In this study
ePS-F4-1	2.8	N.D	3.9	2.3	N.D	1.0	In this study
ePSP-Cv[a]	9.1	N.A	91.4	9.7	N.D	1.0	Lin et al. (2008)
ePSP-LBE[a]	1.0	N.A	42.2	4.9	N.D	4.6	Lin et al. (2008)
PSP-Lb[a]	N.D	N.A	37.8	13.2	24.9	1.0	Lin et al. (2008)

N.D, not detected; N.A, not analyzed.
[a] Crude polysaccharides.

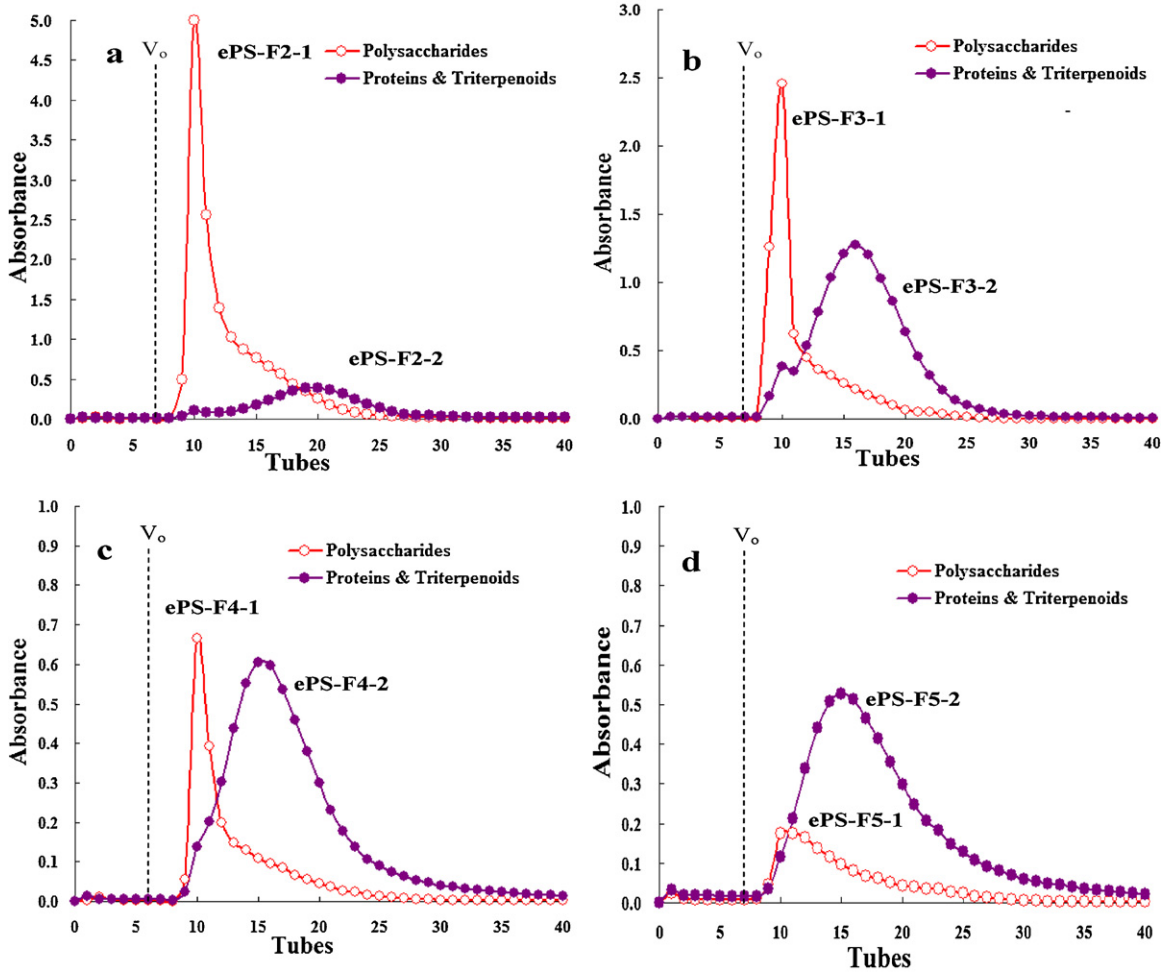

Fig. 3. (a) ePS-F2, (b) ePS-F3, (c) ePS-F4, and (d) ePS-F5 profile on Sephadex™ G-50. The column was eluted with 0.1 M Na_2HPO_4 (pH 9.0) at a flow rate of 0.4 mL/min.

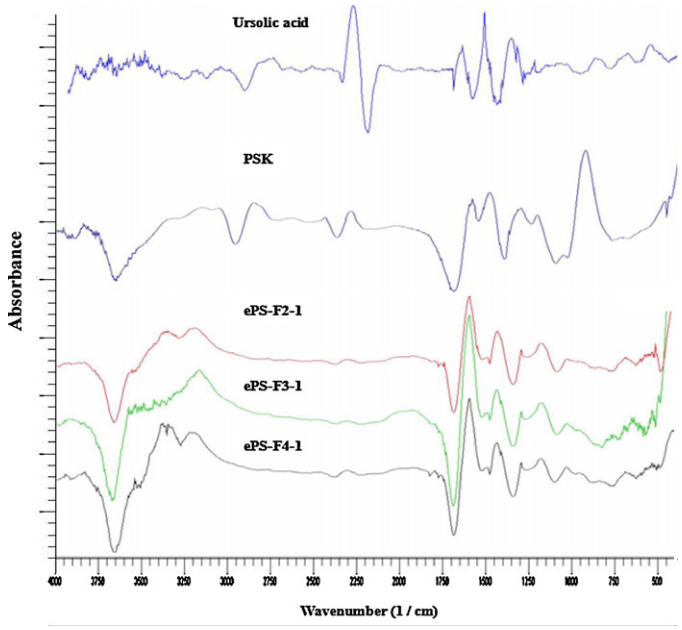

Fig. 4. FTIR spectrum of ePS fractions.

the overlapping of the O—H and N—H stretching (Liu et al., 2011). Also, the stronger absorption bands at 1600 cm^{-1} might be the spectrum of saponin (glycyrrhizinate) (Suo et al., 2010).

Triterpenoids, triterpenoids are not normally water soluble. However, we had found out the triterpenoids with polysaccharopeptide dissolve in the water suggesting that the triterpenoids are bound to the polysaccharides or the polysaccharides and the triterpenoids are carried by free proteins. Hence, the triterpenoids can be dissolved in the culture medium or in the water. After Sevag reagent treatment, some triterpenoids were lost. The missing triterpenoids are carried by free proteins and the present triterpenoids are bonded with polysaccharides. In our studies, saponin might be contained in the samples of PSK, ePS-F2, ePS-F3, ePS-F4, and ePS-F5. However, ePS-F3-1 and ePS-F4-1 are saponins, and ePS-F2-1 is polysaccharopeptides–triterpenoids mixture. The results of above are confirmed by the spectroscopic analysis.

4. Conclusions

In this work, the recovery conditions of polysaccharides from culture medium of *C. versicolor* LH1 were discovered. Results showed that the optimal recovery effect of polysaccharides was observed using 4 vol of 95% ethanol under a concentration of total ePS at 23.3 mg/mL, which had a recovery ratio of crude ePS is 98.7 ± 0.3% and the residue ratio of reducing sugar is 10.0 ± 0.1%.

Three polysaccharide fractions (ePS-F2-1, ePS-F3-1, ePS-F4-1) that demonstrated stronger α-glucosidase inhibition were

separated from the crude ePS of *C. versicolor* LH1 culture medium using a DEAE Sepharose™ CL-6B anion-exchange column and Sephadex™ G-50 column. Results of HPLC and infrared spectra analysis indicated that ePS-F2-1 mainly contained polysaccharide, proteins, and triterpenoids, so we consider it a polysaccharopeptide–triterpenoid mixture. The major molecular weight of ePS-F2-1 was 1.6 kDa, and the monosaccharides components contained D-mannose, L-rhamnose, D-glucose, D-galactose, and D-arabinose. Both ePS-F3-1 and ePS-F4-1 mainly contained polysaccharide and triterpenoids, so we consider them to be saponins. The major molecular weight of ePS-F3-1 and ePS-F4-1 were 3.4 kDa and 5.0 kDa, respectively; the monosaccharides component of ePS-F3-1 contained D-mannose, L-rhamnose, D-glucose, D-galactose, and D-xylose; and ePS-F4-1 contained D-mannose, D-glucose, D-galactose, and D-xylose. According to the characterizations of IR spectra and chemical components, ePS-F3-1 and ePS-F4-1 are saponins, and the ePS-F2-1 is a polysaccharopeptide–triterpenoids mixture.

Two first purified saponins fractions (ePS-F3-1 and ePS-F4-1) exhibited strong α-glucosidase inhibition, and had a higher inhibitory activity than Krestin (PSK), and the crude ePS; ePS-F4-1 showed the best inhibition activity. Although a better structural analysis is required for the polysaccharides present in these fractions, the α-glucosidase inhibition activity may be contributed from the polysaccharides, triterpenoids, or polysaccharide mixture. Further studies are needed to clarify the role of the polysaccharides, triterpenoids, and the polysaccharide mixture in relation to activity of α-glucosidase inhibition. In conclusion, the polysaccharopeptide–triterpenoids mixture and polysaccharide–triterpenoids (saponins) separated and first purified from submerged fermentation culture of *C. versicolor* LH1 could be explored as a novel potential antidiabetic. Further studies should be carried out to clarify the role of the polysaccharides, triterpenoids, and the polysaccharide mixture in relation to activity of α-glucosidase inhibition and to elucidate bioactivity through animal experiments with the purpose of applying the polysaccharide–triterpenoids (saponins) in the food industry.

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