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Separation, purification, and α-glucosidase inhibition of polysaccharides from *Coriolus versicolor* LH1 mycelia

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ABSTRACT

Intracellular polysaccharides (iPs) were separated and purified from *Coriolus versicolor* LH1 mycelia and characterized for their α-glucosidase inhibitory properties. Three iP fractions (iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1) were extracted, separated, and purified from LH1 mycelia using microwave extraction technology, a DEAE-Sepharose™ CL-6B column, a Diaion® HP20 macroporous adsorption column, and a Sephadex™ G-50 gel-permeation column. The principal constituents of iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 were saponins and polyphenoic compound mixtures. The enzyme inhibition activity, IC_{50} values, of these three fractions were 1.7, 1.8, and 0.8 mg/mL, respectively. The α-glucosidase inhibitory properties were related to the presence of α-(1,4) glycosidic linkages in the polysaccharide structure and the total relative percentage of D-glucose and D-galactose in the structure of polysaccharides, other than triterpenoids.

1. Introduction

Diabetes mellitus is a common metabolic disease characterized by high blood glucose levels. This is caused by insulin deficiency or functional disturbance of the receptors, which causes blood glucose to rise and induce disorders in the metabolization of fats and proteins. The number of patients with diabetes mellitus has increased with each year. According to the statistics from International Diabetes Federation (IDF), 6.0% of the world's population had diabetes in 2007. Because the number of patients is increasing every year, the prevalence of diabetes is expected to reach 7.3% by the end of 2025 (Mbanya, Gan, & Allgot, 2006). Thus, there is an urgent need to create new medication for diabetes.

Prior to the incidence of diabetes mellitus, the patient experiences high blood sugar; this can be divided into two types, Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG). These two prediabetes symptoms are important risk factors for diabetes and cardiovascular disease. α-Glucosidase inhibitors have been used to reduce blood glucose by preventing the digestion of carbohydrates and are used in patients with IGT and diabetes (Geng, Jin, Wu, Fang, & Wang, 2011). Some enzyme-inhibitory medicines have been used in clinical practice; for example, Acarbose, Voglibose, and Miglitol have been used effectively to treat type 2 diabetes mellitus (non-insulin dependent) and IGT.

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). Some edible mushrooms can be employed to produce α-glucosidase inhibitors by extraction or fermentation. According to a number of studies, various edible mushroom extracts can help develop immunity to diseases, inhibit tumors, provide antioxidants, stimulate free-radical scavenging activity, fight viruses, inhibit diabetes, and lower blood lipid levels. These extracts are used in health foods and in the development of natural medicines (Lu, Li, Suo, & Li, 2010). Polysaccharides present in several edible mushrooms tend to lower blood sugar. Polysaccharides can be present in fruiting bodies, mycelia, and culture media. They are obtained in different forms, such as polysaccharopeptides (PSP), polysaccharide-proteins, polysaccharide-triterpenoids, and in the original polysaccharide form, through extraction, separation, and purification (Wu, Xiao, Yang, & Zhang, 2009; Yang, Hsu, Lin, Hsu, & Chen, 2012).

Coriolus versicolor (syn. Trametes versicolor), is taxonomically a member of the phylum Basidiomycota, order Polyporales, and family Polyporaceae (Emberger, 2008). It can be used in medicines and health food to treat diseases and to enhance body functions (Ng, 1998). *C. versicolor* has notable antioxidant effects; can lower blood sugar; and has been used in the treatment and prevention of hepatitis B, tumor-related diseases such as cancer of the liver,

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breast, and stomach, and certain immune-deficiency diseases (Li & Xu, 1987; Qian, Xu, & Tang, 1997; Ren, Dai, & Li, 1993).

Recently, a new strain of *C. versicolor* named LH1 was identified. It has been reported that the exopolysaccharopeptides (ePSP) from LH1 exhibit significant immunomodulatory activity; ePSP have proven to be safe in 28-day oral trials in mice (Lai et al., 2011; Lin et al., 2008). Recently, we separated and analyzed extracellular polysaccharide (eP) fractions and reported that they possess significant α-glucosidase inhibitory activity (Yang et al., 2012). Therefore, the objective of this study was to further investigate the α-glucosidase inhibitory effects and chemical properties of LH1 intracellular polysaccharides (iP) separated and purified from *C. versicolor* mycelia.

The Sevag method is commonly used to remove and separate free proteins, which exist in crude polysaccharide extracts. The proteins are precipitated after repeated denaturation by vigorous shaking with a solution of octanol in chloroform (Chen, Zhong, Zeng, & Ge, 2008; Chen et al., 2011; Wang, Chang, & Chen, 2009; Zhang et al., 2009). However, we recently determined that the α-glucosidase inhibitory activity of active fractions could be lost after separation by the Sevag method (Yang et al., 2012). To ensure that the extract constituents were intact during analysis, an extraction procedure using physical processing methods was designed. Diaion® HP20 macroporous adsorption resins were used instead of the Sevag method to denature and remove free proteins during the processing of crude iP extracts, which were then analyzed for activity.

Saponins, which is a kind of glucoside that has aglycon combined with triterpenoids or spirostanol, are a class of naturally occurring, secondary metabolites that are particularly abundant in various plant species (Podolak, Galanty, & Sobolewska, 2010), but have rarely been reported in research on mushrooms (Lu et al., 2008). This study investigated the potential α-glucosidase inhibitory activity of extracts from *C. versicolor* LH1 mycelia. In addition, the chemical compositions (monosaccharides, proteins, triterpenoids, and polyphenol compounds), molecular weights (MW), and FTIR spectra of the isolated iP-fractions were determined.

2. Materials and methods

2.1. Chemicals

The mycelia of *C. versicolor* LH1 were provided by Dr. Hsu Tai-Hao of Da-Yeh University, Changhua County, Taiwan. 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 (BSA), 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). Diaion® HP20 resins were purchased from Tai Young Chemical Co., Ltd. (Japan). All other chemicals were analytical-grade commercial preparations.

Analysis was performed using a spectrophotometer (Spectrophotometer U-3310, Hitachi, Japan). Isolation and purification equipment included a high pressure pump (L-7100, Hitachi, Japan) and three empty chromatography columns (FG-38-40, FG-25.4-30, and FG-19-100, RAY-E Creative Co., Ltd., Taiwan).

2.2. Cultivation of C. versicolor

The culture was maintained on potato dextrose agar (PDA) plates at 25 °C. For seeding, mycelia were cultured in normal 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. Fermentation was 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 ($11{,}000 \times g$) for 30 min. The mycelia of *C. versicolor* LH1 were lyophilized until further use.

2.3. Preparation and quantification of crude iP

2.3.1. Preparation of crude iP

The dried *C. versicolor* LH1 biomass was extracted under optimal conditions using microwave extraction technology. Fifty grams of *C. versicolor* LH1 biomass were extracted in 750 mL distilled water. Extractions were performed at 90 °C for 15 min at 600 W. After paper filtration crude polysaccharides were precipitated from the filtrate with four volumes of 95% ethanol (EtOH) (4:1, v/v) at 4 °C overnight. After precipitation, the supernatant and precipitate (crude iP) were separated by centrifugation at 6500 rpm ($11{,}000 \times g$) for 20 min. The entire crude iP sample was concentrated using an evaporator and freeze dried. The chemical components and the α-glucosidase inhibitory effect of the crude iP were analyzed.

2.3.2. Quantification of polysaccharides

Crude iP content was determined by measuring total sugars using the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The absorbance of total sugars was read after deduction of the blank and sample color.

2.3.3. Quantification of proteins

Proteins were quantified using the Biuret method (Ohnishi & Barr, 1978). Biuret reagent (4.0 mL) was added to a tube containing l.0 mL of sample proteins dissolved in distilled water. After incubation at 37 °C for 30 min the absorbance was read at 540 nm after deduction of the reagent blank. BSA was used as the standard.

2.3.4. Quantification of triterpenoids

Total triterpenoids were quantified by a colorimetric method using vanillin/glacial acetic acid (Fan & He, 2006). The contents of a tube containing 1.0 mL of test sample were evaporated in an oven to remove the water solvent. The solid particles were retained for subsequent analysis. Successive additions of 0.3 mL 5% vanillin/glacial acetic acid (w/v) and 1.0 mL perchloric acid solution were made to each test sample. The reaction was modified by incubating the sample solution at 60 °C for 10 min prior to cooling on ice for 3 min. The sample absorbance was measured at 548 nm after addition of 5.0 mL glacial acetic acid. Ursolic acid was used as the standard.

2.3.5. Quantification of polyphenolic compounds

Total polyphenolic compounds were quantified by the colorimetric method using Folin–Ciocalteu reagent (Lachman, Hosnedl, Pivec, & Orsak, 1998); modified doses of a 0.05 mL sample were mixed with 2.25 mL distilled water and 0.25 mL of 2 N Folin–Ciocalteu reagent. After agitation, 1.25 mL of 20% (w/v) sodium carbonate (Na_2CO_3) solution was added. After 20 min at laboratory temperature, the absorbance of the samples was measured on a spectrophotometer (Spectrophotometer U-3310, Hitachi) at a wavelength of 765 nm after deduction of the blank and sample color. The results were expressed as gallic acid equivalents (GAE) in mg/mL of dry matter.

2.4. Chromatographic fractionations of iP

Before chromatography, crude iP was dialyzed by membrane (MWCO 12–16 kDa) filtration to separate the small molecules of crude intracellular polysaccharides (iPS) for a more accurate chromatogram during ion-exchanger fractionation. Then, the large molecules of crude intracellular polysaccharide (iPL), inside the membrane, were filtered through a 0.45 μm nylon membrane. The filtrate (1.0 g, pH 4.26) 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 increase of 0.1 M for each fraction and 1.0 M NaCl for the final elution. For each tube, 10 mL of eluate was collected by a fraction collector (Advantec SF-2100W, Japan). Absorbance at wavelength 490 nm was measured for detection of polysaccharides after using the phenol–sulfuric acid method, and the ultraviolet absorbance at 280 nm was monitored for the aromatic group of proteins and the phenolic group of triterpenoids or polyphenols. The fractions were further tested to determine the α-glucosidase inhibitory effect.

2.5. Analysis of α-glucosidase inhibitory activity

The method of Gaxiola et al. (2005) was used to analyze α-glucosidase inhibitory activity in vitro, using ρNPG as a substrate and modifying the dosage. The assay mixture (1.6 mL) contained 0.2 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 the reaction was stopped by adding 0.8 mL of 0.2 M Na_2CO_3. The amount of ρNP released was measured on Spectrophotometer U-3310 (Hitachi) at 400 nm.

2.6. Separation by HP20 macroporous adsorption resins

The fractions that showed maximum enzyme inhibition activity during DEAE Sepharose™ CL-6B column elution were desalted using a dialysis membrane (MWCO 12–16 kDa) and freeze-dried. Before separation, the powder fraction was dissolved in distilled water (at a concentration of 10.0 mg/mL), and the sample was filtered through a 0.45-μm nylon membrane. Next, the filtrate (10.0 mL) was applied to a Diaion® HP20 column (25.4 mm × 100 mm), which was eluted with a stepped gradient EtOH aqueous solution (from 0% to 47.5%), with an increasing 1/10 concentration of 95.0% EtOH for each fraction and 95.0% EtOH for the final elution. The eluate was collected in fractions of 10 mL for each tube using a fraction collector. The chemical components and the α-glucosidase inhibitory effects were monitored via the absorbance and were determined as described in Sections 2.3 and 2.5, respectively.

2.7. Purification by gel-permeation chromatography (GPC)

The fraction that showed the highest enzyme inhibition during HP20 macroporous adsorption column elution was concentrated using an evaporator and then freeze-dried. Before purification, the powder fraction was dissolved in 0.1 M Na_2HPO_4 buffer (pH 9.0, at a concentration of 5.0 mg/mL), and the samples were filtered with a 0.45-μm nylon membrane. After that, the filtrate (1.0 mL) was applied to a Sephadex™ G-50 column (19 mm × 760 mm, fractionation range 1.5–30 kDa), which was eluted with a 0.1 M Na_2HPO_4 buffer (pH 9.0) at a 0.3 mL/min flow rate. The eluate was collected in fractions of 9 mL for each tube using a fraction collector.

2.8. 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, and an RI detector (Jasco RI-2031 Plus). The mobile phase used was 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 the 0.1 M Na_2HPO_4 buffer. The volume of sample (1.0 mg/mL) injected 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 (342 Da).

2.9. Analysis of monosaccharide composition

Hydrolysis of polysaccharides to monosaccharides was performed according to the method of Lv et al. (2009); modified doses of 50 mg of iPL-fractions were dissolved in 5.0 mL of 3 M trifluoroacetic acid (TFA) in an ampoule (10 mL). The ampoules were kept in an oven at 110 °C for 2 h to hydrolyze the polysaccharides to monosaccharides. After cooling, the hydrolyzed sample of the iPL-fraction was carried out with 1-phenyl-3-methyl-5-pyrazolone (PMP) as a derivative. Derivation of PMP was also carried out according to the method reported by Lv et al. (2009). The hydrolyzed iPL-fraction was dried using an evaporator. Next, the sample 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; 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.

Analysis was performed using 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 volume: 20 μL; UV detector: WL 235 nm; column temperature: 30 °C.

2.10. 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}.

2.11. Statistical analysis

Results were expressed as mean ± standard deviations (SD) of four replicated determinations. MINITAB® software (Minitab, Inc., USA) was used for data analysis. Significant differences between two means were determined by the least significant difference (LSD) test at a 0.05 significance level. SD and error bars are shown wherever necessary.

Table 1
The activity (IC_{50}) of α-glucosidase inhibition and the comparison of different components.

Processes[b]	Sample name	α-Glucosidase inhibition[e]	Relative percentage comparison of different components (%)[c]			
		IC_{50} (mg/mL)[a]	Polysaccharides	Proteins	Triterpenoids	Polyphenols
	Glucobay (Acarbose)	0.6 ± 0.0				
Extraction	Extract	12.7 ± 0.2	62.89	30.19	4.40	2.52
Precipitation and dialysis	Krestin (PSK)	5.9 ± 0.0	51.81	43.52	2.07	2.59
	iPL	19.6 ± 0.1	80.00	17.60	1.60	0.80
	iPS	22.8 ± 1.2	65.36	30.07	3.27	1.31
DEAE-Sepharose™ CL-6B	iPL-F1	N.D.[d]	100.00	N.D.	0.00	N.D.
	iPL-F2	57.2 ± 1.4*	92.59	5.56	0.93	0.93
	iPL-F3	4.4 ± 0.0*	48.31	42.51	3.38	5.80
	iPL-F4	2.8 ± 0.0	31.65	56.65	5.06	6.65
	iPL-F5	2.9 ± 0.0	25.51	57.40	6.38	10.71
	iPL-F6	3.3 ± 0.0*	44.64	34.38	6.25	14.73
	iPL-F7	4.1 ± 0.0*	81.30	8.94	4.07	5.69
	iPL-F8	N.D.	84.03	N.D.	5.88	10.08
Diaion® HP20 resins	iPL-F4-1	4.2 ± 0.0*	76.92	N.D.	10.77	12.31
	iPL-F4-2	2.6 ± 0.0*	16.29	67.59	5.86	10.26
	iPL-F4-3	2.8 ± 0.0*	16.67	64.50	7.00	11.83
	iPL-F4-4	2.8 ± 0.0*	10.72	71.38	5.57	12.33
	iPL-F4-5	2.3 ± 0.0*	37.59	47.74	12.03	2.63
	iPL-F4-6	3.5 ± 0.0*	59.17	31.95	4.14	4.73
	iPL-F4-7	1.2 ± 0.0	44.64	N.D.	51.79	3.57
	iPL-F5-1	8.8 ± 0.0*	17.24	72.24	3.97	6.55
	iPL-F5-2	1.7 ± 0.0*	24.27	44.17	9.71	21.84
	iPL-F5-3	1.7 ± 0.0*	29.41	36.18	24.41	10.00
	iPL-F5-4	1.6 ± 0.0*	18.35	48.62	9.17	23.85
	iPL-F5-5	1.2 ± 0.0	48.31	N.D.	15.94	35.75
	iPL-F5-6	1.4 ± 0.0*	80.65	N.D.	8.06	11.29
	iPL-F5-7	3.3 ± 0.0*	35.71	N.D.	48.57	15.71
Sephadex G-50	iPL-F5-2-1	1.7 ± 0.0*	54.05	N.D.	15.68	30.27
	iPL-F5-4-1	1.8 ± 0.0*	43.86	N.D.	24.12	32.02
	iPL-F5-5-1	0.8 ± 0.0	45.25	N.D.	24.89	29.86

[a] IC_{50} is defined as the concentration of the solid of samples that resulted in 50% α-glucosidase inhibition. The results are the mean ± SD of four independent replicates ($n = 4$). Values of the fractions were analyzed by one-way analysis of variance (ANOVA) and least significant difference.

[b] Krestin (PSK), large intracellular crude polysaccharide molecules (iPL) and small intracellular crude polysaccharide molecules (iPS) were precipitated by 4 vol of 95% ethanol and then separated by a dialysis membrane (MWCO 12-14 kDa); iPL-F2 to iPL-F8 were NaCl-eluted fractions separated by a DEAE-Sepharose™ CL-6B column, but iPL-F1 was a water-eluted fraction; iPL-F4-2 to iPL-F4-7 and iPL-F5-2 to iPL-F5-7 were EtOH-eluted fractions separated by a HP20 column, but iPL-F4-1 and iPL-F5-1 were water-eluted fractions.

[c] Based of the all analyzed chemical components.

[d] N.D. = not detectable.

[e] α-Glucosidase inhibition was calculated as

$$1 - \frac{(\text{absorbance of sample} - \text{absorbance of sample color})}{(\text{absorbance of reagent} - \text{absorbance of reagent color})} \times 100\%$$

The assay mixture was incubated for 15 min at 37 °C, and absorbance was measured at 400 nm. The values of 50% inhibitory concentration (IC_{50}) are expressed as mean ± SD.

* $P < 0.05$ as compared with the same process group.

3. Results and discussion

3.1. Separation and fractionation of iPL

Before separation, many previous studies on polysaccharides have employed the Sevag method to denature the proteins, and then go on chromatography. To denature and remove free proteins, 5 vol of Sevag reagent (chloroform and n-butyl alcohol in 5:1 (v/v)) was added to the sample, and the precipitated proteins were separated by centrifugation at 6500 rpm (11,000 × *g*) for 10 min. This was repeated four times when Sevag reagent layer was clean. After separation by addition of the Sevag reagent, the samples were dialyzed again by a membrane. However, this method has been shown to change the chemical conformation of crude polysaccharides with potential effects on their bioactivity. Yang et al. (2012) demonstrated that the α-glucosidase inhibitory activity of ePS-F5 was lost after treatment with the Sevag method. In this study, physical methods were designed (two-stage separation and purification strategy) to replace the Sevag method and thus avoid inactivation of α-glucosidase inhibitory activity. Analysis of the iPL fractions revealed the presence of polysaccharides, proteins, triterpenoids, and polyphenols (Table 1). Consequently, the separation techniques employed, namely ion-exchange chromatography and HP20 macroporous adsorption chromatography, exploited the different, intrinsic physical properties of proteins, triterpenoids, and polyphenols. The fraction with the highest α-glucosidase inhibitory activity was purified using a gel permeation chromatography column.

The anion-exchange chromatography results for the iPL fractions are shown in Fig. 1. Separation using a DEAE-Sepharose™ CL-6B column yielded the following fractions: iPL-F1, iPL-F2, iPL-F3, iPL-F4, iPL-F5, iPL-F6, iPL-F7, and iPL-F8 (the percentage of each fraction recovered was 56.6, 29.4, 5.5, 2.4, 1.2, 1.1, 0.8, and 2.6, respectively, relative to the total amount of initial iPL). These fractions were eluted using an increasing salt (NaCl) concentration gradient (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 1.0 M), respectively. Determination of α-glucosidase inhibition revealed that fractions iPL-F4 and iPL-F5 exhibited the highest inhibitory activity (Table 1). These two fractions were purified further using a Diaion® HP20 column.

The results of the fractionation of iPL-F4 and iPL-F5 by Diaion® HP20 column are shown in Fig. 2a and b, respectively. The fractions, iPL-F4-1, iPL-F4-2, iPL-F4-3, iPL-F4-4, iPL-F4-5, iPL-F4-6, and

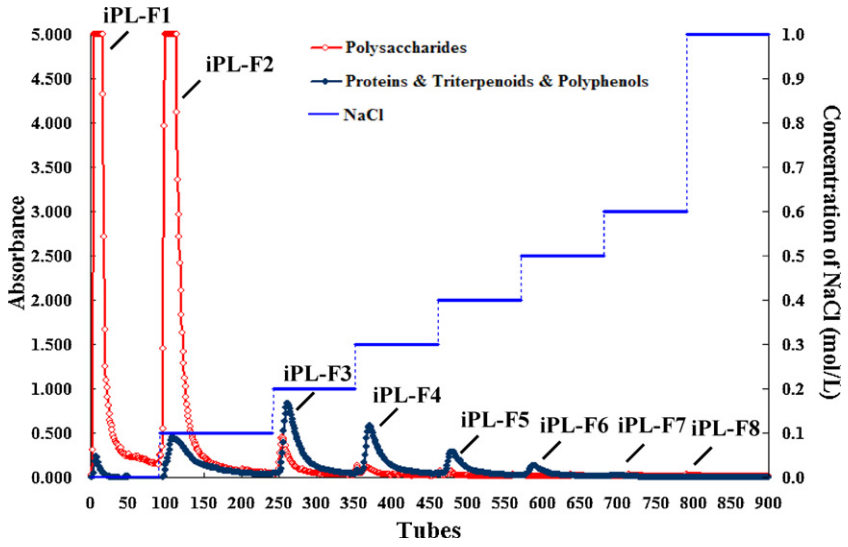

Fig. 1. DEAE-Sepharose™ CL-6B anion-exchange column chromatogram of the crude iPS. The column was eluted with water, NaCl (0.1–0.6 M), and an NaCl (1 M) step gradient at a flow rate of 1.0 mL/min.

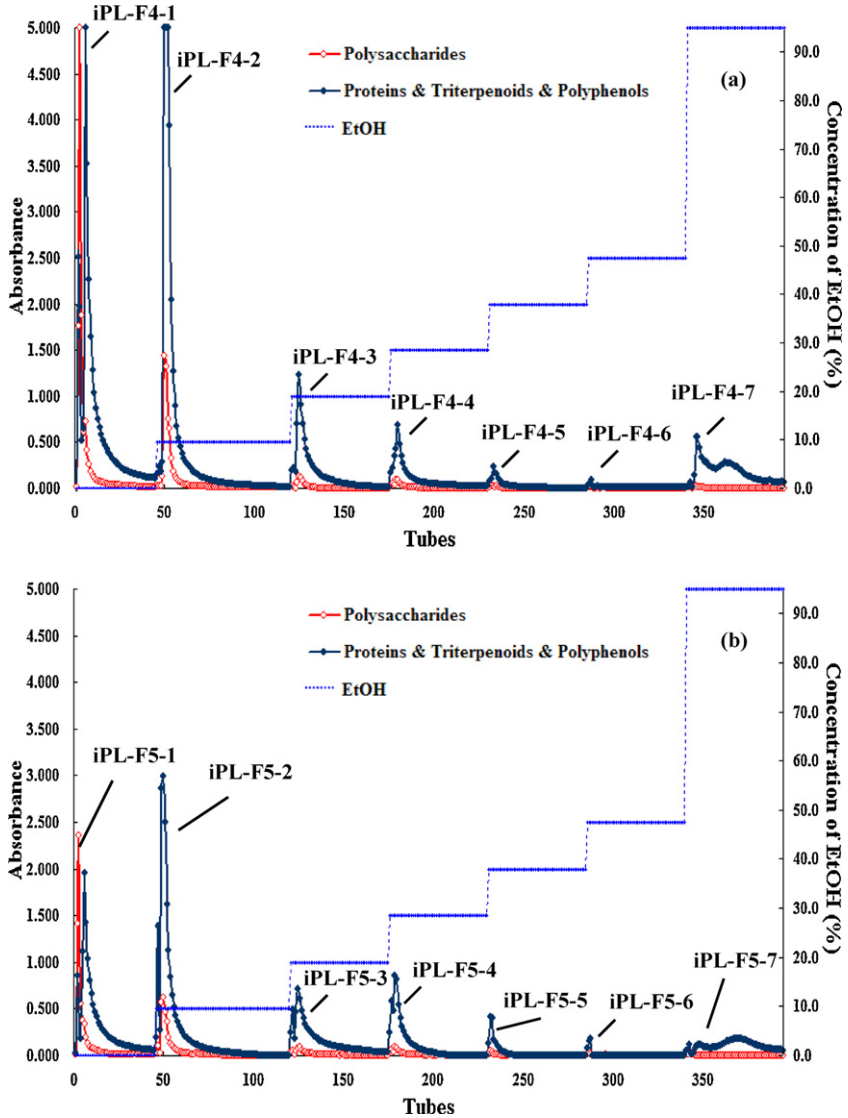

Fig. 2. Diaion® HP20 macroporous adsorption resins column chromatogram of (a) iPL-F4 fraction and (b) iPL-F5 fraction. The column was eluted with water, EtOH (9.5–47.5%), and a 95% EtOH step gradient at a flow rate of 1.0 mL/min.

iPL-F4-7, were eluted using a water–EtOH gradient (9.5%, 19.0%, 28.5%, 38.0%, 47.5%, and 95.0%). The percentage of each fraction recovered was 38.3, 23.4, 7.3, 6.1, 3.5, 5.3, and 16.0, respectively, in relation to the total amount of initial iPL-F4. Furthermore, iPL-F5-1, iPL-F5-2, iPL-F5-3, iPL-F5-4, iPL-F5-5, iPL-F5-6, and iPL-F5-7 were obtained using the same elution conditions as for iPL-F4. The percentage of each fraction recovered was 42.7, 19.7, 7.1, 5.8, 3.4, 3.2, and 18.1, respectively, in relation to the total amount of initial iPL-F5. These results revealed that iPL-F4-7 and iPL-5-7 were impure fractions and that iPL-F4-1 and iPL-F5-1 were the two most-polar fractions. The fractions with the strongest polysaccharide signals were iPL-F4-1 and iPL-F5-1; the fractions with intermediate strength signals were iPL-F4-2 and iPL-F5-2; the fractions with weak polysaccharide signals were iPL-F4-3, iPL-F4-4, iPL-F4-5, iPL-F5-3, iPL-F5-4, and iPL-F5-5; the remaining fractions did not have detectable polysaccharide signals.

3.2. Enzyme inhibition activity of different polysaccharide fractions

Polysaccharides exhibit varying levels of α-glucosidase inhibition. Consistent with this, the different polysaccharide fractions separated and purified from iPL showed a range of enzyme inhibitory activities (Table 1).

Analysis of the enzyme inhibitory activity of the LH1 mycelia extract indicated that the crude iPL and iPs extracts had lower inhibitory activity than those of Krestin (PSK) and Glucobay (Acarbose), but fractions iPL-F3 to iPL-F7 showed higher inhibitory activity (IC_{50} value) than PSK. Analysis of α-glucosidase inhibition revealed that iPL-F4-7, iPL-F5-6, and in particular, iPL-F5-5, displayed higher inhibitory activity than the other fractions. However, iPL-F4-7 will not be discussed any further in this study because the fraction comprised a mixture of components.

The presence of proteins, triterpenoids, and polyphenols was observed in the samples during precipitation and fractionation. Triterpenoids have low polarity and are insoluble in water unless covalently bound with more polar substances (such as polysaccharides) or electrovalently bound with proteins. The samples were purified and the iPL-F5-fractions were obtained because it has a more anionic character with proteins, a less polar character with triterpenoids or polyphenols, and is more soluble in water with polysaccharides. Five, highly active, α-glucosidase-inhibiting fractions (iPL-F5-2 to iPL-F5-6) were purified and analyzed by gel-permeation (Sephadex™ G-50) chromatography.

The relationship between the chemical components of the polysaccharide fractions and α-glucosidase inhibition was investigated (Table 1). The results from the separation by DEAE-Sepharose™ CL-6B demonstrated that an increase in the relative percentage of proteins resulted in higher α-glucosidase inhibitory activity. For example, iPL-F4 and iPL-F5 contained a higher percentage of proteins than the other iPL-fractions (56.65% and 57.40%, respectively, no significant difference between iPL-F4 and iPL-F5 by one-way ANOVA), and possessed higher α-glucosidase inhibitory activity. Therefore, the α-glucosidase inhibitory activity was considered to be associated with the proteins present in the iPL fractions, but not with the triterpenoids or polyphenols. Thus, these two fractions were separated further using HP20 macroporous adsorption resin columns to further investigate the relationship between the relative percentage of proteins in these polysaccharide fractions and α-glucosidase inhibition.

Analysis of the fractions obtained from the HP20 macroporous adsorption resin columns shows that significantly higher levels of α-glucosidase inhibition are displayed by iPL-F4-7 and iPL-F5-2 to iPL-F5-6, which were separated, respectively, from iPL-F4 and iPL-F5 (Table 1). Of these, iPL-F4-7 and iPL-F5-5 showed maximum α-glucosidase inhibitory activity (there was no significant difference between the inhibitory activities of iPL-F4-7 and iPL-F5-5 by one-way ANOVA) without proteins in iPL-F4-7 and iPL-F5-5 when compared with the other fractions of iPL-F4- and iPL-F5-fractions. This result demonstrates that there is no relation between the chemical components of the polysaccharides and α-glucosidase inhibition. This observation led to the proposal that the α-glucosidase inhibitory effect might be dependent on polysaccharide structure. Thus, we continued to separate the fractions containing higher inhibitory activity using gel permeation chromatography. Fraction iPL-F4-7 consisted of a mixture of polysaccharides that proved difficult to purify and it was not investigated further in this study.

The results of separation by Sephadex™ G-50 revealed that iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 comprised pure polysaccharides whereas the iPL-F5-3 and iPL-F5-6 fractions contained a mixture of polysaccharides. The latter two fractions were not investigated further in this study (Fig. 3). The results of α-glucosidase inhibition analysis demonstrate that iPL-F5-5-1 had the maximum enzyme inhibitory activity (IC_{50} value); its activity approached that of Glucobay (Table 1).

α-Glucosidase, one of the starch hydrolysis enzymes in our intestine, can hydrolyze starch to glucose and then absorb glucose into intestinal micromodules. This study demonstrated that the iPL-F5-5-1 fraction extracted from *C. versicolor* LH1 mycelia can strongly inhibit α-glucosidase. Therefore, iPL-F5-5-1 could potentially play an active role during the postprandial phase of diabetes.

3.3. Molecular mass

On the basis of calibration with standard dextrans, the apparent molecular weights of the polysaccharides (iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1) were 9.2 ± 0.1 kDa, 11.3 ± 0.4 kDa, and 14.6 ± 0.1 kDa, respectively. Compared to the polysaccharides PSK-Pro (MW 150 kDa) (Hamanaka et al., 2011), CVE (from *C. versicolor* fruiting bodies, MW 500 kDa) (Zhang, Han, & Pan, 2001), and PSP (MW 100 kDa) (Chan & Yeung, 2006), the three polysaccharides extracted from LH1 mycelia had smaller molecular masses but were larger than the extracellular polysaccharides obtained from LH1-submerged fermentation culture [ePS-F2-1 (MW 1.6 kDa), ePS-F3-1 (MW 3.4 kDa), and ePS-F4-1 (MW 5.0 kDa)] (Yang et al., 2012).

The bioactivity of mushroom polysaccharides can be classified into three groups: (a) antidiabetic activity, with a molecular mass between 3 and 5 kDa; (b) anti-inflammatory activity, with a molecular mass between 10 and 100 kDa; (c) anti-tumor activity, with a molecular mass over 30 kDa (Wang, Chen, & Hua, 1998). According to this classification and the analysis of α-glucosidase inhibition, iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1, show antidiabetic activity but may also have the potential to reduce inflammation.

3.4. Monosaccharide analysis

The sugar content of the polysaccharides was determined using a PMP-derivation method to hydrolyze the polysaccharides (Lv et al., 2009). After hydrolysis with 3 M TFA, three types of polysaccharide were derived by PMP and then detected by HPLC analysis. iPL-F5-2-1 comprised D-mannose, L-rhamnose, D-glucose, D-galactose, D-arabinose, and D-xylose (Table 2). The relative percentages estimated by HPLC were 21.76%, 6.48%, 32.87%, 25.46%, 8.80%, and 4.63%, respective. Fraction iPL-F5-4-1 comprised D-mannose, D-glucose, D-arabinose, and D-xylose. The relative percentages were 11.82%, 57.27%, 21.82%, and 9.09%, respective. Fraction iPL-F5-5-1 comprised D-mannose, D-glucose, D-galactose, and D-arabinose. The relative percentages were 7.09%, 37.59%, 41.13%, and 14.18%, respective. The sugar series of these three polysaccharides differ from the sugar series reported by Lin et al. (2008) and Yang et al. (2012), as shown in Table 2. The relationship

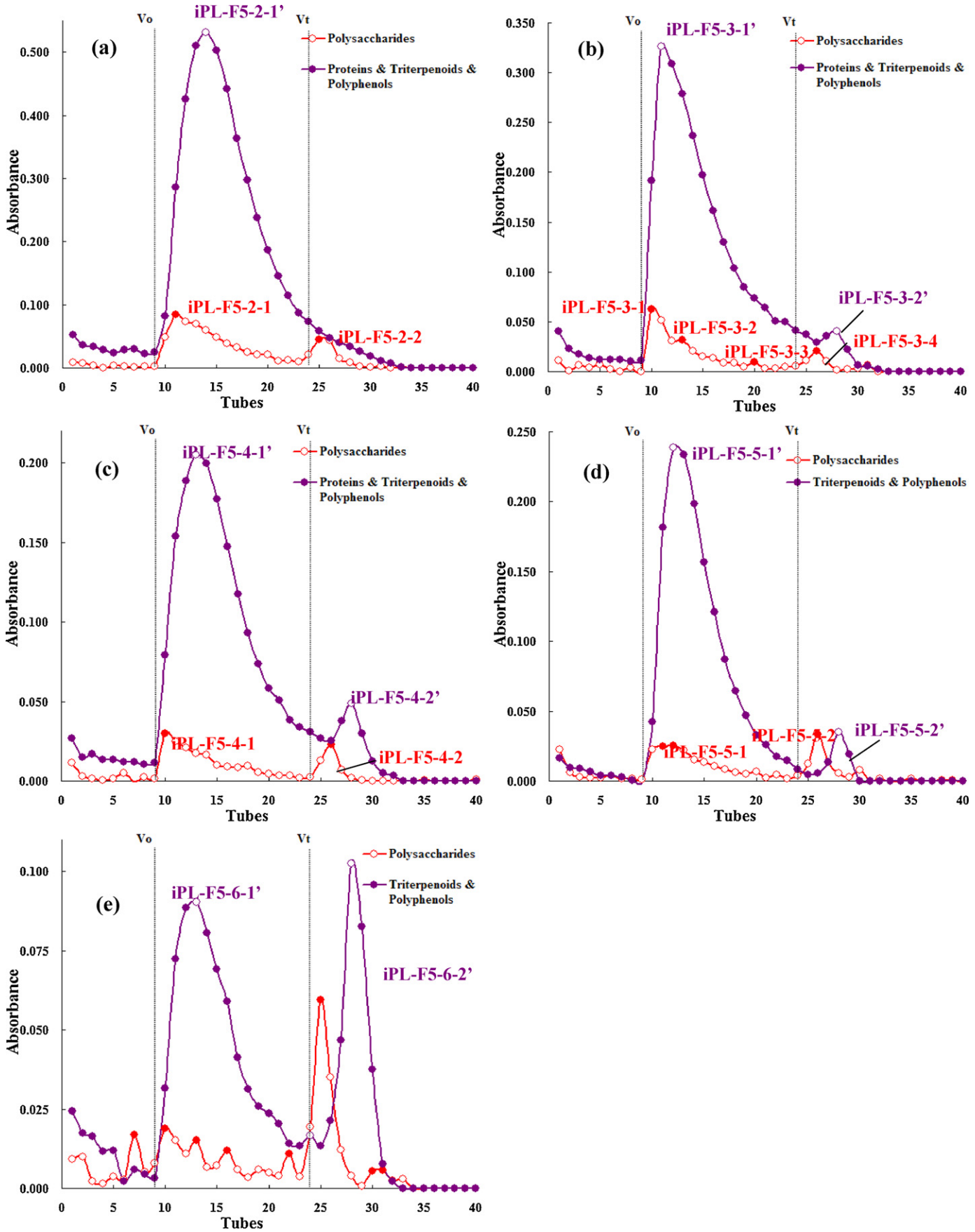

Fig. 3. (a) iPL-F5-2, (b) iPL-F5-3, (c) iPL-F5-4 (d) iPL-F5-5 and (e) iPL-F5-6 profile on Sephadex^{TM} G-50. The column was eluted with 0.1 M Na_2HPO_4 (pH 9.0) at a flow rate of 0.3 mL/min.

Table 2
Sugar composition of polysaccharide fractions.

Samples	Relative percentage (%)[b]						Reference
	Man	Rha	Glc	Gal	Ara	Xyl	
iPL-F5-2-1	21.76	6.48	32.87	25.46	8.80	4.63	In this study
iPL-F5-4-1	11.82	N.D.	57.27	N.D.	21.82	9.09	In this study
iPL-F5-5-1	7.09	N.D.	37.59	41.13	14.18	N.D.	In this study
ePS-F2-1	18.75	26.04	26.04	18.75	10.42	N.D.	Yang et al. (2012)
ePS-F3-1	11.17	5.08	35.03	36.04	N.D.	12.69	Yang et al. (2012)
ePS-F4-1	28.00	N.D.	39.00	23.00	N.D.	10.00	Yang et al. (2012)
ePSP-Cv[a]	8.18	N.A.	82.19	8.72	N.D.	0.90	Lin et al. (2008)
ePSP-LBE[a]	1.90	N.A.	80.08	9.30	N.D.	8.73	Lin et al. (2008)
PSP-Lb[b]	N.D.	N.A.	49.15	17.17	32.38	1.30	Lin et al. (2008)

N.D. = not detected; N.A. = not analyzed.
[a] ePSP-Cv, ePSP-LBE, and PSP-Lb are the crude polysaccharides.
[b] Based of the all analyzed monosaccharides.

between the monosaccharide and polysaccharide fractions and α-glucosidase inhibition was investigated (Table 2). The results of Sephadex™ G-50 separation showed that the total relative percentage of D-glucose and D-galactose linearly correlated with α-glucosidase inhibitory activity. For example, iPL-F5-2-1 (IC_{50} 1.7 mg/mL; 58.33%: total relative percentage of D-glucose and D-galactose), iPL-F5-4-1 (IC_{50} 1.8 mg/mL; 57.27%: total relative percentage of D-glucose and D-galactose), iPL-F5-5-1 (IC_{50} 0.8 mg/mL; 78.72%: total relative percentage of D-glucose and D-galactose), and ePS-F4-1 (IC_{50} 1.0 mg/mL; 62.00%: total relative percentage of D-glucose and D-galactose) (Yang et al., 2012) contained high, total relative percentages of D-glucose and D-galactose and also possessed greater α-glucosidase inhibitory activity.

3.5. Infrared spectral analysis

The infrared (IR) spectra of the purified iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 fractions were found to be similar (Fig. 4); the spectra were recorded at absorbances ranging from 4000 to 400 cm^{-1}. The band between 3400 cm^{-1} and 3300 cm^{-1} represents the stretching vibration of O—H bonds in constituent sugar residues. The band between 3250 cm^{-1} and 3100 cm^{-1} is associated with

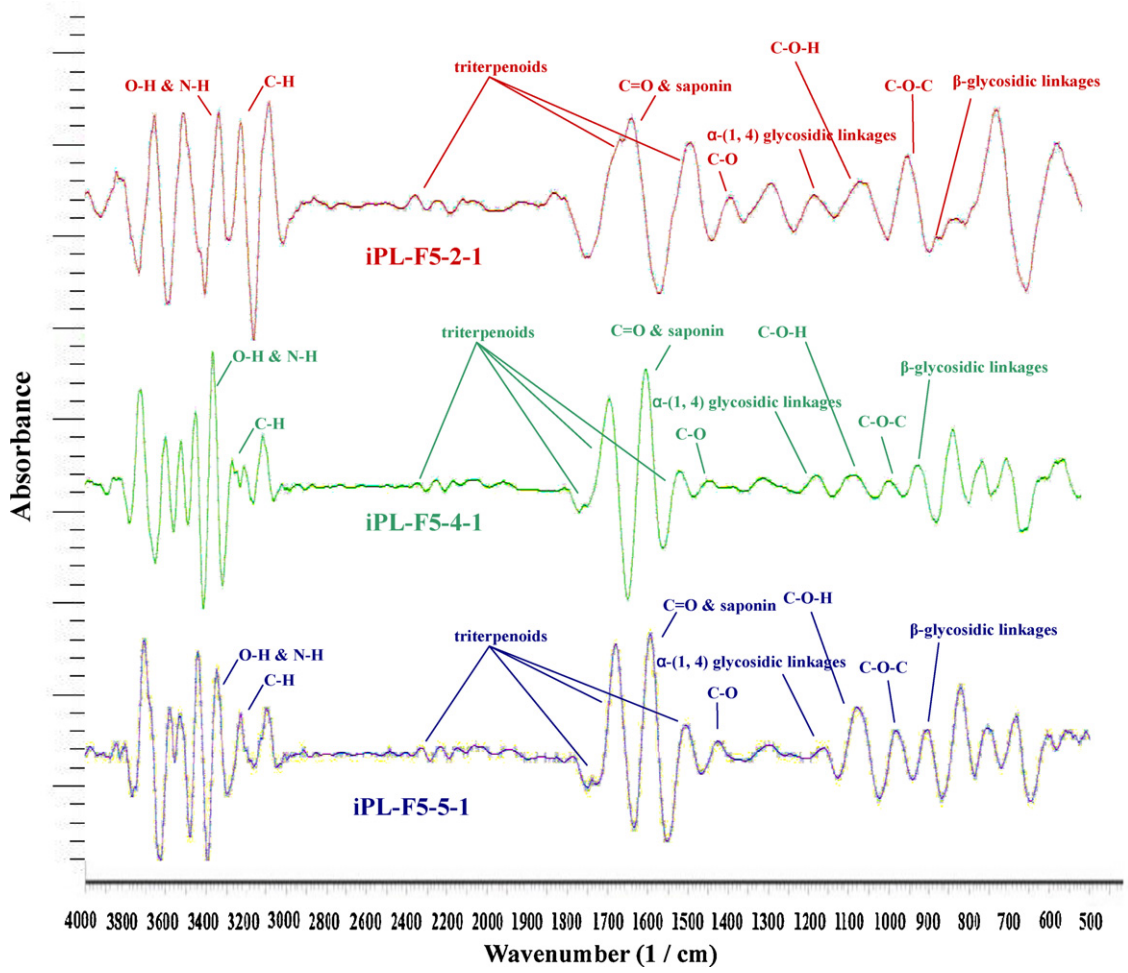

Fig. 4. FTIR spectrum of iPL-F5-fractions.

the stretching vibration of C—H bonds. The characteristic absorption band that appeared between 1700 cm^{-1} and 1600 cm^{-1} was assigned to the stretching of C=O bonds of iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 (Sun, Fang, Goodwin, Lawther, & Bolton, 1998), whereas the band between 1450 cm^{-1} and 1400 cm^{-1} is associated with the stretching vibration of C—O bonds (Yang et al., 2008). The prominent band between 1035 cm^{-1} and 1100 cm^{-1} is representative of C—O—C and C—O—H bonds. The absorption bands between 935 cm^{-1} and 900 cm^{-1} in the FTIR spectra may be associated with β-glycosidic linkages between the sugar units (Yang et al., 2008). The absorption bands at 1151 cm^{-1} in the FTIR spectra may correspond to the antisymmetric α-(1,4) glycosidic linkages stretching between the sugar units (Sekkal, Dincq, Legrand, & Huvenne, 1995). This absorption bands shows correlated on the structure of polysaccharide-triterpenoids with their glycosidic bonds [α-(1,4) glucosidic linkages] for α-glucosidase inhibition.

Acarbose is the competitive type of α-glucosidase inhibition on Baker's yeast α-glucosidase (Kim et al., 1999). It is a pseudotetrasaccharide consisting of an unsaturated cyclitol unit linked α-1,4 to 4-amino-4,6-dideoxy-D-glucopyranose that is attached α-1,4 to maltose (Kim et al., 1999). Owing to the α-glucosidase acting upon the α-1,4-glucosidic linkages (Lee, He, & Withers, 2001), the mechanism of α-glucosidase inhibition analysis of Acarbose is to compete for the activity seat of α-glucosidase with ρ-nitrophenyl-α-D-glucopyranoside (PNPG) (Guo, Jiang, Lv, & Wang, 2010; Lee et al., 2001). For the aforementioned reason, it suggested that the saccharides chain which is presented α-1,4-glucosidic linkages is to compete the activity seat of α-glucosidase with PNPG in the mechanism of α-glucosidase inhibition. Therefore, the correlation between the structure of polysaccharide triterpenoids and their inhibitory ability might result from the α-1,4-glucosidic linkages in the polysaccharides (iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1), which might confer a competitive type of α-glucosidase inhibition.

IR absorption spectra were obtained for the three polysaccharides (iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1). Compared to the spectrum of the ursolic acid standard (Yang et al., 2012), the polysaccharides in this study showed stronger absorption bands between 1800 cm^{-1} and 1700 cm^{-1}. The absorption bands between 1700 cm^{-1} and 1600 cm^{-1}, 1550 cm^{-1} and 1450 cm^{-1}, and 2450 cm^{-1} and 2300 cm^{-1} are typical of triterpenoids (Yang et al., 2012). The presence of bands between 3356 cm^{-1} and 3361 cm^{-1} indicate the presence of saponins, with characteristic overlap of O—H and N—H bond stretching (Liu et al., 2011). The strong absorption bands between 1600 cm^{-1} and 1500 cm^{-1} may indicate of the presence of saponins (glycyrrhizinate) (Suo, Sun, & Wang, 2010).

Because of their physical characteristics, triterpenoids are insoluble in water. However, we had discovered that triterpenoids were soluble in the water (liquor samples). We consider that the triterpenoids, polyphenol compounds, and polysaccharides are bound to each other, and thus the triterpenoids can be dissolved in the water extract. After purification on a Sephadex™ G-50 column, the proteins in iPL-F5-2-1 and iPL-F5-4-1 were removed and the residual triterpenoids and polyphenol compounds were bound to polysaccharides. As shown in previous studies, saponin may be present in the PSK samples (Yang et al., 2012), iPL-fractions, iPL-F4-fractions, and iPL-F5-fractions. This indicates that iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 are saponins. The above results are consistent with the spectrophotometric analysis.

4. Conclusions

In this work, three polysaccharide fractions (iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1) that demonstrated strong α-glucosidase inhibition (iPL-F5-5-1 shows the strongest activity) were separated and purified from an extract of *C. versicolor* LH1 mycelia using a DEAE-Sepharose™ CL-6B anion-exchange column, a Diaion® HP20 macroporous adsorption column, and a Sephadex™ G-50 column. Results from spectrophotometric analysis and infrared spectra analysis indicated that iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 contained polysaccharides, triterpenoids, and polyphenol compounds, and are therefore considered to be saponins; polysaccharides are a component of saponins. The MW of iPL-F5-2-1 was 9.2 ± 0.1 kDa, and its monosaccharide complement consisted of D-mannose, L-rhamnose, D-glucose, D-galactose, D-xylose, and D-arabinose. The MW of iPL-F5-4-1 was 11.3 ± 0.4 kDa, and its monosaccharide complement consisted of D-mannose, D-glucose, D-xylose, and D-arabinose. The molecular weight of iPL-F5-5-1 was 14.6 ± 0.1 kDa, and its monosaccharide complement consisted of D-mannose, D-glucose, D-galactose, and D-arabinose. According to IR spectra analysis and chemical characterization, iPL-F5-2-1, iPL-F5-4-1, and iPL-F5-5-1 are saponins.

The purified saponin fraction (iPL-F5-5-1) exhibited the strongest α-glucosidase inhibition, and had a higher inhibitory activity than PSK, the crude iPL extract, or the other isolated fractions; its activity approached that of Glucobay. Moreover, after analyzing the relationship between the monosaccharides of the polysaccharide fractions and α-glucosidase inhibition after Sephadex™ G-50 separation and FTIR spectral analysis, it was concluded that α-(1,4) glycosidic linkages and the total relative percentages of D-glucose and D-galactose are the important factors in α-glucosidase inhibition.

In this study, we used a physical method (two-stage separation and purification strategy) instead of the Sevag method to obtain purified polysaccharides from the extracts of *C. versicolor* LH1 mycelia. Throughout the experimental procedures, we successfully used the chemical properties of proteins, triterpenoids, and polyphenol compounds to permit fractionation by ion-exchange and macroporous adsorption resin chromatography. The principal findings are that the glycosidic bonds (α-1,4-glucosidic linkages) and the total percentages of D-glucose and D-galactose in the polysaccharides have significant linear correlations with α-glucosidase inhibitory activity. In conclusion, our research suggests that the two-stage separation and purification strategy for polysaccharides from *C. versicolor* LH1 mycelia can substitute for the Sevag method. Furthermore, the iPL fractions have potential to treat diabetes because of their α-glucosidase inhibitory activity.

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References

Chan, S. L., & Yeung, J. H. K. (2006). Effects of polysaccharide peptide (PSP) from *Coriolus versicolor* on the pharmacokinetics of cyclophosphamide in the rat and cytotoxicity in HepG2 cells. *Food and Chemical Toxicology*, *44*, 689–694.

Chen, X., Jin, J., Tang, J., Wang, Z., Wang, J., Jin, L., et al. (2011). Extraction, purification, characterization and hypoglycemic activity of a polysaccharide isolated from the root of *Ophiopogon japonicus*. *Carbohydrate Polymers*, *83*, 749–754.

Chen, X., Zhong, H. Y., Zeng, J. H., & Ge, J. (2008). The pharmacological effect of polysaccharides from *Lentinus edodes* on the oxidative status and expression of VCAM-1mRNA of thoracic aorta endothelial cell in high-fat-diet rats. *Carbohydrate Polymers*, *74*, 445–450.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Emberger, G. (2008). *Fungi growing on wood*. Messiah College. http://www.messiah.edu/Oakes/fungi_on_wood/index.htm

Fan, J. P., & He, C. H. (2006). Simultaneous quantification of three major bioactive triterpene acids in the leaves of *Diospyros kaki* by high-performance liquid

chromatography method. *Journal of Pharmaceutical and Biomedical Analysis*, *41*, 950–956.
Gaxiola, G., Cuzon, G., Garcia, T., Taboada, G., Brito, R., & Chimal, M. E. (2005). Factorial effects of salinity, dietary carbohydrate and moult cycle on digestive carbohydrates and hexokinases in *Litopenaeus vannamei*. *Comparative Biochemistry and Physiology*, *140*, 29–39.
Geng, D. F., Jin, D. M., Wu, W., Fang, C., & Wang, J. F. (2011). Effect of alpha-glucosidase inhibitors on the progression of carotid intima-media thickness: A meta-analysis of randomized controlled trials. *Atherosclerosis*, *218*, 214–219.
Guo, L. P., Jiang, T. F., Lv, Z. H., & Wang, Y. H. (2010). Screening α-glucosidase inhibitors from traditional Chinese drugs by capillary electrophoresis with electrophoretically mediated microanalysis. *Journal of Pharmaceutical and Biomedical Analysis*, *53*, 1250–1253.
Hamanaka, T., Sakasegawa, Y., Ohmoto, A., Kimura, T., Ando, T., & Doh-ura, K. (2011). Anti-prion activity of protein-bound polysaccharide K in prion-infected cells and animals. *Biochemical and Biophysical Research Communications*, *405*, 285–290.
Kim, M. J., Lee, S. B., Lee, H. S., Lee, S. Y., Baek, J. S., Kim, D., et al. (1999). Comparative study of the inhibition of α-glucosidase, α-amylase, and cyclomaltodextrin glucanosyltransferase by Acarbose, Isoacarbose, and Acarviosine-Glucose. *Archives of Biochemistry and Biophysics*, *371*(2), 277–283.
Lachman, J., Hosnedl, V., Pivec, V., & Orsak, M. (1998). Polyphenols in cereals and their positive and negative role in human and animal nutrition. In *Proceedings of the conference cereals for human health and preventive nutrition* Czech Republic, Brno 7–11, July, (pp. 118–125).
Lai, C. H., Teng, J. F., Hsu, T. H., Lin, F. Y., Yang, P. W., & Lo, H. C. (2011). 28-Day oral safety evaluation of extracellular polysaccharopeptides produced in submerged culture from the turkey tail medicinal mushroom *Trametes versicolor* (L.:Fr.) Pilát LH-1 in mice. *International Journal of Medicinal Mushroom*, *13*(3), 227–236.
Lee, S. S., He, S., & Withers, S. G. (2001). Identification of the catalytic nucleophile of the family 31 α-glucosidase from *Aspergillus niger* via trapping of a 5-fluoroglycosyl–enzyme intermediate. *Biochemical Society*, *359*, 381–386.
Li, X. M., & Xu, L. Z. (1987). Comparison of anti-cancer effect of two polysaccharopeptide from *Coriolus versicolor* in vitro. *Acta Academiae Medicinae Shanghai*, *14*(5), 326–329.
Lin, F. Y., Lai, Y. K., Yu, H. C., Chen, N. Y., Chang, C. Y., Lo, H. C., et al. (2008). Effects of *Lycium barbarum* extract on production and immunomodulatory activity of the extracellular polysaccharopeptides from submerged fermentation culture of *Coriolus versicolor*. *Food Chemistry*, *110*, 446–453.
Lin, X., Jia, J. F., Huang, L. J., & Wang, Z. F. (2006). Study on the use of RP-HPLC in analysis of aloe polysaccharide composition. *Food Science*, *27*(4), 192–195.
Liu, Z. F., Zeng, G. M., Zhong, H., Yuan, X. Z., Jiang, L. L., Fu, H. Y., et al. (2011). Effect of saponins on cell surface properties of *Penicillium simplicissimum*: Performance on adsorption of cadmium (II). *Colloids and Surfaces B: Biointerfaces*, *86*, 364–369.
Lu, F., Li, B., Suo, X. M., & Li, B. (2010). Research advances in hypoglycemic activity of edible fungi. *Food Research and Development*, *31*(2), 189–192.
Lu, Y. Y., Ao, Z. H., Lu, Z. M., Xu, H. Y., Zhang, X. M., Dou, W. F., et al. (2008). Analgesic and anti-inflammatory effects of the dry matter of culture broth of *Termitomyces albuminosus* and its extracts. *Journal of Ethnopharmacology*, *120*, 432–436.
Lv, Y., Yang, X. B., Zhao, Y., Ruan, Y., Yang, Y., & Wang, Z. Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection. *Food Chemistry*, *112*, 742–746.
Mbanya, J. C., Gan, D., & Allgot, B. (2006). *Diabetes atlas* (3rd ed.). Belgium: International Diabetes Federation.
Ng, T. B. (1998). A review of research on the protein-bound polysaccharide (polysaccharopeptide, PSP) from the mushroom *Coriolus versicolor* (Basidiomycetes: polyporaceae). *General Pharmacology*, *30*, 1–4.
Ohnishi, S. T., & Barr, J. K. (1978). A simplified method for quantitating protein using the Biuret and phenol reagent. *Analytical Biochemistry*, *86*, 193–200.
Podolak, I., Galanty, A., & Sobolewska, D. (2010). Saponins as cytotoxic agents: A review. *Phytochemistry Reviews*, *9*(3), 425–474.
Qian, Z. I., Xu, M. F., & Tang, P. L. (1997). Polysaccharide peptide (PSP) restores immunosuppression induced by cyclophosphamide in rats. *The American Journal of Chinese Medicine*, *25*(1), 27–35.
Ren, B. Z., Dai, R. Z., & Li, B. B. (1993). Influence of PSP on immune function of tumor patients. *Chinese–German Journal of Clinical Oncology*, *20*(5), 348–349.
Sekkal, M., Dincq, V., Legrand, P., & Huvenne, J. P. (1995). Investigation of the glycosidic linkages in several oligosaccharides using FT-IR and FT Raman spectroscopies. *Journal of Molecular Structure*, *349*, 349–352.
Sun, R., Fang, J. M., Goodwin, A., Lawther, J. M., & Bolton, A. J. (1998). Fractionation and characterization of polysaccharides from abaca fibre. *Carbohydrate Polymers*, *37*(4), 351–359.
Suo, J. X., Sun, S. Q., & Wang, W. Q. (2010). Application of FTIR spectroscopy to the identification of *Glycyrrhiza Uralensis Fisch*. *Spectroscopy and Spectral Analysis*, *30*(5), 1218–1223.
Wang, B. C., Chen, C. J., & Hua, J. (1998). *Cultivation and application of edible and medicinal mushrooms*. Taipei: Food Industry Research and Development Institute., pp. 112–187.
Wang, C. C., Chang, S. C., & Chen, B. H. (2009). Chromatographic determination of polysaccharides in *Lycium barbarum* Linnaeus. *Food Chemistry*, *116*, 595–603.
Wang, Y., & Chang, H. (2009). Research progress of α-glucosidase inhibitors. *Journal of Strait Pharmaceutical*, *21*(9), 4–6.
Wu, Q. P., Xiao, C., Yang, X. B., & Zhang, J. M. (2009). Hypoglycemic effects of components extracted from edible and medicinal Fungi and their mechanisms of action. *Acta Edulis Fungi*, *16*(3), 80–86.
Yang, C., He, N., Ling, X., Ye, M., Zhang, C., Shao, W., et al. (2008). The isolation and characterization of polysaccharides from longan pulp. *Separation and Purification Technology*, *63*, 226–230.
Yang, J. P., Hsu, T., Lin, F., Hsu, W., & Chen, Y. (2012). Potential antidiabetic activity of extracellular polysaccharides in submerged fermentation culture of *Coriolus versicolor* LH1. *Carbohydrate Polymers*, *90*, 174–180.
Zhang, J. S., Han, W. W., & Pan, Y. J. (2001). Studies on chemical structure of polysaccharide of *Coriolus Versicolor* (CVP) III. *Mycasystema*, *20*(4), 531–535.
Zhang, X., Yu, L., Bi, H., Li, X., Ni, W., Han, H., et al. (2009). Total fractionation and characterization of the water-soluble polysaccharides isolated from *Panax ginseng C. A. meyer*. *Carbohydrate Polymers*, *77*, 544–552.