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Comparison of the Biological Activity of Crude Polysaccharide Fractions Obtained from *Cedrela sinensis* Using Different Extraction Methods

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Four preparations of crude polysaccharides were obtained from *Cedrela sinensis* using four methods of extraction, and finally precipitation with ethanol. Hot-water and processes assisted by ultrasound and enzymes (Shearzyme or Viscozyme) were carried out. The yield of polysaccharide fraction extracted with the enzyme-assisted method using Shearzyme (P-EAS) was the highest at 10.53%. All crude saccharides were composed of six monosaccharides including arabinose, galactose, glucose, mannose, rhamnose, and xylose. Crude polysaccharide fraction extracted by hot water showed the highest DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging activities as well as α-amylase inhibitory activity. All fractions showed higher retardation effects on glucose and bile acid absorption using the *in vitro* dialysis system compared to the control (without polysaccharide fraction); specifically, the glucose retardation index of P-EAS was similar to that of carboxymethylcellulose. Overall, crude polysaccharide fractions obtained from *C. sinensis* can be used as functional ingredients owing to their excellent functional properties.

INTRODUCTION

Cedrela sinensis, which belongs to the Meliaceae family, is a tall tree that grows in Korea and China and has been used to treat enteritis, dysentery, and itch in oriental medicine [Oh *et al*., 2015]. Young *C. sinensis* leaves and sprouts have also been used as functional foods in Korean cuisine for decades. Tree buds, which appear in springtime, are known to contain an abundance of nutrients and antioxidant constituents, such as limonoids, flavonoids and other phenolic compounds, and phytol derivatives [Luo *et al*., 2000; Park *et al*., 1996]. In addition, the crude extract of *C. sinensis* was shown to have apoptosis-inducing effects on cancer cells as well as anti-obesity activity due to enhanced lipolysis of differentiated 3T3-L1 adipocytes [Hsu *et al*., 2003].

Polysaccharides, which are an abundant group of biopolymers, have recently attracted attention owing to their biological effects on living organisms and have been used in the pharmaceuitcal, feed, and food industries [Wang *et al*., 2012b]. Natural polysaccharides play important roles in numerous biological processes, such as cell–cell communication, embryonic development, and infection of bacteria. Polysaccharides are potentially natural pharmaceuticals and targets for drug design because they have specific biological activities, such as antibacterial activity, anticancer activity, and immunoenhancing effects [Luo *et al*., 2012]. Bioactive polysaccharides have also been widely studied as radical scavengers in the prevention of oxidative damage in human cells [Wang *et al*., 2012a].

In general, polysaccharides are bound to other components like protein, lipids, lignin, and some inorganic minerals in cells. Thus, polysaccharide extraction and isolation from various sources is the most important task in the investigation and application of bioactive polysaccharides. The extraction technique used may significantly influence the yield, physical characteristics, and biological activities of the polysaccharides. Hot-water extraction (HWE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction, enzyme-assisted extraction (EAE), maceration, and heat reflux with solvents are the main methods for polysaccharide extraction from natural resources [Fan *et al*., 2015]. HWE is traditionally used because it is a convenient, fast, environment-friendly, and economical technology [Liu *et al*., 2016]. UAE has been widely used to extract bioactive components from various plant materials based on mass transfer intensification, cell collapse, increased penetration, and capillary effects [Yin *et al*., 2016]. EAE can be used to release natural functional compounds from plant materials, owing to cell wall degradation that facilitates polysaccharide dissolution into the solvent [Pan *et al*., 2015].

In this study, crude polysaccharides were extracted from *C. sinensis* by various extraction methods and their health-related properties were measured in order to improve their availability to functional food materials. The crude polysaccharide fractions from *C. sinensis* were extracted using hot water, ultrasonic-assisted, and enzyme-assisted methods. Biological activities such as antioxidant activity, α-amylase in-

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hibitory activity, and glucose and bile acid retardation effects of the polysaccharides isolated using four extraction methods were compared in this study.

MATERIAL AND METHOD

Materials

C. sinensis was purchased from the National Agricultural Cooperative Federation (Sangju, Korea) and freeze-dried. It was ground to a fine power using a food mixer (M-1211; Starion, Busan, South Korea) and then stored in a deep freezer (MDF-435; Sanyo, Tokyo, Japan) at –40°C. Shearzyme Plus and Viscozyme L (Novozymes A/S, Bagsvaerd, Denmark) are both commercial enzymes which were used in this study for enzyme-assisted extraction.

Hot-water extraction (HWE)

A dried ground sample of *C. sinensis* (5 g) was mixed with 100 mL of distilled water in an Erlenmeyer flask. The mixture was placed in a shaking water bath (BS-11, JeioTech, Seoul, Korea) at 80°C for 3 h. After extraction, the suspension was filtered and centrifuged at 12,000×*g* for 20 min in a Supra-21K Centrifuge (Hanil, Incheon, Korea). The supernatant was collected and the residue was processed again twice as described above, and the extract was lyophilized with the use of FD-8512 Freeze Dryer (IlshinBioBase, Gyeonggi-do, Korea).

Ultrasonic-assisted extraction (UAE)

A ground sample of *C. sinensis* (5 g) was extracted with distilled water (1:20, w/v) in an Erlenmeyer flask (500 mL). The flask was placed in an ultrasonic bath (5510-DTH, Brason, Danbury, CT, USA), which was circulated by water, set at 45°C, and an ultrasound power of 495 W for 45 min. The mixture was centrifuged at 12,000×*g* for 20 min, and supernatant was collected and freeze-dried.

Enzyme-assisted extraction (EAE)

A dried ground sample of *C. sinensis* (5 g) was mixed with 100 mL of 0.1 N acetate buffer (pH 4.5), and 1% (v/w) of the enzyme preparation (Viscozyme or Shearzyme) was added to the mixture. The mixture was incubated in a shaking water bath (BS-11, JeioTech, Seoul, Korea) at 100 rpm and 50°C for 120 min, and the temperature was then rapidly increased to 100°C for 10 min to inactivate the enzyme. The reaction mixture was centrifuged at 12,000×*g* for 20 min, and supernatant was collected and freeze-dried.

Preparation of crude polysaccharide fractions

The crude polysaccharide fractions were separated from each extract obtained by different methods as previously described from He *et al*. [2016] with slight modification. The freeze-dried extract was dissolved in 95% ethanol and placed at room temperature for 5 h. The polysaccharide precipitate was obtained by centrifugation at 12,000×*g* for 20 min, washed three times with ethanol, and freeze-dried. Crude polysaccharide fractions obtained by HWE, UAE, and EAE using Shearzyme and Viscozyme were named as P-HW (crude polysaccharide fraction extracted by hot water), P-UA (crude polysaccharide fraction obtained by UAE), P-EAS (crude polysaccharide fraction obtained by EAE using Shearzyme), and P-EAV (crude polysaccharide fraction obtained by EAE using Viscozyme), respectively. The yield of crude polysaccharide fraction was calculated as a percentage of the weight of *C. sinensis* powder used in the extraction.

Monosaccharide composition

Carbohydrate analysis was performed as described by Gulbrandsen *et al*. [2015] with slight modification. A polysaccharide fraction (0.2 g) was hydrolyzed with 3 mL of 72% H_2SO_4 at 45°C for 1 h and then diluted with 84 mL of distilled water. The hydrolysates were rehydrolyzed at 121°C and neutralized to pH 7 with 2 N sodium hydroxide solution. The resulting supernatant was filtered (0.2 μm filter). Thus obtained sample was analyzed by a high-performance anion-exchange chromatography system (HPAEC, Dionex ICS-5000, Thermo Scientific, Sunnyvale, CA, USA) equipped with a CarboPac PA-1 column (250 × 4 mm, Dionex, Thermo Scientific). The monosaccharides were eluted from column with 18 mM NaOH at a flow rate of 1.0 mL/min. Arabinose, galactose, glucose, mannose, rhamnose, and xylose used as standards were purchased from Sigma Chemical Co. (USA). The content of each monosaccharide was calculated from the calibration curve obtained using each standard.

Antiradical activity – DPPH assay

The ability of crude polysaccharide fraction to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined as described by Kim *et al.* [2013]. Each sample (0.5 mL) was mixed with 1 mL of 0.2 mM DPPH solution in a test tube. The mixtures were then vortexed and incubated for 20 min at 37°C and DPPH radical scavenging activity was calculated as a percentage of DPPH discoloration by measuring the absorbance at 520 nm (U-2900, Hitachi, Tokyo, Japan).

Antiradical activity – ABTS assay

The ability of crude polysaccharide fraction to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radicals was measured using the method described by Kim *et al.* [2013]. Each extract (50 μL) was added to 3 mL of $ABTS^{\bullet+}$ solution, which was prepared by mixing 0.7 mM $ABTS^{\bullet+}$ solution and 2.45 mM potassium persulfate. The mixtures were incubated in the dark for 6 min at room temperature, and absorbance was then measured at 734 nm using a spectrophotometer (U-2900, Hitachi, Tokyo, Japan). The $ABTS^{\bullet+}$ radical scavenging activity was calculated as follows:

$$ABTS^{\bullet+} \text{ radical scavenging activity } (\%) = = (1\text{-}A_{sample}/A_{control}) \times 100 \quad (1)$$

where: A_{sample} is the absorbance with the test sample and A_{blank} is the absorbance with distilled water.

α-Amylase inhibitory activity

α-Amylase inhibitory activity of crude polysaccharide fraction was determined according to Telagari & Hullatti [2015], with some modifications. The reaction time and temperature were changed accordingly due to the increase in the amount of sample solution. In other words, a reaction mixture con-

taining 40 μL of the polysaccharide solution, at varying concentrations, 50 μL phosphate buffer (100 mM, pH 6.8), and 100 μL α-amylase (1 unit/mL; Sigma Chemical Co.) was preincubated at 20°C for 10 min. Then, 100 μL of 1% soluble starch (100 mM phosphate buffer, pH 6.8) was added as a substrate and the mixture was further incubated for 10 min at 20°C. DNS color reagent solution (100 mL; 96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium phosphate tartrate in 2 M NaOH) was then added and the mixture was boiled at 90°C for 15 min. Finally, 900 μL of distilled water was added and the mixture was vortexed. The absorbance was measured at 540 nm using a microplate reader (EPOCH, BioTek Instrument, Inc., Winooski, VT, USA). Acarbose (Sigma Chemical Co.) at various concentrations was used as a positive control. The change of absorbance was monitored before and after incubation. Percent inhibitory activity was calculated using the following formula:

$$\text{Inhibitory activity (\%)} = (1\text{-}A_{sample}/A_{blank}) \times 100 \quad (2)$$

where: A_{sample} is the absorbance with the test sample and A_{blank} is the absorbance with distilled water.

Determination of the glucose retardation index (GRI)

GRIs were evaluated as previously described by Im & Yoon [2015]. Dialysis membrane (Sigma, D7884), with a cut off molecular weight of 1,200 Da, was immersed in a 0.1% sodium azide solution for 10 h and filled with 6 mL of 0.1% sodium azide solution containing 36 mg of glucose with or without (control) 0.2 g of the polysaccharide fraction; each sample had previously been hydrated in 0.1% sodium azide solution for 14 h. Each bag was transferred into a reservoir containing 100 mL of a 0.1% sodium azide solution, and dialysis was performed at 37°C for 2 h. Dialysate (1 mL) was collected after 30, 60, 90, and 120 min in order to determine the glucose content. Glucose content in the dialysate was analyzed using the dinitrosalicylic acid (DNS) method previously described by Miller [1959]. The GRI values were then calculated according to the following equation (3):

$$\text{GRI value (\%)} = 100 - \left(\frac{\text{Total glucose diffused from bag with crude polysaccharide}}{\text{Total glucose diffused from bag without crude polysaccharide}} \right) \times 100 \quad (3)$$

To compare the effect of crude polysaccharide fractions on glucose with standard dietary fiber, carboxymethylcellulose (CMC) (Sigma Chemical Co.) was used as a positive control. Sodium azide solution containing glucose without the polysaccharide fraction was used as a negative control.

Determination of bile acid retardation index (BRI)

Bile acid dialysis retardation indices (BRIs) were used to monitor the effect of crude polysaccharide fractions on sterol metabolism. BRI values were measured using the same method as that used for GRIs described above [Im & Yoon, 2015]. Dialysis bags were loaded with phosphate buffer (50 mM, pH 7.0), containing 0.1% sodium azide and 15 mM taurocholic acid (control) or 0.2 g of sample, and hydrated for 14 h in buffered taurocholate-containing solution. Bags were transferred into reservoirs containing phosphate buffer (50 mM, pH 7.0) with 0.1% sodium azide and dialysis was performed at 37°C for 5 h with stirring. Dialysate (2 mL) was collected after 30 min, and 1, 2, and 5 h in order to determine bile acid content. Bile acid content in the dialysate was determined by measuring taurocholic acid content as described by Boyd *et al*. [1966]. BRI values were calculated using the following equation (4):

$$\text{BRI value (\%)} = 100 - \left(\frac{\text{Total bile acid diffused from bag with crude polysaccharide}}{\text{Total bile acid diffused from bag without crude polysaccharide}} \right) \times 100 \quad (4)$$

To compare the effect of crude polysaccharide fractions on bile acid with standard dietary fiber, carboxymethylcellulose (CMC) (Sigma Chemical Co.) was used as a positive control. Sodium azide solution containing taurocholic acid without the polysaccharide fraction was used as a negative control.

Statistical analysis

Results were expressed as mean ± standard deviations of triplicate experiments. Multivariate analysis of variance (ANOVA) was performed using SPSS ver. 18.0 (Chicago, IL, USA). Significant differences between mean values were identified using Duncan's multiple range test. The level of significance was at $P<0.05$.

RESULTS AND DISCUSSION

Yield of crude polysaccharide fractions and their monosaccharide compositions

The yield of crude polysaccharide fractions obtained by the four extraction methods and composition of polysaccharides are shown in Table 1. Yields of P-HW, P-UA, P-EAS, and P-EAV were 9.7±2.1, 7.7±1.8, 10.5±1.3, and 6.0±0.3%, respectively. The significantly higher yield of P-EAS than that of P-EAV ($P<0.05$) suggested that Shearzyme degraded the cell wall of *C. sinensis* more efficiently than Viscozyme. Shearzyme, from *Aspergillus oryzae*, mainly shows endo-1,4--β-xylanase activity and may also have cellulase, xylanase, and β-glucanase activities, while Viscozyme is a multifunctional enzyme that contains hemicellulase, β-gluconase, arabinase, and xylanase [Oh *et al*., 2015].

All crude saccharides were composed of six monosaccharides, including rhamnose, arabinose, galactose, glucose, mannose, and xylose. Galactose content was the highest in the polysaccharides (24.6±2.8, 25.8±1.2, 25.4±2.4, and 24.0±0.6 g/100 g of P-HW, P-UA, P-EAS, and P-EAV, respectively), followed by arabinose (17.0±3.6, 15.2±0.8, 21.4±2.8, and 12.4±0.2 g/100 g of P-HW, P-UA, P-EAS, and P-EAV, respectively), with xylose content being the lowest. The major monosaccharides of polysaccharides extracted from *Hohenuehelia serotine* using the same extraction methods as in the present study, were glucose and mannose

TABLE 1. The yield of crude polysaccharide fractions of *C. sinensis* and monosaccharide composition of polysaccharides.

Fraction	Yield (%)	Monosaccharide composition (g/100 g)					
		Arabinose	Galactose	Glucose	Mannose	Rhamnose	Xylose
P-HW	9.7±2.1^{a}	17.0±3.6^{bB}	24.6±2.8^{bA}	14.6±1.6^{aB}	1.6±0.4^{aC}	2.4±0.3^{cC}	0.2±0.0^{bC}
P-UA	7.7±1.8^{ab}	15.2±0.8^{cB}	25.8±1.2^{aA}	2.8±0.1^{cC}	1.4±0.1^{D}	2.6±0.2^{bC}	0.2±0.0^{bE}
P-EAS	10.5±1.3^{a}	21.4±2.8^{aB}	25.4±2.4^{abA}	2.8±0.8^{cCD}	0.6±0.3^{bD}	5.2±0.4^{cC}	0.1±0.0^{cD}
P-EAV	6.0±0.3^{b}	12.4±0.2^{dB}	24.0±0.6^{bA}	3.2±0.4^{bD}	0.8±0.2^{bE}	7.2±0.2^{aC}	0.4±0.1^{aE}

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasonic-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extract using Shearzyme; P-EAV, crude polysaccharide fraction after enzyme-assisted extraction using Viscozyme.

Data are expressed as the mean ± standard deviation (n=3). Values with different small letters in the same column are significantly different ($P<0.05$). Values with different capital letters in the same row are significantly different ($P<0.05$).

[Li & Wang, 2016], which was inconsistent with the present results. Zhu *et al*. [2016] reported that polysaccharides from *Cordyceps gunnii* mycelia were mainly composed of mannose, glucose, and galactose, with galactose content being the highest regardless of the extraction method used, which was consistent with the results of the present study. These results suggest that the different methods of extraction did not affect monosaccharide composition of the crude polysaccharide fractions, although there was a slight difference in monosaccharide contents. The results also suggest the cell wall of *C. sinensis* is mainly composed of arabinogalactan. Arabinogalactans are one of the major components of the plant cell wall and are present in a wide range of plants [Bartels *et al*., 2017]. As a result of their potent biological activity, immunopotentiation properties, and unique solution properties, this peculiar dietary fiber has received increased attention as a clinically useful nutraceutical material [Kelly, 1999].

Antioxidant activity

Numerous methods are used to evaluate antioxidant activities of natural compounds in foods or biological systems. ABTS and DPPH assays are commonly used to assess antioxidant activity of phenolic compounds as well as natural plant extracts *in vitro* [Shalaby & Shanab, 2013; Li *et al*., 2017]. Both assays were also successfully used to determine the antioxidant activity of polysaccharide fractions isolated from edible plants [Rout & Banerjee, 2007; Lin *et al*., 2009].

DPPH assay was used to determine the proton-scavenging activity of the polysaccharide fractions (P-HW, P-UA, P-EAS, and P-EAV) extracted from *C. sinensis* using the four methods. The dose-response curves for the four tested samples are shown in Figure 1A. P-HW showed the highest radical scavenging activity at all concentrations. At a concentration of 800 μg/mL, the DPPH radical scavenging activities of P-HW, P-UA, P-EAS, and P-EAV were 62.3±1.2, 47.7±1.7, 55.8±3.9, and 50.3±3.6%, respectively. Fu *et al*. [2010] reported that polysaccharides extracted with hot water showed higher DPPH radical scavenging activity than those extracted by ultrasonic treatment, which was consistent with our results. This suggests that hot water treatment induces the extraction of substances with antiradical activity, such as polyphenols as well as soluble polysaccharides, and is consistent with find-

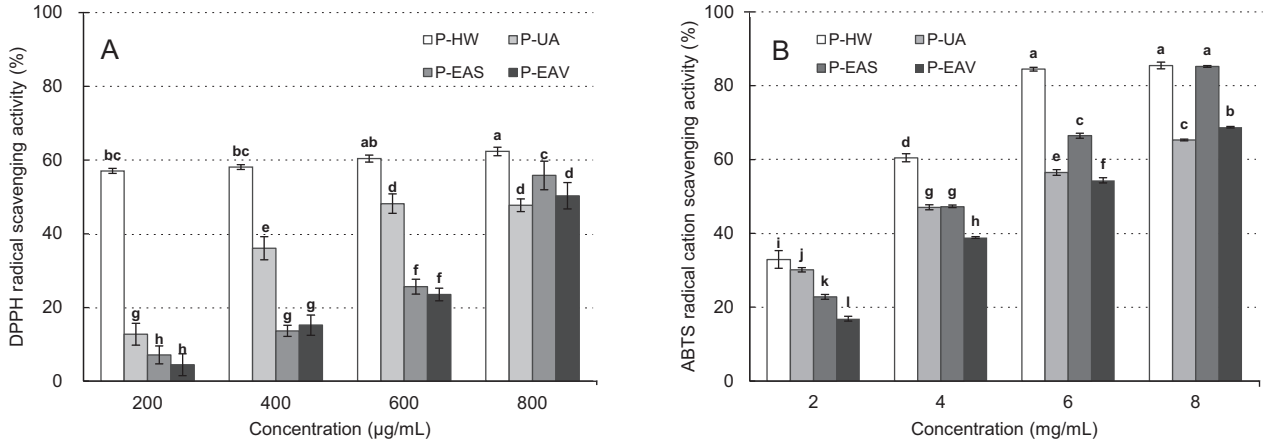

FIGURE 1. Antiradical activity of the crude polysaccharide fractions extracted from *C. sinensis* determined using DPPH (A) and ABTS assay (B).

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasonic-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extract using Shearzyme; P-EAV, crude polysaccharide fraction after enzyme-assisted extraction using Viscozyme.

The results represent mean and standard deviation (n=3). Values with different letters are significantly different ($P<0.05$).

ings of other authors [Oh & Yoon, 2017; Sangeethapriya & Siddhuraju, 2014].

The ABTS assay is a useful method for measuring the antioxidant activities of natural compounds in food or biological systems. Blue-green ABTS radical cation is decolorized by antioxidants and the extent of decolorization is proportional to the antioxidant activity. Figure 1B shows the scavenging activity towards ABTS radical cation. All crude polysaccharide fractions exhibited scavenging activity towards $ABTS^{\bullet+}$ at all tested concentrations in a dose-dependent manner. The $ABTS^{\bullet+}$ scavenging activity of all samples was more than 50% at 6 mg/mL; at a concentration of 8 mg/mL, $ABTS^{\bullet+}$ scavenging activities of P-HW, P-UA, P-EAS, and P-EAV were 85.5±0.9, 65.3±0.2, 85.2±0.2, and 68.8±0.2%, respectively. Among the four polysaccharide fractions, antioxidant activity of P-HW was significantly higher than that of the others at all concentrations (except for P-EAS at 8 mg/mL), while P-EAV showed the lowest radical scavenging activity at all concentrations tested. The results suggest that hot water is most effective at extracting functional substances which contribute to the $ABTS^{\bullet+}$ scavenging ability, which is in accordance with findings of other authors [Shalaby & Shanab, 2013].

The radical scavenging abilities of the polysaccharide fractions were generally related to the presence of reductones, which may react with free radicals by donating hydrogen atoms [Chen *et al*., 2012]. This suggests that the radical scavenging activity of the polysaccharides extracted from *C. sinensis*, particularly P-HW, may be attributable to a strong hydrogen-donating ability.

α-Amylase inhibitory activity

In the digestive tract, α-amylase enzyme starts the process of carbohydrate digestion by hydrolysis of 1,4-glycosidic bonds of alpha-linked polysaccharides (starch, glycogen) to maltose and glucose, leading to postprandial hyperglycemia. An α-amylase inhibitor is thus useful for controlling hyperglycemia as it delays carbohydrate digestion, reducing postprandial plasma glucose levels [Telagari & Hullatti, 2015]. Acarbose is an inhibitor of α-amylase and commonly used to decrease glucose absorbance by reducing the production of α-amylase in the small intestine. However, acarbose shows some disadvantages, such as an increased risk of gastrointestinal problems, inconvenient dosing, and high cost [Shori, 2015]. Many medicinal plants have been investigated for their ability to inhibit glucose production from carbohydrates in the intestine or glucose absorption in the gut [Horii *et al*., 1986].

The α-amylase inhibitory activity of the four polysaccharide fractions is shown in Table 2. The inhibitory activity of crude polysaccharides increased significantly at an increasing concentration of fractions; from 18.06±0.46––53.78±0.20% (0.5 mg/mL) to 52.26±0.23–86.92±0.58% (2.0 mg/mL). At all concentrations, P-HW showed the highest inhibitory activity among the samples; more specifically the activity of P-HW was 86.92±0.58% at a concentration of 2.0 mg/mL, which was equivalent to 95% of that of the positive control, acarbose.

Ou *et al*. [2001] reported that fibers from natural food could decrease postprandial blood glucose levels by hindering glucose diffusion, retarding glucose absorption, and inhibiting carbohydrate-hydrolyzing enzymes. Natural enzyme inhibitors may offer an effective strategy for treating postprandial hyperglycemia due to minimized abdominal side effects induced from excessive inhibition of α-glucosidase by synthetic drugs [Sangeethapriya & Siddhuraju, 2014]. Our results therefore indicate that polysaccharides obtained from *C. sinensis*, particularly P-HW, may decrease the α-amylase activity, and may thus be potential therapeutic agents for the management of postprandial hyperglycemia.

Inhibitory effect of crude polysaccharide fractions on glucose diffusion

The GRI is a valuable *in vitro* index for predicting the effect of dietary fiber on the retardation of glucose absorption in the gastrointestinal tract. Table 3 presents the effects of crude polysaccharide fractions extracted from *C. sinensis* by different methods on glucose diffusion relative to the effects of CMC and a control (without crude polysaccharide fraction). As dialysis time was extended from 30 to 120 min, glucose concentrations in the dialysates containing crude polysaccharide fractions increased from 5.32±0.50–5.48±0.45 mg/100 mL (at 30 min) to 16.68±1.52–23.22±1.08 mg/100 mL (at 120 min). Glucose concentrations in the dialysates of CMC and control reached 5.28±0.38 and 6.08±0.50 mg/100 mL (at 30 min) and 18.07±2.56 and 27.87±1.76 mg/100 mL (at 120 min), respectively. When compared with the control, all crude polysaccharide fractions significantly decreased the amount of diffused glucose in dialysate across the dialysis bag ($P<0.05$). In turn, for P-EAS (after 30 min of dialysis) and P-EAS and P-EAV (after 60 min of dialysis), and P-EAV (after 90 min of dialysis) there were no significant differences in glucose concentrations in the dialysates when compared to the CMC.

The GRIs of crude polysaccharides were 9.87–12.50% after 30 min of dialysis, and the GRI values of all samples except P-HW reached a maximum after 60 min of dialysis. GRI showed the highest value for P-EAS with 63.76% followed by CMC (52.16±0.63%), P-EAV (51.48±2.02%), P-UA (47.50±2.20%),

TABLE 2. α-Amylase inhibitory activity of crude polysaccharide fractions extracted from *C. sinensis*.

Fraction	Sample concentration (mg/mL)			
	0.5	1.0	1.5	2.0
P-HW	53.78 ± 0.20^{bD}	68.92 ± 0.20^{bC}	78.62 ± 0.30^{bB}	86.92 ± 0.58^{bA}
P-UA	18.06 ± 0.46^{eD}	39.38 ± 0.12^{dC}	49.20 ± 0.35^{dB}	53.85 ± 0.12^{dA}
P-EAS	39.38 ± 0.46^{cD}	42.63 ± 0.20^{cC}	54.12 ± 0.12^{cB}	60.69 ± 0.12^{cA}
P-EAV	31.27 ± 0.35^{dD}	33.40 ± 0.12^{eC}	42.90 ± 0.12^{eB}	52.26 ± 0.23^{eA}
Acarbose	75.83 ± 0.12^{aD}	79.81 ± 0.30^{aC}	82.94 ± 0.12^{aB}	91.70 ± 0.23^{aA}

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasonic-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extract using Shearzyme; P-EAV, crude polysaccharide fraction after enzyme-assisted extraction using Viscozyme.

Data are expressed as the mean ± standard deviation (n=3). Values with different small letters in the same column are significantly different ($P<0.05$). Values with different capital letters in the same row are significantly different ($P<0.05$).

TABLE 3. Retarding effect of crude polysaccharide fractions extracted from *C. sinensis* on dialysis membrane transport of glucose.

Fraction	Dialysis time (min)							
	30		60		90		120	
	Glucose in dialysate (mg/100 mL)	GRI (%)	Glucose in dialysate (mg/100 mL)	GRI (%)	Glucose in dialysate (mg/100 mL)	GRI (%)	Glucose in dialysate (mg/100 mL)	GRI (%)
Control	6.08±0.50^{a}	–	16.18±1.39^{a}	–	22.38±2.61^{a}	–	27.87±1.76^{a}	–
CMC	5.28±0.38^{c}	13.16±0.56^{a}	7.74±0.59^{d}	52.16±0.63^{b}	10.95±1.57^{e}	51.07±1.22^{a}	18.07±2.56^{d}	35.16±2.23^{a}
P-HW	5.45±0.63^{b}	10.36±0.98^{c}	13.85±1.41^{b}	14.40±0.96^{d}	16.29±2.36^{c}	27.21±4.52^{c}	20.74±0.91^{c}	25.58±1.78^{b}
P-UA	5.47±1.23^{b}	10.03±1.00^{cd}	11.75±4.60^{c}	47.50±2.20^{c}	18.78±9.65^{b}	16.09±7.18^{d}	23.22±1.08^{b}	16.68±1.52^{c}
P-EAS	5.32±0.50^{c}	12.50±0.23^{b}	8.11±1.05^{d}	63.76±1.12^{a}	14.1±1.82^{d}	37.00±2.05^{b}	20.70±1.46^{c}	25.73±2.65^{b}
P-EAV	5.48±0.45^{b}	9.87±0.02^{d}	7.85±1.17^{d}	51.48±2.02^{b}	11.36±1.60^{e}	49.24±3.45^{a}	20.19±1.16^{c}	27.56±3.25^{b}

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasonic-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extract using Shearzyme; P-EAV, crude polysaccharide fraction after enzyme-assisted extraction using Viscozyme; CMC, Carboxymethylcellulose.

Sodium azide solution containing glucose without the crude polysaccharide fraction was used as a control. GRI, Glucose retardation index. Data are expressed as the mean ± standard deviation (n=3). Values with different letters in the same column are significantly different (P<0.05).

and P-HW (14.40±0.96%) after 60 min of dialysis. The retardation effects of the sample decreased with longer dialysis time, but the GRI of P-HW increased steadily for up to 90 min of dialysis. After 120 min of dialysis, GRIs of crude polysaccharide fractions were 16.6–27.56% and the GRI of CMC was 35.16%. The GRI value of high dietary fiber powder from lime residues reached a maximum after 30 min of dialysis and decreased after a prolonged dialysis time [Peerajit *et al*., 2012], which was inconsistent with our results. This discrepancy may be due to differences in physical characteristics, such as water holding capacity, gelling property, and the viscosity of the solution. Crude polysaccharide fractions showed higher inhibitory effects on glucose diffusion compared to the control, although crude polysaccharide fractions retarded glucose diffusion less than CMC. A previous study showed that the delay of glucose diffusion and absorption by fiber is affected by viscosity of the intestinal digesta [Edwards *et al*., 1987] and that viscous polysaccharides may inhibit the intestinal uptake of nutrients by augmenting the apparent thickness of the unstirred water layer [Dongowski, 2007]. Based on these results, crude polysaccharide fractions, particularly P-EAS and P-EAV, are likely to dissolve and create network linkages in water, thereby retarding glucose diffusion and preventing glucose absorption in the gastrointestinal tract.

Inhibitory effect of crude polysaccharides on bile acid diffusion

Table 4 presents the effects of crude polysaccharide fractions extracted from *C. sinensis* by different methods on bile acid diffusion relative to the effects of CMC and a control

TABLE 4. Retarding effect of crude polysaccharide fractions extracted from *C. sinensis* on dialysis membrane transport of bile acid.

Fraction	Dialysis time (h)							
	0.5		1		2		5	
	Bile acid in dialysate (μmol/L)	BRI (%)	Bile acid in dialysate (μmol/L)	BRI (%)	Bile acid in dialysate (μmol/L)	BRI (%)	Bile acid in dialysate (μmol/L)	BRI (%)
Control	184.7±11.6^{a}	–	300.9±5.4^{a}	–	396.6±16.7^{a}	–	503.9±5.6^{a}	–
CMC	111.3±1.5^{c}	39.7±0.5^{a}	176.3±7.0^{c}	41.5±4.5^{a}	203.7±5.7^{c}	49.6±2.8^{a}	247.0±8.1^{d}	59.3±5.8^{a}
P-HW	174.7±10.0^{ab}	5.4±2.5^{c}	273.7±8.7^{a}	9.0±4.9^{c}	356.3±11.0^{b}	10.2±1.8^{b}	457.0±7.2^{c}	9.1±2.2^{b}
P-UA	181.7±3.2^{ab}	2.2±1.4^{c}	281.8±53.2^{a}	5.7±2.8^{c}	380.8±7.7^{ab}	4.0±1.7^{c}	481.0±5.6^{b}	4.6±2.1^{c}
P-EAS	179.3±14.6^{ab}	3.2±2.7^{c}	281.3±18.9^{a}	6.4±3.8^{c}	367.3±10.5^{b}	7.4±1.9^{b}	451.7±19.4^{c}	10.3±2.4^{b}
P-EAV	164.3±11.4^{b}	11.4±4.1^{b}	208.3±8.1^{b}	30.9±6.7^{b}	374.0±11.4^{b}	5.6±2.5^{bc}	477.0±6.0^{b}	5.4±1.9^{c}

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasonic-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extract using Shearzyme; P-EAV, crude polysaccharide fraction after enzyme-assisted extraction using Viscozyme; CMC, Carboxymethylcellulose

Sodium azide solution containing taurocholic acid without the crude polysaccharide fraction was used as a control. BRI, bile acid retardation index. Data are expressed as the mean ± standard deviation (n=3). Values with different letters in the same column are significantly different (P<0.05).

(without crude polysaccharide fraction). Taurocholic acid concentrations in the dialysate containing crude polysaccharide fractions ranged from 164.3±11.4–181.7±3.2 μmol/L after 30 min, to 451.7±19.4–481.0±5.6 μmol/L after 5 h. Taurocholic acid levels of the control and CMC in the dialysate were 184.7±11.6 and 111.3±1.5 μmol/L after 30 min of dialysis, and 503.9±5.6 and 247.0±8.1 μmol/L after 5 h of dialysis, respectively. Compared to the control, crude polysaccharide fractions reduced the amount of taurocholic acid that transferred into the dialysate, but only P-EAS significantly prevented the diffusion of bile acid out of the dialysis membrane.

The BRIs of crude polysaccharide fractions ranged from 2.2–11.4% after dialysis for 30 min. P-EAV showed the highest BRI value, which was however much lower than that of CMC (39.7%). The BRIs of P-EAS and CMC steadily increased during dialysis, with the BRIs of CMC and ASP being 59.6% and 10.3%, respectively, after 5 h of dialysis. In contrast, most samples, except of P-EAS, showed the maximum BRI after dialysis for 1 or 2 h, and the BRIs of P-HW, P-UA, and P-EAV were 9.0%, 5.7%, and 30.9%, after dialysis for 1 h respectively. Water-soluble dietary fibers produced from Chinese cabbage and buckwheat hulls by enzymatic hydrolysis also showed greater bile acid inhibitory effects than CMC [Im & Yoon, 2015; Park & Yoon, 2015]. In the present study, crude polysaccharide fractions showed slight absorption inhibitory effects and these observations are not consistent with those made in the previous studies.

CONCLUSIONS

In the presented study, we compared the antioxidant activity, α-amylase inhibitory activity, and retarding effects on dialysis membrane transport of glucose and bile acid of crude polysaccharide fractions extracted from *C. sinensis* using four extraction methods. Data from this study demonstrated the feasibility of producing polysaccharides with biological activity. Especially, the crude polysaccharide (P-HW) extracted by hot-water showed not only high yield but also DPPH and ABRS radical scavenging activities and α-amylase inhibitory activity. In addition, P-HW effectively hindered outward diffusion of glucose and bile acid through the dialysis membrane. Therefore, the crude polysaccharide fractions from *C. sinensis* are a practical new material with antioxidant activity, antidiabetic activity, and hypoglycemic effect that may be used in the food industry to prepare functional foods and nutraceutical products. It is undoubtedly comprehensible that a more detailed investigation for producing functional polysaccharides on a larger scale is needed to prove its values.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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