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

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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 pharmaceutical, 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-
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 (MD-435; Sanyo, Tokyo, Japan) at -40°C. Shearzime 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 (Hamil, 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, Brandon, 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 Shearzime) 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 modifications. 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 Shearzime 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 Shearzime), 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 modifications. A polysaccharide fraction (0.2 g) was hydrolyzed with 3 mL of 72% H2SO4 at 45°C for 1 h and then diluted with 84 mL of distilled water. The hydrolysates were rehydroyzed 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•⁺) radicals was measured using the method described by Kim et al. [2013]. Each extract (50 μL) was added to 3 mL of ABTS•⁺ solution, which was prepared by mixing 0.7 mM ABTS•⁺ 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•⁺ radical scavenging activity was calculated as follows:

\[
\text{ABTS}^{\text{•+}} \text{ radical scavenging activity (\%)} = (1 - \frac{A_{\text{sample}}}{A_{\text{control}}}) \times 100
\]

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

**α-Amylase inhibitory activity**  
α-Amylase inhibitory activity of crude polysaccharide fraction was determined according to Telagari & Hullati [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:

\[
\text{Inhibitory activity (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100
\]

where: \(A_{\text{sample}}\) is the absorbance with the test sample and \(A_{\text{blank}}\) 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 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 absorbance was monitored before and after incubation. Percent inhibitory activity was calculated using the following formula:

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\]

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

**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-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-glucanase, 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 *Hohenwetella serotina* using the same extraction methods as in the present study, were glucose and mannose.

To determine the BRI of the crude polysaccharide fractions and standard dietary fibers, 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):

\[
\text{BRI value (\%)} = 100 - \left(\frac{\text{Total bile acid diffused from bag}}{\text{Total bile acid diffused from bag with crude polysaccharide}} \times 100\right) \times 100
\]

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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (%)</th>
<th>Arabinose</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Mannose</th>
<th>Rhamnose</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-HW</td>
<td>9.7±2.1a</td>
<td>17.0±3.6ab</td>
<td>24.6±2.8Ab</td>
<td>14.6±1.6b</td>
<td>1.6±0.4c</td>
<td>2.4±0.3c</td>
<td>0.2±0.0c</td>
</tr>
<tr>
<td>P-UA</td>
<td>7.7±1.8d</td>
<td>15.2±0.8d</td>
<td>25.8±1.2a</td>
<td>2.8±0.1c</td>
<td>1.4±0.1d</td>
<td>2.6±0.2c</td>
<td>0.2±0.0a</td>
</tr>
<tr>
<td>P-EAS</td>
<td>10.5±1.3d</td>
<td>21.4±2.8d</td>
<td>25.4±2.4a</td>
<td>2.8±0.8c</td>
<td>0.6±0.3cd</td>
<td>5.2±0.4c</td>
<td>0.1±0.0d</td>
</tr>
<tr>
<td>P-EAV</td>
<td>6.0±0.3b</td>
<td>12.4±0.2b</td>
<td>24.0±0.6a</td>
<td>3.2±0.4cd</td>
<td>0.8±0.2b</td>
<td>7.2±0.2c</td>
<td>0.4±0.1d</td>
</tr>
</tbody>
</table>

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).

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-

[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].
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•⁺ at all tested concentrations in a dose-dependent manner. The ABTS•⁺ scavenging activity of all samples was more than 50% at 6 mg/mL; at a concentration of 8 mg/mL, ABTS•⁺ 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•⁺ scavenging activity, which is in accordance with findings of other authors [Chen et al., 2012].

The radical scavenging abilities of the polysaccharide fractions were generally related to the presence of reducing sugars, which may react with free radicals by donating hydrogens [Chen et al., 2014]. M.H. Ou & K.Y. Yoon (2013) reported that α-glucosidase 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-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%), and P-EAS (42.63±0.20%) for 30 min of dialysis. In the next 90 min of dialysis, 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%), and P-EAS (42.63±0.20%) for 120 min of dialysis.

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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Sample concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>P-HW</td>
<td>53.78±0.20b</td>
</tr>
<tr>
<td>P-UA</td>
<td>18.06±0.46b</td>
</tr>
<tr>
<td>P-EAS</td>
<td>39.38±0.46b</td>
</tr>
<tr>
<td>P-EAV</td>
<td>31.27±0.35b</td>
</tr>
<tr>
<td>Acarbose</td>
<td>75.83±0.12b</td>
</tr>
</tbody>
</table>

P-HW, crude polysaccharide fraction extracted by hot water; P-UA, crude polysaccharide fraction after ultrasound-assisted extraction; P-EAS, crude polysaccharide fraction after enzyme-assisted extraction 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).
and P-HW (14.40±0.96%) after 60 min of dialysis. The retardation effects of the sample decreased 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.
(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 effect 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, anti-diabetic 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.

REFERENCES


