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Potential antioxidant activities in vitro of polysaccharides extracted from ginger (*Zingiber officinale*)

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ABSTRACT

Polysaccharides extracted from ginger are a group of hetero polysaccharides, and their antioxidant activities were investigated employing various established in vitro systems. Available data obtained with in vitro models suggested that among the three samples, G2 (extract with acid solution) showed significant inhibitory effects on superoxide radical, hydroxyl and DPPH radical; its reducing power and iron(II) chelation activity was also the strongest. These results clearly establish the possibility that polysaccharides extracted from ginger could be effectively employed as an ingredient in health or functional food, to alleviate oxidative stress. However, comprehensive studies need to be conducted in experimental animal models.

1. Introdution

It has been recognized that polysaccharides from microorganisms or higher plants possess bioactivities, and the antioxidant activities are most deeply studied. Antioxidant activities mean inhibitory effects on reactive oxygen species (ROS), which are capable of causing damage to DNA. They have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Patel et al., 1999). Thus, it is essential to develop and utilize effective antioxidants which can protect the human body from free radicals and retard the progress of many chronic diseases (Nandita and Rajini, 2004). In our previous reports, the polysaccharides extracted from plants and seaweed has been found possessing antioxidant activities (Zhang et al., 2009).

Ginger (*Zingiber officinale*) has been used as a spice for over 2000 years (Bartley & Jacobs, 2000). Its roots and the obtained extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen, Kuo, Wu, & Ho, 1986; Herrmann, 1994). It has been shown previously that long term dietary feeding of ginger has hypoglycemic and hypolipidemic effects in rats and the antioxidant effect of this dietary constituent is as effective as ascorbic acid (Ahmed, Seth, & Banerjee, 2000; Ahmed & Sharma, 1997). Hypolipidemic and antiatherosclerotic effects of ginger extract were also demonstrated in cholesterol fed rabbits (Sharma, Gusain, & Dixit, 1996). The superoxide scavenging and tyrosinase inhibitory activity of ginger is well documented (Khanom, Kayahara, Hirota, & Tadasa, 2003; Masuda, Kikuzaki, Hisamoto, & Nakatani, 2004). However, there was little report about polysaccharide from ginger and its activities. Given its long history of use as a food, studying the role of its products on ROS is not only very interesting but also of practical importance. Hence, the present study was designed to evaluate the effect of polysaccharides from ginger by comparing its antioxidant activity and chemical characteristics. The antioxidant activities of polysaccharides were evaluated based on the ability to scavenge superoxide, DPPH and hydroxyl radicals, and to bind to Fe(II) ions and reducing power.

2. Experimental

2.1. Materials

Ginger (*Z. officinale*) was collected in Huzhou city, China. The fresh root was soon washed, sun dried and kept in plastic bags at room temperature for use.

Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide-reduced (NADH), ethylene diamine tetra-acetic acid (EDTA) and ferrozine were purchased from Sigma Chemical Co. Ascorbic acid, sodium citrate and other reagents were of analytical grade. Dialysis membranes were produced by Spectrum Co., and molecular weight was cut off at 3600 Da.

2.2. Analytical methods

Total sugar content was determined by phenol–sulfuric acid method using D-glucose as standard (Dubois, Gillis, Hamilton,

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Rebers, & Smith, 1956). Uronic acid was estimated in a modified carbazole method using D-glucuronic acid as standard (Bitter & Muir, 1962). The protein was determined by Bradford assay as previously described (Pirie, 1987). Infrared spectrums were measured by a Nicolet Magna-Avatar 360 with KBr disks.

Molecular weight of all samples was determined by HP-GPC on a Waters 515 GPC system at 35 °C, where 0.7% Na_2SO_4 solution was used as mobile phase with a flow rate of 0.5 mL/min. TSK G3000 column (300 mm × 7.8 mm) and 2140 refractive index detector was used. A series of different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) were used as standard.

2.3. Neutral sugar analysis

2.3.1. Hydrolysis of polysaccharide

Polysaccharide sample (15.0–20.0 mg) was dissolved in 2 M trifluoroacetic acid (2.0 mL) in a 10 mL ampoule. The ampoule was sealed in a nitrogen atmosphere and incubated for 4 h at 110 °C. Following incubation, the ampoule was cooled to room temperature. The reaction mixture was then neutralized to pH 7 with 2 M sodium hydroxide, and we added 2.0 mL of the internal standard solution. The mixture was shaken well, diluted to 10 mL and filtered. The filtrate was retained for PMP (1-phenyl-3-methyl-5-pyrazolone) determination.

2.3.2. Derivatization with PMP

0.5 M methanolic solution of PMP (100 μL) and 0.3 M aqueous sodium hydroxide (100 μL) were added to the monosaccharide reference solution or a reducing polysaccharide solution (100 μL each). The mixture was incubated at 70 °C for 30 min. The reaction mixture was then cooled at 8 °C, and neutralized with 0.3 M hydrochloric acid. We then added 1 mL of chloroform to the solution. The mixture was shaken well and centrifuged at 5000 rev/min for 10 min at 6–8 °C. The chloroform layer was discarded and the aqueous layer was extracted twice with chloroform. The final aqueous layer was analyzed directly by HPLC.

2.3.3. Chromatography

Chromatographic conditions were generally as follows: column, YMC-Pack ODS-AQ (250 mm × 4.6 mm, 5 μm); temperature, 25 °C; solvent A, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (9:1); solvent B, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (4:6); gradient, 10–14% in 9 min, 14–64% from 9 min to 30 min, 64% during the next 5 min at 1 mL/min. The eluate was monitored at 245 nm.

2.4. Preparation of natural polysaccharide

2.4.1. Extraction with water

The air-dried specimen was extracted at 90 °C for 3 h with 15 portions of water. The hot aqueous solution was separated from the shoot residues by successive filtration through gauze and siliceous earth. The solution was dialyzed against tap water for 48 h and against distilled water for 24 h, and then the solution was concentrated under reduced pressure. The concentrated extract was lyophilized to give product (G1) as a white powder.

2.4.2. Extraction with sulfuric acid

Air dried fronts were heated in 40 vol. of 1.25% sulfuric acid at 85 °C for 30 min. The hot aqueous solution was separated from the residues by successive filtration through gauze, neutralized with 1 M sodium hydroxide, and then filtrated through siliceous earth. The solution was dialyzed, concentrated and then lyophilized to give product (G2) as a white powder.

2.4.3. Extraction with sodium hydroxide

Air dried fronts were heated in 40 vol. of 0.1 M sodium hydroxide at 85 °C for 4 h. The hot aqueous solution was separated from the residues by successive filtration through gauze, neutralized with 1 M hydrochloric acid, and then filtrated through siliceous earth. The solution was dialyzed, concentrated and then lyophilized to give product (G3) as a white powder.

2.5. Antioxidant activity

2.5.1. Superoxide radical assay

The superoxide radical-scavenging abilities of all the samples were assessed by the method of Nishimiki, Rao, and Yagi (1972). In this experiment, superoxide anion radicals were generated in 4.5 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 mL of NBT (300 μM) solution, 0.5 mL of NADH (468 μM) solution and one sample (0.5–50.0 μg/mL). The reaction was started by adding 0.5 mL of PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm by a spectrophotometer against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide radical-scavenging activity. The capability of scavenging the superoxide anion radicals was calculated using the following equation:

$$\text{Scavenging effect} = \left(1 - \frac{A_{\text{sample 560}}}{A_{\text{control 560}}}\right) \times 100$$

where $A_{\text{control 560}}$ is the absorbance of the control (Tris–HCl buffer, instead of sample).

2.5.2. Hydroxyl radical assay

The reaction mixture, containing all the samples (0.6–7.0 mg/mL), was incubated with EDTA-Fe^{2+} (2 mM), saffron (360 μg/mL), and H_2O_2 (3%) in potassium phosphate buffer (150 mM, pH 7.4), and was incubated for 30 min at 37 °C (Wang et al., 1994). The absorbance was read at 520 nm against a blank. Hydroxyl radical bleached the saffron, so decreased absorbance of the reaction mixture indicated a decrease in hydroxyl radical-scavenging ability. The capability of scavenging hydroxyl radical was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control (without samples) and A_1 is the absorbance of the mixture containing samples.

2.5.3. Reducing power assay

The reducing power was determined as described previously by Yen and Chen (1995). Briefly, 1.0 mL different concentration of samples (0.47–6.0 mg/mL) in phosphate buffer (0.2 M, pH 6.6) was mixed with 1.0 mL potassium ferricyanide (1%, w/v), and was incubated at 50 °C for 20 min. Afterwards, 2.0 mL trichloroacetic acid (10%, w/v) was added to the mixture to terminate the reaction. Then the solution was mixed with 1.2 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated increased reducing power.

2.5.4. DPPH radical-scavenging assay

The effect of porphyrans on scavenging DPPH-radical was studied employing the modified method described earlier by Yamaguchi, Takamura, Matoba, and Terao (1998). Briefly, 1 mL of DPPH solution (0.1 mM, in 50% ethanol solution) was incubated

Table 1
Yield and chemical composition of the samples (% w/w of dry weight).

Samples	Yield (%)	Total sugar (%)	Protein (%)	Uronic acids (%)	Neutral sugar (mole ratio)[a]					
					Gal	Glc	Ara	Rha	Xyl	Man
G1	4.32	83.4	6.49	4.20	15.5	54.7	1.21	11.4	14.9	6.46
G2	2.50	86.7	5.11	7.45	18.3	63.3	0.93	13.75	11.2	5.51
G3	3.14	76.9	3.62	1.92	12.0	48.0	3.67	11.70	17.3	3.04

[a] Gal: galactose; Glc: glucose; Ara: arabinose; Rha: rhamnose; Xyl: xylose; Man: mannose.

with varying concentrations of the sample. The reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left(1 - \frac{A_{\text{sample}\,517}}{A_{\text{control}\,517}}\right) \times 100$$

2.5.5. Iron(II) chelation activity

The chelating of ferrous ions by the extracts and reference antioxidant was estimated by the method of Dinis, Madeira, and Almeida (1994). Samples in different concentrations (0.18–1.94 mg/mL) were mixed with $FeCl_2$ (0.05 mL, 2 mM) and ferrozine (0.2 mL, 5 mM), shook well, stayed still for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm. In the control, sample was substituted with EDTA. The ferrous ion-chelating activity was given by the following equation:

$$\text{Chelating ability}\,(\%) = \left(1 - \frac{A_{\text{sample}\,562\,\text{nm}}}{A_{\text{control}\,562\,\text{nm}}}\right) \times 100$$

2.5.6. Statistical analysis

Results were expressed as the mean ± standard deviation of triplicate analysis. Statistical comparisons were performed using the Student's t test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical analysis

The chemical compositions of three natural polysaccharides are shown in Table 1. The result showed that the main chemical components of these samples were total sugar and protein, along with uronic acid.

Neutral monosaccharide constitutions of the polysaccharides were analyzed by HPLC. Results showed that glucose was the main sugar form in all the samples. In addition, the more content of monosaccharides was xylose and arabinose. The other common monosaccharides were also seen in these samples, which show that chemical property may have great influence on antioxidant activities.

The FT-IR spectrum of the products was shown in Fig. 1. Infrared spectroscopy analysis indicated that all the three samples showed typical peaks of polysaccharide. The peaks at 1640–1650 cm^{-1} are caused by the bending vibration of C–O of uronic acids. Signals at 3420–3450 and 1050–1070 cm^{-1} correspond to stretching vibration of O–H and C–O, respectively.

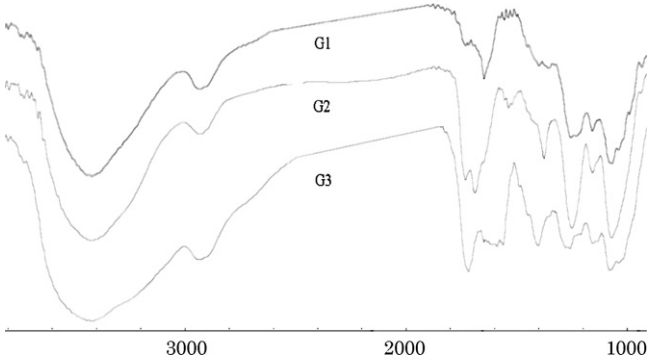

Fig. 1. The IR spectrum of three polysaccharides.

3.2. Superoxide radical assay

The superoxide radical is a highly toxic species that is generated by numerous biological and photochemical reactions (Banerjee, Dasgupta, & De, 2005). Fig. 2 showed that the inhibitory effect of all samples on superoxide radicals was marked and concentration related. Significant scavenging of superoxide radical was evident at all the tested concentrations of all products. Results showed that EC_{50} of G1, G2 and G3 were 18, 16 and 24 μg/mL, respectively. When the concentration was over 85 μg/mL, the scavenging abilities of all the samples were parallel.

It was reported that addition of electron-withdrawing groups to the pyrrole enhanced antioxidant activity (Yanagimoto, Lee, Ochi, & Shibamoto, 2002). Although superoxide was a relatively weak oxidant, it decomposed to form stronger, reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Furthermore, superoxides were also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Duh, Du, & Yen, 1999). Based on this, the antioxidant activities of all the samples were also related to their ability to scavenge superoxide radical.

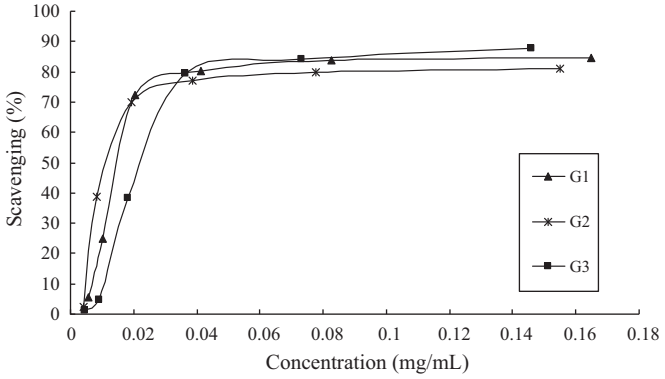

Fig. 2. Scavenging effects of the samples on superoxide radical. Values are mean ± S.D. ($n = 3$).

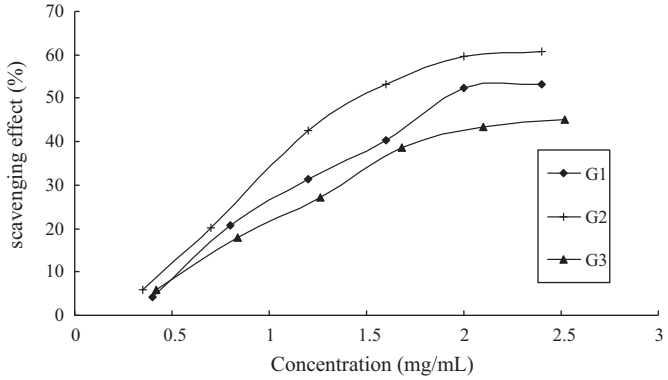

Fig. 3. Scavenging effects of the samples on hydroxyl radical. Values are mean ± S.D. (n = 3).

3.3. Hydroxyl radical assay

The hydroxyl radical, known to be generated through the Fenton reaction in this system, was scavenged by polysaccharide samples. The scavenging effect of all samples was shown in Fig. 3. For all the samples, the effects of scavenging hydroxyl radicals were in a concentration-dependent manner. With regard to the EC_{50} values, the acid extract G2 (1.41 ± 0.43 mg/mL) was a considerably less effective ($p < 0.05$) hydroxyl radical scavenger compared to G1 (1.88 ± 0.13 mg/mL) and G3.

For hydroxyl radical, there were two types of antioxidation mechanism; one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated. In the former, the antioxidant activity may ligate to the metal ions which react with H_2O_2 to give the metal complexes. The metal complexes thus formed cannot further react with H_2O_2 to give hydroxyl radicals (Ueda, Saito, Shimazu, & Ozawa, 1996). Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases. Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation, and reduction of Fe^{2+} concentrations in the Fenton reaction would protect against oxidative damage. In the present study, all the samples contain the high content of total sugar, which may be the main factor on the scavenging activities on the hydroxyl radical. The mechanism on the hydroxyl radicals needs to be further investigated.

3.4. Reducing power assay

The reducing power of all samples was shown in Fig. 4. As shown in the figure, the reducing power of the samples correlated well with increasing concentrations except that of G2, which showed

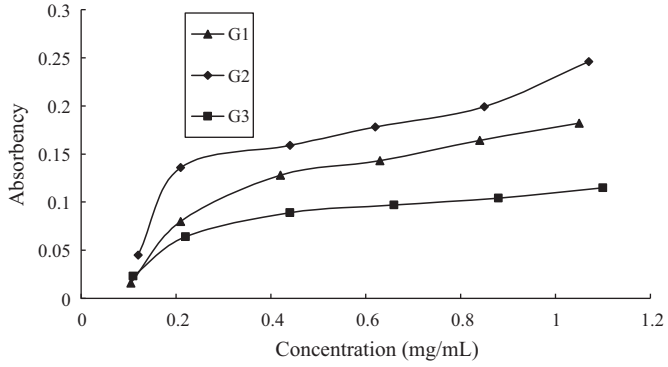

Fig. 4. Reducing power assay of the samples. Values are mean ± S.D. (n = 3).

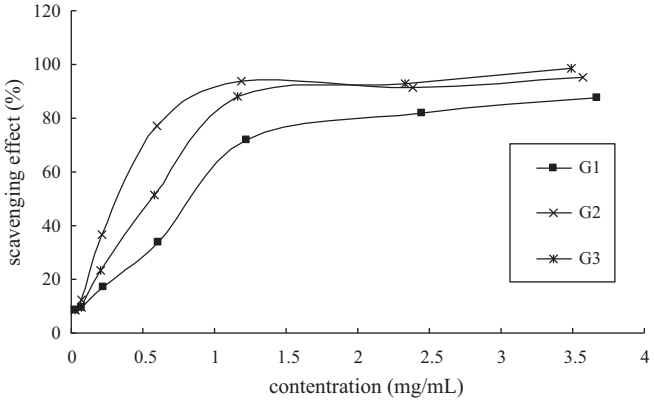

Fig. 5. Scavenging effects of the samples on DPPH radical. Values are mean ± S.D. (n = 3).

extremely strong reducing power even at higher concentration. It has been previously reported that there was a direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductant, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductant is also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, G2 contain the more content of uronic acid, which resulted in the increase of the reducing power.

3.5. DPPH radical-scavenging assay

The extracts showed maximum hydrogen-donating ability in the presence of DPPH stable radicals at high concentrations. As shown in Fig. 5, the scavenging activities of the extracts on DPPH radicals were similar to the results of the scavenging activities on hydroxyl radicals. The scavenging activity of the acid extract G2 was significantly ($p < 0.05$) lower (0.21 ± 0.93%) at 23 mg/mL and, at a concentration of 1.17 mg/mL, reached a plateau of 88.5 ± 0.16%. The DPPH scavenging activities of the extracts, expressed as an EC_{50} value, ranged from 0.34, 0.56 to 0.87 mg/ml. G2 and G3 exhibited the strongest antioxidant activity than G1 at the high concentration. These results suggested that the acid and sodium hydroxide extracts contained the strongest free radical scavenging compounds.

3.6. Iron(II) chelation activity

By forming a stable iron(II) chelate, an extract with high chelating power reduces the free ferrous ion concentration and thus decreases the extent of the Fenton reaction which is implicated in many diseases (Halliwell & Gutteridge, 1984). All the extracts demonstrated an ability to chelate iron(II) ions in a dose-dependent manner (Fig. 6). Three samples G1, G2 and G3 chelated ferrous ions by 35.4 ± 0.56%, 56.8 ± 0.12% and 57.1 ± 1.83% at 1.14 μg/mL, respectively. From the figure, it can be seen that the most effective iron(II) chelating extract was acid extract (96.3%), followed by water extract (82.4%) and sodium hydroxide extract (62.5%), in decreasing order.

The results of the present work indicated that all samples possessed antioxidant activities in certain assays. Of the three samples, G2 had the strong radical scavenging effect in five systems of assay. The reason was possible that G2 has the higher total sugar content, the higher uronic acid content and the lower molecular weight. These results were consistent with our previous reports. G2 may have a use as a possible supplement in the food and pharmaceutical

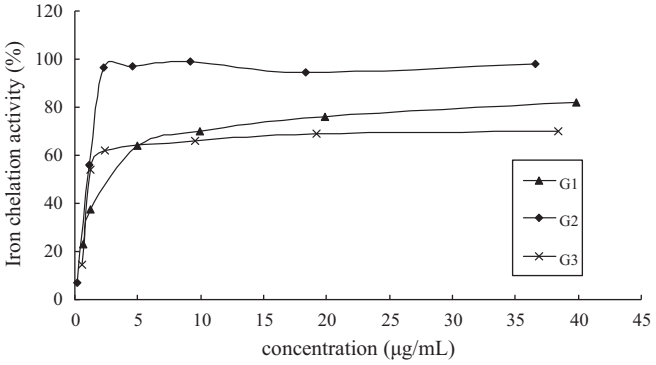

Fig. 6. Iron(II) chelation activity of the samples. Values are mean ± S.D. ($n = 3$).

industries. The radical scavenging effect was stable at high temperatures so that these samples may be used as resources of medicine. However, factors effecting and attributing to radical scavenging effect need to be further studied.

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