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# Antioxidant activities of water-soluble polysaccharide extracted from mung bean (*Vigna radiata* L.) hull with ultrasonic assisted treatment

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ABSTRACT

Ultrasonic assisted extraction and antioxidant activity of water-soluble polysaccharide (MSP) from mung bean (*Vigna radiata* L.) hull were investigated. After deproteinization, MSP was isolated and purified by a DEAE-cellulose anion-exchange column and a Sephadex G-100 gel-permeation column. Two acid polysaccharide fractions, MP1 and MP2, were characterized by FT-IR, GC, and gel-permeation chromatography (GPC). Results indicated that MP1 and MP2 were acid heteropolysaccharide with 9.9% and 36.4% of uronic acid contents respectively. The main composition of MP1 was mannose, whereas MP2 consisted of rhamnose and galactose. The molecule weights of MP1 and MP2 were 83 kDa and 45 kDa, respectively. Although, MP2 showed higher hydroxyl radical-scavenging activity, MP1 exhibited higher reducing power and stronger scavenging capacity for superoxide radical and DPPH radicals as well as higher inhibition on self-oxidation of 1,2,3-phentriol than that of MP2. Thus, MP1 should be explored as a potential antioxidant.



## 1. Introduction

Reactive oxygen species (ROS), like superoxide anion, hydroxyl radical, hydrogen peroxide, are generated in living organisms through many pathways (Fridovich, 1978). The generation of free radicals is beneficial, e.g. when cells of the immune system attack tumours, bacteria, fungi and virus-infected cells (Ndhlala et al., 2008). However, the uncontrolled generation of oxygen-derived free radicals will cause damage to the cell membrane (Staniek & Nobl, 1999) and may be associated with cancer (Knight, 1995), arteriosclerosis (Witztum, 1994) and diabetes mellitus (Dandona, Thusu, & Cook, 1996). To counteract the oxidative damage from ROS, antioxidant defence systems are coevolved with aerobic metabolism. Antioxidants play an important role to maintain the optimal cellular and systematic health, and scavenge excessive free radicals (Percival, 1998).

Antioxidants can delay or prevent oxidation of cellular oxidative substrates. Synthetic antioxidants are widely used since they are effective and cheaper than natural ones. However, the safety and toxicity of synthetic antioxidants have brought great concerns (Imaida et al., 1983). Thus, it is essential to develop and utilize effective and natural antioxidant to protect the body from ROS damage. In this respect, the water-soluble polysaccharides have received great attention as free radical scavenger, inhibitors of lipid peroxidation and metal chelators (Costa et al., 2009; Wang & Luo, 2007).

Compared with soybean and kidney bean, mung bean is a richer source of vitamins, minerals as well as proteins with its essential amino acid profile (Mubarak, 2005). The previous research has reported that mung bean consumption slightly increase the blood glycemic index in human, making it an attractive option for diabetic patients (Bomet, Fontvieille, & Rizkalla, 1989). The seed hulls act as a defensive role to protect the cotyledon and hypocotyl from damage. There have been some researchers focusing on the antioxidant activities of plant hulls (Asamarai, Addisd, Epley, & Krick, 1996; Duh, Yeh, & Yen, 1992), and indicated that antioxidant components may exist in the plant hulls. Although the mung bean hull methanol extracts (MBHME) has been demonstrated to have free radicals scavenging activity and act as inhibitor of lipid peroxidation and non-lipid oxidative damage (Duh, Du, & Yen, 1999; Duh, Yen, Du, & Yen, 1997), there is no investigation carried out on the antioxidant activity of water-soluble polysaccharide isolated and purified from mung bean hull.

Recently, ultrasonic method is widely employed to extract polysaccharide from plant material due to its high extraction efficiency (Yang, Jiang, Zhao, Shi, & Wang, 2008), furthermore, ultrasonic treatment can change the structure and other properties of the polysaccharides to some extent (Mislovicova, Masarova, Bendzalova, Soltes, & Machova, 2000). In the present study, the water-soluble polysaccharide was extracted from mung bean (*Vigna radiata* L.) hull by ultrasonic treatment. The physicochemi-

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cal and antioxidant properties of crude and purified polysaccharide fractions were also investigated.

## 2. Materials and methods

### 2.1. Materials

Mung bean hull was supplied by Zhenqiao starch factory (Hebei, China).

Monosaccharide standard (rhamnose, fucose, galactose, mannose, xylose, arabinose), galacturonic acid, dextrans with various molecular weight, *m*-hydroxydiphenyl, 1,1-diphenyl-2-picrylhydrazyl (DPPH), bovine serum albumin (BSA), butylated hydroxyanisole (BHA), ethylene diamine tetra-acetic acid (EDTA), trichloroacetic acid (TCA), thiobarbituric acid (TBA) and deoxyribose were purchased from Sigma Chemical Co. (St Louis, MO, USA). DEAE-52 cellulose and Sephadex G-100 were obtained from GE Healthcare Bio-Sciences Co. (Piscataway, NJ, USA). Nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS) and nitroblue tetrazolium (NBT) were purchased from E. Merck (Darmstadt, Germany). Glucose and inositol were from Bo'ao Biological Technology Co. (Shanghai, China). Phenol and sulfuric acid were from the Guangzhou Reagent Co. (Guangzhou, China). All other chemicals were of the analytical grade available.

### 2.2. Isolation and purification of water-soluble polysaccharide from mung bean hull

The water-soluble polysaccharide was extracted from mung bean hull according to Yang et al. (2008) with some modifications. Briefly, mung bean hull was ground in a sample mill to pass a 0.25 mm sifter after oven drying for 2 days at 60 °C. The powdered material was refluxed in 80% ethanol for 6 h. Then the cooled extract was discarded and the residue was washed with 95% ethanol, anhydrous ethyl alcohol, acetone and diethyl ether respectively. The residue was dried at room temperature for 24 h prior to extraction. Subsequently, the extraction was carried out using an ultrasonic cleaner (KQ-300VDE, Kunshan Ultrasonic Instruments Co. Ltd.) at a power of 150 W for 30 min at room temperature without additional stirring. After that, the syrup was centrifuged at 7500 × *g* for 15 min, and the residue was re-extracted under the same conditions. The combined supernatant fluids were concentrated to minimum volume using a rotary evaporator (Biochemical Equipment Co., Ltd., Shanghai) at 60 °C under low pressure. After the protein in the concentrated solution was removed by Sevag reagent (chloroform and n-butanol in 4:1 ratio) (Staub, 1965), the extract was dialyzed again with tap water and deionized water for 24 h, respectively. In order to obtain the crude polysaccharide, the extract was precipitated with 4 volumes anhydrous ethanol at 4 °C for overnight and the precipitation was centrifuged at 4500 × *g* for 15 min. The precipitate was dissolved in distilled water, collected, frozen and freeze-dried, then the crude polysaccharide MSP was obtained.

The sample MSP (500 mg) was dissolved in distilled water, loaded on a DEAE-52 cellulose column (2.6 cm × 18 cm) and eluted with 300 mL distilled water and 300 mL of 0.5 M NaCl solution, respectively, at a flow rate of 1.0 mL/min. The sugar was assayed through phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the uronic acid content was measured by the sulfuric acid carbazole assay (Dische, 1947). Fractions of two peaks with positive response were pooled together separately, concentrated, dialyzed with tap water and deionized water for 36 h, respectively, and then lyophilized. The fractions were dissolved in 0.05 M phosphate buffer (pH 6.8), then purified by Sephadex G-100 (1.0 cm × 45 cm) gel filtration column, at 0.4 mL/min. Two purified polysaccharide fractions were collected, dialyzed, freeze-dried, and produced MP1 and MP2, respectively.

### 2.3. Uronic acid, protein and total sugar content

The contents of uronic acid of purified fractions were determined colorimetrically by m-hydroxydiphenyl (Blunenkrantz & Asboe-Hansen, 1973), and the galacturonic acid was used to make a standard curve. Absorbance of the samples was measured at 525 nm. Protein content was determined according to the method of Bradford (1976), using bovine serum albumin as standard. Total sugar content of purified samples was measured by phenol–sulfuric acid, using glucose as standard.

### 2.4. Monosaccharide composition

MP1 and MP2 were treated with 2 mol/L trifluoroacetic acid (TFA) at 120 °C for 6 h, after that, TFA was evaporated under reduced pressure. Then, methanol (1.5 mL) was added, and evaporated to dryness, which were repeated for four times. The hydrolyzed polysaccharide sample with rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glu) as monosaccharide standard, and inositol as internal standard were dissolved in distilled water (0.5 mL), reduced by sodium borohydride ($NaBH_4$, ~20 mg, 2 h, with oscillation in every 30 min), treated with glacial acetic acid (AcOH), and dried. Methanol (1~2 mL) was added to vessel, dried, and repeated 5 times to avoid the influence of AcOH, then dried at 100 °C for 15 min in the drying cabinet. After the derivatization (Zhang, 2001) with pyridine and acetic anhydride (the ratio was 1:1, reacted in sealed condition at 100 °C for 2 h) and filtrated by 0.22 μm filter membrane, the derivatives were analyzed by GC-2014 gas chromatograph (Shimadzu, Japan) equipped with flame ionization detector (FID) on 30QC2-AC20 (Shimadzu, Japan) capillary column (30 m × 0.22 mm × 0.25 μm). 1 μL samples was injected into GC analyzer and the column temperature was programmed as follows: the initial temperature was 180 °C, hold for 2 min, then increased from 180 °C to 230 °C at rate of 5 °C/min with a 3 min hold at 230 °C. Injector and detector temperature were 230 °C and 250 °C, respectively. Nitrogen gas was used as the carrier gas with a flow rate at 30 mL/min. The peak areas of monosaccharide were compared with inositol peak area (internal standard) to calculate the content of neutral sugars.

### 2.5. Molecular weight determination of MP1 and MP2

The purified polysaccharides dissolved in 0.02 M phosphate buffer ($KH_2PO_4$, pH 6.0) (final concentration was 2 mg/mL) were characterized by gel-permeation chromatography (GPC), using Waters 515 instrument (USA) equipped with TSK G – 5000 PW xL gel column (Tosoh, Japan) and Waters 2414 Refractive index Detector (Waters, USA). The dextrans with various molecular weight (5200, 11,600, 23,800, 48,600, 148,000, 273,000, 410,000, 668,000, 1,482,000) were used as standard to calibrate the column and fit the regression curve. 20 μL samples were injected by Waters 717 Plus Autosampler into the gel column, then eluted with 0.02 M $KH_2PO_4$ at 35 °C and a flow rate of 0.6 mL/min. The molecular weight ($M_w$) was obtained from the calibration curve. All samples were filtrated through a 0.22 μm pore diameter membrane (Millipore, USA) prior to analysis.

### 2.6. Infrared spectroscopy

After grinding the polysaccharide in KBr-disk, FT-IR spectra were determined using the Nexus Euro Fourier transform infrared

(FT-IR) spectrometer (Nexus TEC Ltd., London, UK) at the range of 400–4000 $cm^{-1}$.

### 2.7. Antioxidant activity

#### 2.7.1. Reducing ability

The reductive potential of MP1 and MP2 was measured according to the method of Oyaizu (1986). 0.5 mL of sample (at different concentration), 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were mixed and incubated at 50 °C for 20 min. Afterwards, 1.25 mL of 10% TCA was added to the mixture, and centrifuged at 3000 × *g* for 10 min. The supernatant (2.5 mL) was mixed with 0.25 mL of 0.1% ferric chloride ($FeCl_3$) and the absorbance was determined at 700 nm. Increased absorbance of the reaction mixture indicated the increase of reduction capability.

#### 2.7.2. Superoxide radical-scavenging activity

The superoxide radical-scavenging activity was determined by the means of Xu et al. (2009) with a minor modification. A 0.1-mL sample solution of polysaccharide at different concentration (0–500 μg/mL) were mixed with 425 μM NADH, 90 μM NBT, 38 μM PMS which were dissolved in 1 mL of Tris–HCl buffer (16 mM, pH 8.0), respectively. The reacting mixture was incubated at room temperature for 5 min and the absorbance was measured at 560 nm against a blank. The superoxide radical-scavenging activity was indicated by the decrease in the absorbance of the reacting mixture. In the control, sample was substituted with Tris–HCl buffer and BHA was used as a positive control. The scavenging activity of superoxide radical was calculated by scavenging effect (%) = $(1 - A_{\text{sample }560}/A_{\text{control }560}) \times 100$.

#### 2.7.3. Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging ability of polysaccharide was measured by the deoxyribose assay described by Halliwell, Gutteridge, and Aruoma (1987). Samples were dissolved in distilled water to form final concentrations of 0, 0.1, 0.5, 1, 2, 5 mg/mL. 0.1 mL of sample aqueous solution was mixed with 0.6 mL of reaction reagent [containing 50 mM phosphate buffer (pH 7.4), 2.7 mM deoxyribose and 0.15 mM EDTA], 0.2 mL of 0.2 mM ferrous sulfate, 0.05 mL of 1.5 mM Vc, and 0.05 mL of 20 mM hydrogen peroxide ($H_2O_2$). After incubation for 30 min at 37 °C, 1.0 mL of 1% TBA, as well as 1.0 mL of 1.5% TCA were added. The mixture was heated in boiling water for 15 min and cooled by ice water. The absorbance of the mixture was read at 532 nm against a blank. Scavenging activity of hydroxyl radical was determined by using the formula of $(1 - A_{\text{sample }532}/A_{\text{control }532}) \times 100$.

#### 2.7.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH radical-scavenging effect of crude polysaccharide MSP and purified fractions MP1 and MP2 was investigated according to the method of previous literature (Blois, 1985) with some modifications. Briefly, polysaccharide samples were dissolved in methanol. One milliliter of sample solution with different concentration (0, 50, 100, 200, 400 and 800 μg/mL) was added to 3 mL of a 75 μM DPPH methanol solution. The mixture was kept at room temperature for 30 min, and the absorbance was recorded at 517 nm. The DPPH radical effect was calculated as the following formula:

$$\text{scavenging activity}\,(\%) = 1 - \frac{A_{sample+DPPH} - A_{sample}}{A_{DPPH}},$$

where $A_{DPPH}$ was the absorbance of DPPH without sample, $A_{sample}$ was the absorbance of sample without DPPH, and $A_{sample+DPPH}$ was the absorbance of sample and DPPH.

**Table 1**
Yield, total sugar contents, protein contents, uronic acid contents, and molecule weight of MSP, MP1 and MP2.

| Sample | MSP | MP1 | MP2 |
|---|---|---|---|
| Yield (%)[a] | 7.6 ± 0.4 | 2.1 ± 0.2 | 4.5 ± 0.3 |
| Carbohydrate (%)[a] | 93.6 ± 1.0 | 97.9 ± 1.9 | 99.1 ± 0.9 |
| Protein (%)[a] | 1.3 ± 0.1 | nd[b] | nd |
| GalA (%)[a] | 22.2 ± 1.3 | 9.9 ± 0.3 | 36.4 ± 2.1 |
| $M_w$ (Da) | –[c] | 83,000 | 45,000 |
| Sugar component (mol%)[d] | | | |
| Rha | 11.6 | nd | 31.8 |
| Fuc | 1.2 | nd | 3.5 |
| Ara | 11.2 | 8.3 | 16.7 |
| Xyl | 3.6 | 2.2 | 4.6 |
| Man | 43.7 | 67.2 | 11.7 |
| Gal | 23.5 | 20.1 | 29.1 |
| Glu | 5.1 | 2.3 | 2.5 |

[a] Data are shown as mean ± standard deviation, $n = 3$.
[b] nd, not detected.
[c] –, not measured.
[d] Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; GalA, galacturonic acid.

#### 2.7.5. Inhibitory effect on self-oxidation of 1,2,3-phentriol

The scavenging capacity for self-oxidation of 1,2,3-phentriol of all polysaccharide samples were measured according to the method of Marklund and Marklund (1974) with a minor modification. 1 mL of samples solution (at various concentrations), 2 mL of 0.05 M Tris–HCl buffer (pH 8.0) containing 1 mM EDTA and 0.5 mL of 6 mM 1,2,3-phentriol were mixed together, then shaken rapidly at room temperature. The absorbance of the mixture was determined at 325 nm in every 30 s for 4 min against a blank, and the slope was calculated as absorbance/min. The scavenging activity for self-oxidation of 1,2,3-phentriol was calculated by using the equation (1 − slope of sample/slope of control) × 100%. BHA was used for comparison.

### 2.8. Data analysis

All data were analyzed by using the SPSS 13.0 software with one-way ANOVA. Significant differences between two means were determined by LSD multiple-range tests. *P*-Values < 0.05 were regarded as significant.

## 3. Results and discussion

### 3.1. Isolation, purification and compositions of water-soluble polysaccharide

The crude polysaccharide (MSP) was isolated from mung bean (*V. radiata* L.) hull by ultrasonic assisted extraction with a yield of 6.4%. After deproteinization and decolourization, MSP was fractionated on a DEAE-52 cellulose anion-exchange column. The total sugar and uronic acid content of each tube was determined by phenol–sulfuric acid and sulfuric acid–carbazole assay, respectively. The tubes with phenol–sulfuric acid positive reaction were pooled together, collected and further purified by gel chromatography on Sephadex G-100 column. Two fractions (MP1 and MP2) were eluted with distilled water and NaCl solution, respectively (Fig. 1a and b). Furthermore, the uronic acid content of MP2 was higher than MP1, suggesting that MP2 was acid polysaccharide. As shown in Fig. 1b, both MP1 and MP2 had only one symmetrical peak, and appeared as a single spot on cellulose acetate pellicle electrophoresis, indicating that purified fraction was a homogeneous polysaccharide.

The extraction yield, total carbohydrate content, protein content, uronic acid content, average molecule weight and sugar compositions of MSP, MP1 and MP2 were shown in Table 1. The

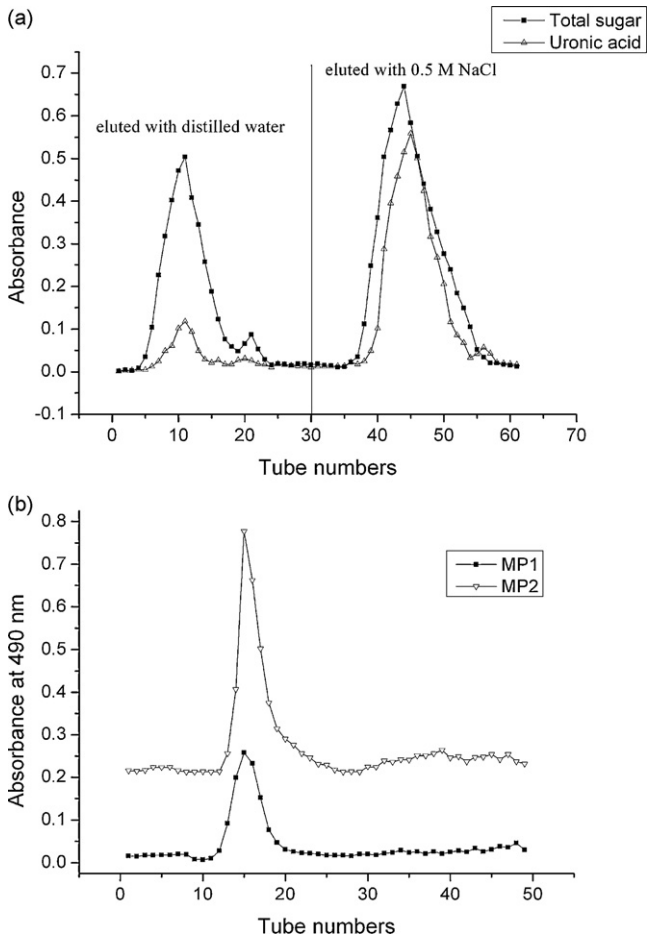


**Fig. 1.** (a) Elution curve of crude polysaccharide extracted from mung bean hull on a DEAE-52 cellulose anion-exchange column. (b) Purification of polysaccharide fractions MP1 and MP2 on a Sephadex G-100 column.

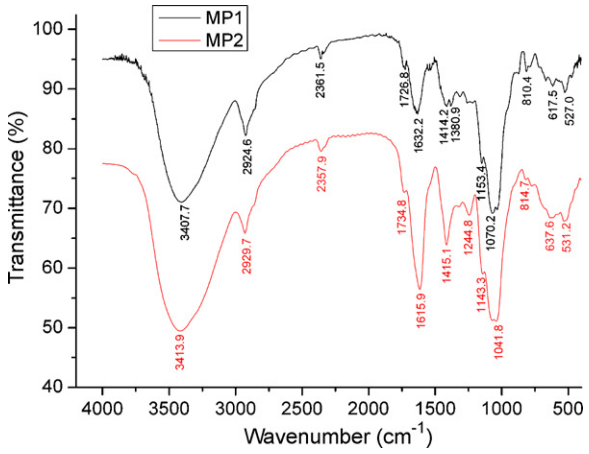


**Fig. 2.** FT-IR spectra of purified polysaccharide fractions MP1 and MP2.

polysaccharide content of crude polysaccharide MSP and purified fractions (MP1 and MP2) was 93.6%, 97.9% and 99.1%, respectively. The protein content of MSP was 1.3%, but no protein was detected in MP1 and MP2. These indicated that the protein existing in mung bean hull polysaccharide was free. According to the calibration curve, $\log M_w = 6.15 - 1.68t + 1.69t^2 - 0.05t^3$ ($t$ was the elution time), the average molecule weight of MP1 and MP2 was calculated to be 83,000 and 45,000 Da.

### 3.2. Neutral sugar composition

The GC results indicated that the crude polysaccharide MSP and purified polysaccharide MP1 mainly consisted of mannose and galactose, whereas the major neutral sugar composition of MP2 was galactose and rhamnose, respectively. After hydrolyzed by 2 M TFA, GC was used to determine the contents of sugars due to its accuracy in polysaccharide analysis. The molar percentage of neutral sugars of samples was summarized in Table 1. The contents of uronic acid were more than one fifths in MSP and MP2, indicating that the uronic acid is very important to form the main chains of polysaccharide.

### 3.3. IR spectroscopy

The IR spectra of MP1 and MP2 were recorded at the range of 4000–400 $cm^{-1}$ (Fig. 2). Both of them displayed a broad and intense peak nearby 3410 $cm^{-1}$, which were due to the hydroxyl groups stretching vibration. The bands in the region of 2925 $cm^{-1}$ were the characteristic of C–H antisymmetrical stretching vibration. The weak absorption around 2360 $cm^{-1}$ indicated the existence of aliphatic C–H bonds. The bands around 1730 $cm^{-1}$ and 1625 $cm^{-1}$ represented the ester carbonyl groups (C=O) and carboxylate (COO–) stretching band (Gnanasambanda & Proctor, 2000), respectively, indicating that there were esterified and free carboxyl groups presentin the polysaccharides from mung bean hull. It also validated the presence of uronic acids. The peak absorption around 1415 $cm^{-1}$ was due to carboxylate groups symmetric stretching. The absorption band from 1300 $cm^{-1}$ to 800 $cm^{-1}$, called "finger print" region, was related to conformation and surface structure of molecule. Although these bands are hard to explain (Gnanasambanda & Proctor, 2000), the peaks at 950–1200 $cm^{-1}$ suggested the presence of C–O–C and C–OH link bonds (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). From the spectrum, we proposed that the absorbance between 1000 $cm^{-1}$ and 1200 $cm^{-1}$ was due to the pyranose ring. The bands of 810 $cm^{-1}$ and 814 $cm^{-1}$ were the characteristic of mannose (Mathlouthi & Koenig, 1986).

### 3.4. Antioxidant activity

#### 3.4.1. Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Kallithraka, Bakker, & Clifford, 2001). The present study investigated the reducing capacity of crude and purified polysaccharide samples. As shown in Fig. 3, the polysaccharides and BHA exhibited effective reducing ability which increased with an increased concentration of samples. Higher absorbance value means stronger reducing ability of samples. At the concentration of 0.5 mg/mL, the reducing capacity of MSP, MP1 and MP2 was 0.06, 0.23 and 0.02, respectively, which is much lower than the methanolic extract from mung bean hull (Duh et al., 1997). Compared to the reducing power of BHA (0.91) at 1 mg/mL, MSP, MP1 showed a lower reducing power of 0.08–0.12, however, MP2 exhibited a relatively higher reducing ability of 0.27. The reducing power of polysaccharide samples increased rapidly from 0.10–0.38 at 2 mg/mL to 0.63–1.03 at 20 mg/mL. It has been reported that there was a direct correlation between antioxidant activities and reducing power (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004; Duh et al., 1999; Qiao et al., 2009). Our data on the reducing capacity of polysaccharides indicated that there might be a direct correlation between antioxidant activities and reducing power in MSP and purified fractions MP1 and MP2.

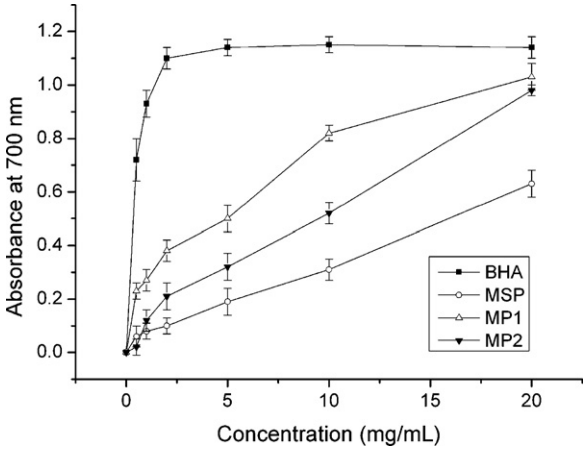


**Fig. 3.** Reducing power of MSP, MP1 and MP2 from mung bean hull. Results are shown as mean ± standard deviation ($n = 3$).

### 3.4.2. Superoxide radical-scavenging activity

Superoxide anion is reduced from oxygen molecular by receiving one electron. It is considered as an initial free radical, formed from mitochondrial electron transport systems, to create other cell-damaging free radicals, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (Bloknina, Virolainen, & Fagerstedt, 2003). In our study, the superoxide anion radicals were generated from dissolved oxygen by the PMS/NADH coupling reaction by reducing NBT in a PMS/NADH system (Luo & Fang, 2008). Fig. 4a shows the scavenging activity of crude and purified polysaccharide samples by comparison with the same concentration of BHA.

As shown in Fig. 4a, MP1 exhibited stronger superoxide radicals scavenging ability than that of MSP and MP2. The scavenging effect of polysaccharide was increased with the concentration increasing. At the concentration of 500 μg/mL, the scavenging capacity of MP1 was 91.7%, which was comparable to BHA (95.3%). These results indicated that the phenolic hydroxyl groups in the molecule may play an important role on the strong scavenging ability of polysaccharide samples (Li, 2002). Although superoxide radical was a weak oxidant in most organisms, it could produce other free radicals. Superoxide radical and all other free radicals may cause the damage to DNA and membrane of cell (MacDonald, Galley, & Webster, 2003). Our data suggest that the polysaccharide extracted from mung bean hull has a noticeable effect on scavenging superoxide radical.

### 3.4.3. Hydroxyl radical-scavenging activity

Hydroxyl radicals, which are well known as the most reactive free radical, can react with almost all the biomacromolecules functioning in living cells in the form of abstracting hydrogen atoms, addition reaction and electron transportation. They can be formed from superoxide anion and hydrogen peroxide in the presence of copper or iron, and they are also thought to initiate cell damage *in vivo* (Rollet-Labelle et al., 1998). Although hydroxyl radical formation can occur in several ways, the most important mechanism *in vivo* is the Fenton's reaction, in which a transition metal is involved as pro-oxidant in the catalysed decomposition of superoxide and hydrogen peroxide (Stohs & Baguchi, 1995).

Fig. 4b depicted the hydroxyl radical-scavenging ability of polysaccharide samples isolated from mung bean hull. The crude polysaccharide and its fractions were found to have the ability to scavenge hydroxyl radicals at concentrations between 0.1 mg/mL and 5 mg/mL compared to the same concentration of BHA. Purified fractions had a higher scavenging effect of hydroxyl radical than that of crude polysaccharide MSP, especially the MP2. At a concentration of 0.1 mg/mL, the scavenging ability of MSP, MP1 and MP2 on hydroxyl radicals was at a range of 10.4–25.1%, whereas scavenging capacity of BHA was 82.8% which is similar to the report of Ningappa, Dinesha, and Srinivas (2008). When the concentration of samples increased to 2 mg/mL, the hydroxyl radical-scavenging activity of MP2 reached to 84.4%. MSP has a moderate hydroxyl radical-scavenging ability at the concentration of 0.1–5 mg/mL. The scavenging activity of all polysaccharide samples is more effective than Vc at the dosage of 0–5 mg/mL (Sun, Zhang, Zhang, & Niu, 2010). Previous studies on antioxidant activity have suggested that the hydroxyl radical-scavenging activity was not due to the direct scavenging but the inhibition of hydroxyl radical generation by chelating ions such as $Fe^{2+}$ and $Cu^{2+}$ (Qi et al., 2005; Simic, 1998). These results indicate that the polysaccharide may have $Fe^{2+}$ chelating ability which can reduce the generation of hydroxyl radical.

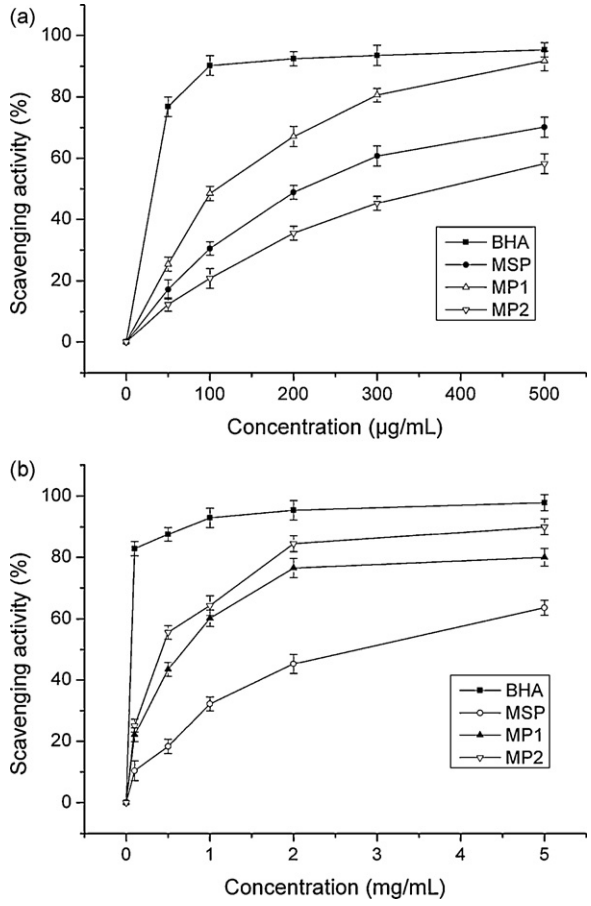


**Fig. 4.** (a) Scavenging activity of superoxide radical by MSP, MP1 and MP2 from mung bean hull. Results are shown as mean ± standard deviation ($n = 3$). (b) Hydroxyl radical-scavenging activity of MSP, MP1 and MP2 from mung bean hull. Results are shown as mean ± standard deviation ($n = 3$).

### 3.4.4. DPPH free radical-scavenging assay

DPPH, a relatively stable free radical, has been widely used to examine the free radical-scavenging ability of tested samples. The samples as antioxidant donate electrons or hydrogen atoms to free radical, leading to non-toxin species and therefore to inhibit the propagation phase of lipid oxidation (Li, Jiang, Zhang, Mu, & Liu, 2008; Naik et al., 2003). It is noticeable as a color change from purple to yellow induced by antioxidants in the reaction. The results of DPPH free radical-scavenging ability of crude and purified polysaccharides are shown in Fig. 5a.

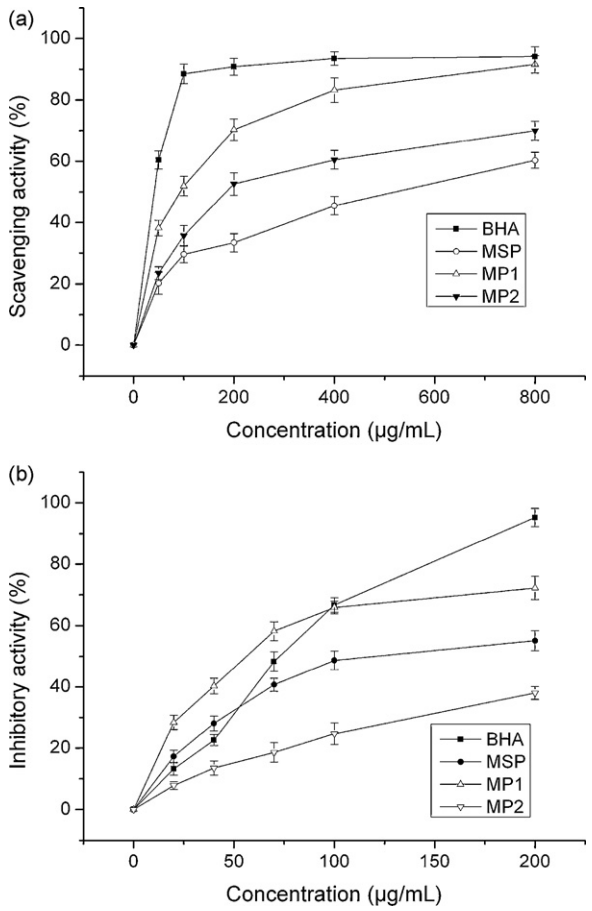


**Fig. 5.** (a) DPPH radical-scavenging capacity of MSP, MP1 and MP2 from mung bean hull. Results are shown as mean ± standard deviation ($n = 3$). (b) Inhibitory effects of MSP, MP1 and MP2 on self-oxidation of 1,2,3-phentriol. Results are shown as mean ± standard deviation ($n = 3$).

The DPPH free radical-scavenging effects of polysaccharide samples increase in a concentration-dependent manner. MP1 exhibited the highest DPPH radical-scavenging activity of 91.6% at 0.8 mg/mL, which is much higher than that of MSP (60.3%) and MP2 (70.2%). Meanwhile, the DPPH free radical-scavenging activity of BHA is 94.1%. This implies that MP1 can be a more effective free radical scavenger than MSP, MP1, but comparable to BHA. The possible mechanism of the polysaccharides acting as an antioxidant may be attributed to their electron donation power to the free radicals, thereby terminating the radical chain reaction further.

### *3.4.5. Scavenging activity of self-oxidation of 1,2,3-phentriol of polysaccharide samples*

Fig. 5b illustrates the scavenging effect for self-oxidation of 1,2,3-phentrol of crude and purified polysaccharide samples. The scavenging power of samples is correlated well with the increasing concentration of polysaccharide. MP1 exhibits stronger scavenging capacity than those of MP2 and MSP, and even stronger than that of BHA at the concentration below than 100 μg/mL. However, when the concentration was increased to 200 μg/mL, the scavenging ability of self-oxidation of 1,2,3-phentriol of polysaccharide samples become lower than BHA. These results indicated that MP1 has a strong scavenging activity of self-oxidation of 1,2,3-phentriol at low dosage. Thus, MP1 should be explored as potential natural antioxidants.

## 4. Conclusions

On the basis of the above results, it could be concluded that the water-soluble polysaccharides extracted from mung bean (*V. radiata* L.) hull by ultrasonic assisted treatment, predominantly consisted of two polysaccharide fractions (MP1, and MP2). The purification of MP1 and MP2 achieved very high purity by using DEAE-cellulose and Sephadex G-100 chromatography. MP1 and MP2 were defined as acid heteropolysaccharides with different content of uronic acids as well as the ratio of neutral sugars.

MP1 showed strong antioxidant potential according to the *in vitro* evaluation of its reduction power, free radical-scavenging activity and self-oxidation of 1,2,3-phentriol inhibitory activities in comparison with BHA. Moreover, both MP1 and MP2 had a strong reduction power, which was equivalent with that of BHA at the dosage of 20 mg/mL. Although MP2 exhibited stronger hydroxyl radical-scavenging activity, it was less effective in scavenging superoxide radical, DPPH radical and self-oxidation of 1,2,3-phentriol than MP1. MP1 possess excellent antioxidant properties, thus, it can be developed as novel potential antioxidants.

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## References

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, *84*, 551–562.

Asamarai, A. M., Addisd, P. B., Epley, R. J., & Krick, T. P. (1996). Wild rice hull antioxidants. *Journal of Agricultural and Food Chemistry*, *44*, 126–130.

Blois, M. S. (1985). Antioxidant determination by the use of a stable radical. *Nature*, *181*, 1199–1200.

Bloknina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals Botany*, *91*, 179–194.

Blunenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic adids. *Analytical Biochemistry*, *54*, 484–489.

Bomet, F., Fontvieille, A., & Rizkalla, S. (1989). Insulin and glycemic responses in healthy humans to native starches processed in different ways: Correlation with *in vitro* α-amylase hydrolysis. *American Journal of Clinical Nutrition*, *50*, 315–323.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Chemistry*, *72*, 248–254.

Costa, L. S., Fidelis, G. P., Cordeiro, S. L., Oliveira, R. M., Sabry, D. A., Camara, R. B. G., et al. (2009). Biological activities of sulfated polysaccharides from tropical seaweeds. *Biomedicine & Pharmacotherapy*, doi:10.1016/j.biopha.2009.03.005

Dandona, P., Thusu, K., & Cook, S. (1996). Oxidative damage to DNA in diabetes mellitus. *Lancet*, *347*, 444–445.

Dische, Z. (1947). A new specific color reaction of hexuronic acids. *The Journal of Biological Chemistry*, *167*, 189–198.

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.

Duh, P. D., Du, P. C., & Yen, G. C. (1999). Action of methanolic extract of mung bean hull as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology*, *37*, 1055–1061.

Duh, P. D., Yeh, D. B., & Yen, G. C. (1992). Extraction and identification of an antioxidantive component from peanut hulls. *Journal of American Oil Chemistry Society*, *68*, 814–818.

Duh, P. D., Yen, W. J., Du, P. C., & Yen, G. C. (1997). Antioxidant activity of mung bean hulls. *Journal of American Oil Chemistry Society*, *74*, 1059–1063.

Fridovich, I. (1978). The biology of oxygen radicals. *Science*, *201*, 875–880.

Gnanasambanda, R., & Proctor, A. (2000). Determinations of pectin fegree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry*, *68*, 327–332.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. *Analytical Chemistry*, *165*, 215–219.

Imaida, K., Fukushima, S., Shicai, T., Ohtani, M., Nakanish, K., & Ito, N. (1983). Promoting activities of butylated hydroxyanisole and butylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis*, *4*, 885–889.

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N., & Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, *43*, 195–203.

Kallithraka, S., Bakker, J., & Clifford, M. N. (2001). Correlations between saliva protein composition and some T-I arameters of astringency. *Food Quality and Preference*, *12*, 145–152.

Knight, J. A. (1995). Diseases related to oxygen-derived free radicals. *Annals Clinical and Laboratory*, *25*, 111–121.

Li, G. R. (2002). Study on isolation of *Lycium barbarum* polysaccharide and its effects on anti-active oxygen free radicals. *Chinese Modern Applied Pharmacy*, *19*, 94–96 (in Chinese).

Li, Y. H., Jiang, B., Zhang, T., Mu, W. M., & Liu, J. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry*, *106*, 444–450.

Luo, D. H., & Fang, B. S. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant acitivities. *Carbohydrate Polymers*, *72*, 376–381.

MacDonald, J., Galley, H. F., & Webster, N. R. (2003). Oxidative stress and gene expression in sepsis. *British Journal of Anaesthesia*, *90*, 221–232.

Mathlouthi, M., & Koenig, J. (1986). Vibrational spectra of carbohydrate. *Advances in Carbohydrate Chemistry and Biochemistry*, *44*, 7–89.

Marklund, S., & Marklund, G. (1974). Involvement of superoxide anion radicals in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, *47*, 469–471.

Mislovicova, D., Masarova, J., Bendzalova, K., Soltes, L., & Machova, E. (2000). Sonication of chitin-glucan, preparation of water-soluble fractions and characterization by HPLC. *Ultrasonics Sonochemistry*, *7*, 63–68.

Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus arueus*) as affected by some home traditional processes. *Food Chemistry*, *89*, 489–495.

Naik, G. H., Priyadarsini, K. I., Satav, J. G., Banavalikar, M. M., Sohoni, P. P., & Biyani, M. K. (2003). Comparative antioxidant activity of individual herbal components used in *Ayurvedic* medicine. *Phytochemistry*, *63*, 97–104.

Ndhlala, A. R., Chitindingu, K., Mupure, C., Murenje, T., Ndhlala, F., Benhura, M. A., et al. (2008). Antioxidant properties of methanolic extracts from *Diospyros mespiliformis* (jackal berry), *Flacourtia indica* (Batoka plum), *Uapaca kirkiana* (wild loquat) and *Ziziphus mauritiana* (yellow berry) fruits. *International Journal of Food Science and Technology*, *43*, 284–288.

Ningappa, M. B., Dinesha, R., & Srinivas, L. (2008). Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chemistry*, *106*, 720–728.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Journal of Nutrition*, *44*, 307–315.

Percival, M. (1998). Antioxidant. *Clinical Nutrition Insight*, *31*, 1–4.

Qi, H. M., Zhang, Q. B., Zhao, T. T., Chen, R., Zhang, H., & Niu, X. Z. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. *International Journal of Biological Macromolecules*, *37*, 195–199.

Qiao, D. L., Ke, C. L., Hu, B., Luo, J. G., Ye, H., Sun, Y., et al. (2009). Antioxidant activities of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*, *78*, 199–204.

Rollet-Labelle, E., Grange, M. J., Elbim, C., Marquetty, C., Gougerot-Pocidalo, M. A., & Pasquier, C. (1998). Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Radical Biology and Medicine*, *24*, 563–572.

Simic, M. G. (1998). Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis. *Mutation Research*, *202*, 377–386.

Staniek, K., & Nobl, H. (1999). $H_2O_2$ detection from intact mitochondria as a measure for on electron reduction of dioxygen requires a non-invasive assay system. *Biochimica Biphysica Acta*, *1413*, 70–80.

Staub, A. M. (1965). Removeal of protein-Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.

Stohs, S. J., & Baguchi, D. (1995). Oxidative mechanism in the toxicity of metal ions. *Free Radical Biology and Medicine*, *18*, 321–336.

Sun, Z. W., Zhang, L. X., Zhang, B., & Niu, T. G. (2010). Structural characterisation and antioxidant properties of polysaccharides from the fruiting bodies of *Russula virescens*. *Food Chemistry*, *118*, 675–680.

Wang, Z. J., & Luo, D. H. (2007). Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum Makino*. *Carbohydrate Polymers*, *68*, 54–58.

Witztum, J. L. (1994). The oxidation hypothesis of atherosclerosis. *Lancet*, *344*, 793–795.

Xu, W. T., Zhang, F. F. F., Luo, Y. B., Ma, L. Y., Kou, X. H., & Huang, K. L. (2009). Antioxidant activity of a water-soluble polysachcharide purified from *Pterdium aquilinum*. *Carbohydrate Research*, *344*, 217–222.

Yang, B., Jiang, Y. M., Zhao, M. M., Shi, J., & Wang, L. Z. (2008). Effects of ultrasonic extraction on the physical and chemical properties of polysaccharide from longan fruit pericarp. *Polymer Degradation and Stability*, *93*, 268–272.

Zhang, W. J. (2001). *Technology of biochemical research on compound polysaccharide* (2nd ed.). Hangzhou: Zhejiang University Press., pp. 108–111 (in Chinese).