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Full Length Research Paper

Antioxidant and anti-tumor activities of purified polysaccharides with low molecular weights from *Magnolia officinalis*

Minbo Lan[1,2], Jing Guo[1], Hongli Zhao[1] and Huihui Yuan[1]*

[1]Shanghai Key Laboratory of Functional Materials Chemistry, and Research Center of Analysis and Test, Shanghai 200237, P. R. China.
[2]Key Laboratory for Ultrafine Materials of Ministry of Education, East China University of Science and Technology, Shanghai 200237, P. R. China.

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Magnolia officinalis **is an important Chinese traditional herb and the *M. officinalis* polysaccharides are considered as their main active ingredients in the decoction. In this study, *M. officinalis* polysaccharides were extracted by hot water and divided into three fractions. The molecular weights of the three polysaccharides fractions ranged from 3.9 to 4.2 kDa, 4.7 to 5.4 kDa and 5.6 to 9.4 kDa, respectively. And the polysaccharides were composed of fucose, xylose, mannose, glucose, galactose and arabinose. The antioxidant assays of the polysaccharides, including scavenging activity of superoxide anion and hydrogen peroxide, 2,2-diphenyl-1-picryl-hydrazyl, reducing power, chelating effect on metal ion and the total antioxidant activity were carried out *in vitro*. Moreover, their anti-tumor activity *in vitro* was determined by MTT assay with human breast carcinoma model cell line (MCF-7). The results indicated that the polysaccharides fractions with molecular weight 3.9 to 4.2 kDa exhibited the highest antioxidant activities and the strongest cytotoxic activity in MCF-7 cells.**

Key words: Polysaccharides, *Magnolia officinalis,* low molecular weight, antioxidant activity.

INTRODUCTION

Free radicals can react with biomolecules, causing extensive damage to DNA, protein, and lipid, which are considered to be related to aging (Oliveira et al., 2010), degenerative diseases of aging (Gey, 1990; Oberley, 2002), cancer (Sangeetha et al., 1990) and neurodegenerative disorders as well (Good et al., 1996). To suppress this reaction induced by free radicals, a number of synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have been proposed and synthesized in recent years. Unfortunately, these antioxidants often exhibited undesirable effects on some enzymes of human organs (Shahidi, 1997; Valentao et al, 2002). Therefore, it is necessary to find a safe and highly active natural anti-oxidant agent for scavenging free radicals or preventing the formation of free radicals.

Due to safety and high activities, edible plants have attracted much attention nowadays. Substantial investigations have found that some plant polysaccharides have strong antioxidant (Wang and Luo, 2007; Jiang et al., 2008), anticancer (Shi et al., 2007) and antiaging activities (Li et al., 2007). Furthermore, the plant polysaccharides with low molecular weights are proven to possess higher antioxidant activity than the polysaccharides with high molecular weights, such as polysaccharides extracted from *Porphyridium cruentum* and rice bran (Sun et al., 2009; Zha et al., 2009).

Magnolia officinalis, a traditional herb in Chinese medicine soups, has been widely used to treat thrombotic stroke, gastrointestinal complaints, anxiety and nervous disturbance (Patočka et al., 2006). The existing studies indicated that the polysaccharides were the major bioactive ingredients of *M. officinalis* (Guo et al., 2004). However, up to date, no study has reported about the antioxidant and anti-tumor activities of the

*Corresponding author. E-mail: yuanhuihui@ecust.edu.cn. Tel: +86-21-64252055. Fax: +86-21-64250624.

polysaccharides of *M. officinalis*.

Herein, the objective of this research was to study the antioxidant activity and the potential anti-tumor activity of the *M. officinalis* polysaccharides with low molecular weight and the related effect of the molecular weight. Three polysaccharides fractions with molecular weights ranged from 5.6 to 9.4 kDa, 4.7 to 5.4 kDa and 3.9 to 4.2 kDa were extracted and isolated from *M. officinalis* by the traditional hot water process. The monosaccharide composition and molecular weight of the extracts were analysed by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC). The antioxidant activities of the polysaccharides fractions were evaluated by scavenging hydrogen peroxide, superoxide anion, 2,2-diphenyl-1-picryl-hydrazyl, chelating effect on ferrous ion and reducing power *in vitro*. Finally, the anti-tumor activity of the polysaccharides derived from *M. officinalis* was evaluated with human breast carcinoma model cell line by MTT assay.

MATERIALS AND METHODS

The bark of *M. officinalis* originally from Sichuan, was purchased from a local herb market (Shanghai, China). Adsorption resins ADS-7 were purchased from the chemical plant of Nankai University (Tianjin, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH), trolox, nitroblue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH) and ferrozine were purchased from Sigma (USA). 2,2-azino-bis (3-ehylbenzthiazoline-6-sulphonate) (ABTS) and phenazine methosulfate (PMS) were purchased from Shanghai Biotechnology Co. Ltd. (Shanghai, China). Human breast carcinoma cell line was obtained from Institute of Biochemistry and Cell Biology (Shanghai, China). Trypsin, dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS) and methyl thiazolyl tetrazolium (MTT) were purchased from GIBCO (USA). Standard dextrans with molecular weight of 1000, 5000, 12000, 80000 were purchased from Fluka (USA). The standard monosaccharides, fucose, xylose, mannose, glucose, galactose and arabinose, were purchased from Sigma (USA). All other chemicals were of analytical grade.

Extraction of polysaccharides

Prior to extraction, the bark of *M. officinalis* was first ground to a fine powder and incubated in 95% EtOH at room temperature for 2 days to remove the EtOH-soluble dyes, oligosaccharides and other small molecules. The pretreated plants were dried at 40°C for 48 h and then mixed with distilled water to a final concentration of 100 mg/ml. After that, the mixture was heated at 95 to100°C for 3 h, and the extract was concentrated to certain volume and centrifuged at a speed of 8000 r/min for 45 min to obtain water-soluble polysaccharides. And then, anhydrous alcohol was added to the polysaccharides solution to get a final concentration of 40% (v/v) solution. The suspension was centrifuged and the precipitated was crude polysaccharides 1. Then, anhydrous alcohol was added to the supernatant of 40% ethanol solution to obtain a final concentration of 60% (v/v). The precipitated was crude polysaccharides, 2. Similarly, anhydrous alcohol was added to the supernatant of 60% ethanol solution to get a final concentration of 80% (v/v). The precipitated was crude polysaccharides, 3. Proteins were removed by Sevage method. And, the crude polysaccharides (1 g) dissolved in water were chromatographed on the adsorption resins ADS-7 column and successively eluted with water to yield the corresponding PM1, PM2 and PM3. The eluted rate was 1 ml/min.

Analysis of monosaccharide composition

PM1, PM2 and PM3 (5 mg) were hydrolyzed with 2 M trifluoroacetic acid solution (TFA, 2 ml) at 100°C in sealed tubes for 3 h. After that, excess TFA was removed by evaporation on a water bath at 40°C and co-distilled with MeOH (2 ml × 3), and then the residue was dissolved in deionized water for derivatization. 20 mg of sodium borohydride was added in residue solution to oximate at room temperature for 1 h. Acetic acid was added into the previous mixture to neutralize excess sodium borohydride. And then 2 ml of acetic anhydride was added into the mixture for acetylation, reacting at 100°C for 1 h. After cooling down to room temperature, 2 ml of chloroform was added into the mixture for extracting the derivative, then they were washed with 2 ml deionized water 6 times. Subsequently, the organic phase was collected and evaporated to remove chloroform. The derivative was dissolved in 100 µl of chloroform and 1 µl of the derivative sample was injected into GC for analysis (Chang et al., 2010).

Gas chromatography-mass spectrometry (GC-MS), Agilent 6890, was used to identify and quantify the monosaccharide composition in three polysaccharides fractions. GC-MS analysis was fitted with a fused silica capillary column of HP-5 (30 m × 0.25 mm × 0.25 µm). The column oven was started at 150°C for 3 min, and then the temperature was raised to 250°C at 5°C /min and maintained for 10 min.

Determination of molecular weight

Molecular weight ranges of the PM1, PM2 and PM3 obtained were determined by a high performance liquid chromatography (HPLC), Agilent 1100 with TSK gel column (TSK G3000 PWXL, 7.8 × 300 mm). The standard curve was determined by standard dextrans with molecular weight of 1000, 5000, 12000 and 80000. The sample concentration was 1 mg/ml, and its injection volume was 20 µl. The eluent was NaCl solution (0.2 M), and the flow rate was 0.5 ml /min.

Assay of antioxidant activities of the polysaccharides fractions

Scavenging of superoxide anion

The activity of scavenging superoxide anion was measured according to the method reported by Zou et al. (2010) with some modifications. The superoxide radicals were generated in PMS-NADH system and assayed by the reduction of NBT (Liu et al., 2007). 1 ml of NBT solution (156 µM of NBT in 0.1 M phosphate buffer, pH 7.4), 1 ml of NADH solution (468 µM of NADH in 0.1 M phosphate buffer, pH 7.4) and 1 ml of sample solution were mixed. The reaction was started by adding 1 ml of PMS solution (60 µM of PMS in 0.1 M phosphate buffer, pH 7.4) to the mixture. The mixture was incubated at 25°C for 5 min, and the absorbance was measured at 560 nm. The scavenging effect was calculated by the following formula:

Inhibition rate (%) = $[1-A_1/ A_0]*100$

where A_0 is the absorbance of the control (water instead of sample solution), A_1 is the absorbance of the polysaccharides solution.

Scavenging of hydrogen peroxide

The activity of scavenging hydrogen peroxide was measured

according to the method reported by Liu et al. (2007). Reaction mixture contained 2.4 ml of phosphate buffer (0.1 M, pH 7.4), 0.6 ml of H_2O_2 solution (0.04 M) and 1 ml of sample solution. Absorbance at 230 nm was measured after 10 min. The scavenging effect was calculated by the following formula:

Inhibition rate (%) = $[1-A_1/ A_0]*100$

where A_0 is the absorbance of the control (water instead of sample solution), A_1 is the absorbance of the sample.

Scavenging of DPPH radicals

The activity of scavenging DPPH was measured according to the method reported by Koksal et al. (2011) with minor modifications. Reaction mixture containing 0.2 ml of DPPH solution (0.4 mM in absolute ethanol), 1 ml of sample solution and 2 ml water was shaken and kept for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. The scavenging effect was calculated by the following formula:

Inhibition rate (%) = $[1-A_1/ A_0]*100$

where A_0 is the absorbance of the control (water instead of sample solution), A_1 is the absorbance of the sample.

Reducing power

The reduction potential was determined based on the method reported by Lin et al. (2009). Reaction mixture containing 1 ml of phosphate buffer (0.2 M, pH 6.6), 0.5 ml potassium ferricyanide (1%, w/v) and 1 ml of sample was incubated at 50°C for 20 min. After cooling down, 1 ml of trichloroacetic acid (10%, w/v) and 0.2 ml of fresh ferric trichloride ($FeCl_3$, 0.1%, w/v) were added to the mixture. The mixture was shaken well and its absorbance was measured at 700 nm. Reduction power was calculated as follows:

Reducing power = (A_1-A_2)

where A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of $FeCl_3$ solution.

Chelating effect on metal ion

The chelating effect on metal ion was measured according to the method reported by Zou et al. (2010) with some modifications. Briefly, 1 ml sample of solution, 0.05 ml of ferrous chloride ($FeCl_2$) solution (2 mM), 0.2 ml of ferrozine solution (5 mM) and 1 ml water were mixed, and the mixture was shaken well and incubated at room temperature for 10 min. The absorbance was measured at 562 nm. The chelating effect was calculated by the following formula:

Chelating rate (%) = $[1-A_1/ A_0]*100$

where A_0 is the absorbance of the control (water instead of sample solution), A_1 is the absorbance of the sample.

Total antioxidant activity

The experiment was carried out according to the method reported by Re et al. (1999) and Li et al. (2005). This assay cannot directly determine the antioxidant ability of the tested sample, but it can reflect the reaction capacity of the tested sample with ABTS radicals of tested sample by TEAC value. Reaction mixture including 5 ml of ABTS solution (7 mM) and 88 µl of potassium persulphate ($K_2S_2O_8$) solution (140 mM) was incubated at room temperature for 12 to 16 h in the dark to produce ABTS radicals. The ABTS radical solution was diluted by phosphate buffer (0.1 M, pH 7.4) to obtain an absorbance of 0.7±0.02 at 734 nm measured at 30°C. Sample solution (200 µl) and 3 ml of ABTS radical solution were mixed and left for 7 min at room temperature in the dark. The absorbance was measured at 734 nm at 30°C. They produced 20 to 80% inhibition of the blank absorbance. A standard curve of Trolox ranged from 8 to 1500 µM. The antioxidant ability was expressed as mmol/L Torlox equivalents / mg/ml polysaccharides.

Cytotoxicity assay

Cell lines and culture

Human breast carcinoma cell line (MCF-7) was cultured in DMEM containing 10% (v/v) heat inactivated fetal bovine serum (FBS) supplemented with penicillin (100 U/ ml) and streptomycin (100 µg/ ml) in an incubator (37°C, 5% CO_2).

After reaching desirable confluence, cells were detached with 0.25% trypsin/0.03% ethylenediamine tetraacetic acid (EDTA) and re-suspended in medium at the desired density for later experiments.

MTT assay

Cytotoxicity was evaluated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay. MCF-7 cells were plated into 96-well plate at a density of 2.0×10^4 cells per well in 200 µl culture medium. After 24 h incubation, the growth media was removed and the cells were washed with PBS (2×), then each well was added 200 µl of DMEM containing PM1, PM2 and PM3 with various concentrations, ranging from 25 to 150 µg/ml, and the 200 µl DMEM in the absence of polysaccharides was used as a negative control. After 48 h exposure, the DMEM media in the 96-well plate were all substituted by 200 µl of fresh DMEM and 200 µl sterile filtered 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide stock solution (5 mg/ml) in PBS pH 7.4 was added to each well and the cells were further incubated for 4h at 37°C to allow the yellow dye to be transformed into blue crystals. Then, the unreacted dye was removed by aspiration, and 200 µl of DMSO was added to each well to dissolve the dark blue crystal. Finally, the optical density was measured by microtitre reader at wavelength of 590 nm with a THERMO Multiskan MK3. Eight duplicates were prepared for each condition. The cell cytotoxicity was calculated by the following formula:

Inhibition rate (%) = $[1-(OD_{treated}/OD_{control})]*100$

where $OD_{control}$ is obtained in the absence of polysaccharides and $OD_{treated}$ is obtained in the presence of polysaccharides.

Statistical analysis of data

All the data were expressed as means ± standard deviation (SD) of three independent replications and analyzed by one-way analysis of variance (ANOVA). Differences were considered to be statistically significant if $P < 0.05$.

Table 1. The composition of different polysaccharides.

Parameter	Fuc (%)	Xyl (%)	Man (%)	Glc (%)	Gal (%)	Ara (%)
PM1	1.54	3.89	17.86	54.29	14.95	
PM2	1.78	7.24	10.51	64.93	14.76	
PM3	2.94	4.31	21.97	33.97	33.65	3.09

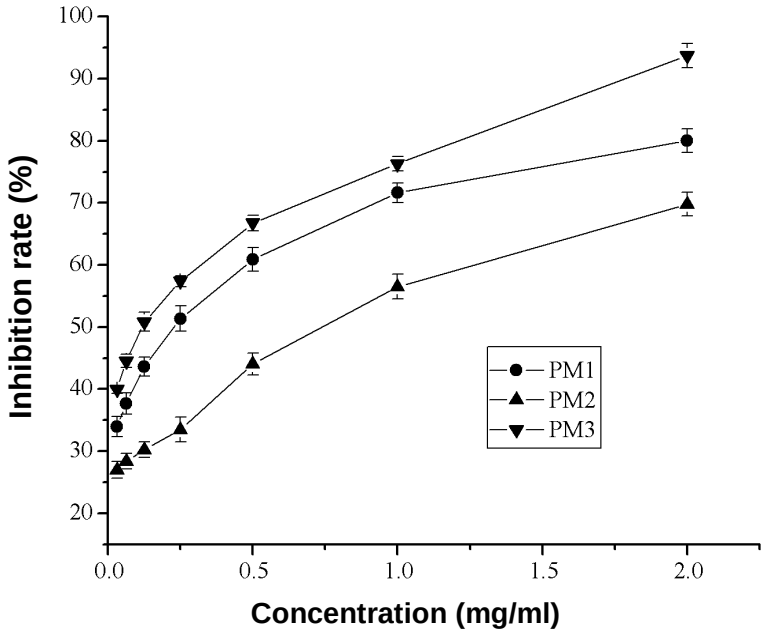

Figure 1. Superoxide anion scavenging activities of different polysaccharides. Data are presented as mean ± SD (n = 3).

RESULTS AND DISCUSSION

Composition and molecular weight of the polysaccharides

Guo et al. (2004) has found that the content of polysaccharide obtained by the hot water process could be modulated by the parameters of the hot water process. So, in the study, to achieve a maximum extraction of the polysaccharides, the main experimental conditions, such as the volume ratio of sample to solvent, precipitation temperature and time were first optimized and under the optimal extraction conditions, the yield of PM1, PM2 and PM3 were 0.036, 0.146 and 0.64%, respectively. No proteins could be detected in the three polysaccharides fractions by Lowry and ultraviolet (UV) scanning method. Table 1 shows the monosaccharide composition and the content of various compositions in PM1, PM2 and PM3. It can be seen that PM1 and PM2 were mainly composed of Fuc, Xyl, Man, Glc and Gal. But for PM3, besides the previous compositions, Ara was also detected. According to the standard curve of different molecular weight dextrans, the molecular weight distributions of PM1, PM2 and PM3 ranged from 5.6 to 9.4 kDa, 4.7 to 5.4 kDa, and 3.9 to 4.2 kDa, respectively.

In vitro antioxidant activities

Scavenging activity of superoxide anion

Superoxide anion is proposed as a signaling molecule in arteries and in the central nervous system. Due to the instability and poor membrane permeability, superoxide anion speculated to be an intracellular messenger (Saran and Bors, 1994). It is accepted that superoxide anion cannot only be converted to hydrogen peroxide by the enzyme superoxide dismutase (SOD), but also allow neutrophils to oxidize chloride ions into hypochlorous aids, providing additional cytotoxic activities (Macdonald et al., 2003). So in this study, the scavenging capacity of superoxide anion of polysaccharides fractions was first evaluated.

Figure 1 illustrates the capability of scavenging

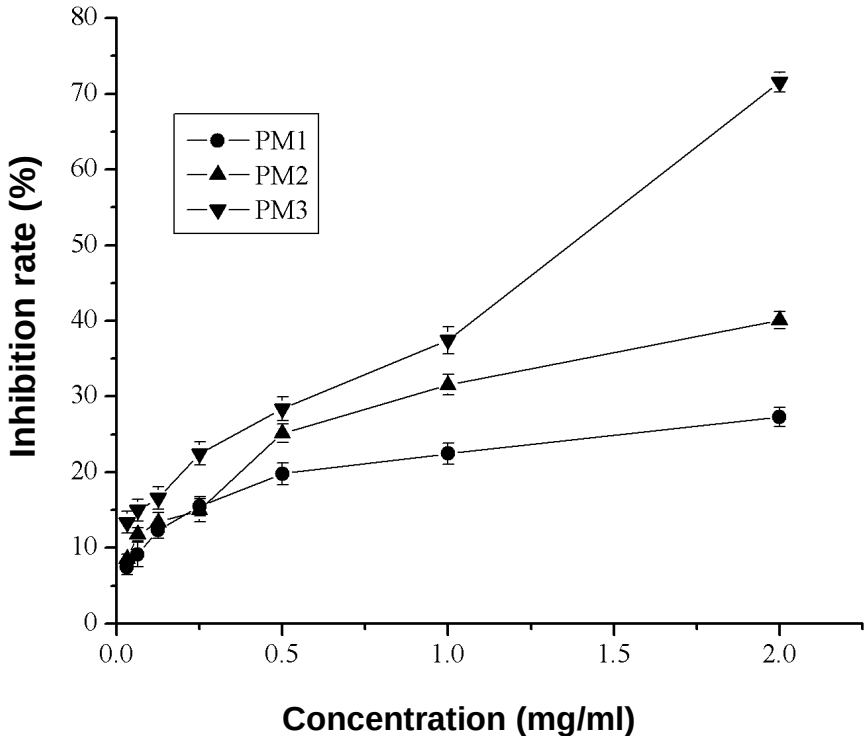

Figure 2. Hydrogen peroxide scavenging activities of different polysaccharides. Data are presented as mean ± SD (n = 3).

superoxide anions of three polysaccharides fractions. As shown, at relative low concentration (0.031 mg/ml), the polysaccharides fractions with various molecular weight all exhibited moderate scavenging activity ranging from 27.0 to 40.0%. With the increase of the concentrations of the polysaccharides from 0.031 to 2 mg/ml, the scavenging activity was correspondingly enhanced, thereby exhibiting a typical concentration-dependent manner. And at the concentration of 2 mg/ml, the inhibiting rate of PM1, PM2 and PM3 were attained 80.0, 69.8 and 93.7%, respectively. In comparison, in the whole concentration range studied, PM3 showed the top level of inhibiting rate followed by PM1 and PM2.

Scavenging activities of hydrogen peroxide

Hydrogen peroxide is not very active, and it is a precursor to incontestable radical species such as peroxyl radical, hydroxyl revolutionary, and superoxide. However, it can be fairly stable in the absent of transition metal ions.

The hydrogen peroxide scavenging abilities of PM1, PM2 and PM3 are shown in Figure 2. A low inhibition rate can be found from 7.5 to 13.4% at a low concentration of 0.031 mg/ml. With the increase of the concentration from 0.031 to 2 mg/ml, the inhibition rate of the three polysaccharides fractions were all enhanced. Under the same concentration at 2 mg/ml, PM3 showed the highest scavenging effect (71.6%), followed by PM2 (40.1%) and PM1 (27.2%).

Scavenging activities of DPPH

DPPH is a stable free radical, and its assay is simply and widely used to evaluate the antioxidant activities of natural compounds (Naik et al., 2003; Hu et al., 2004). The abilities of scavenging DPPH radicals of PM1, PM2 and PM3 are exhibited in Figure 3. Just as the previous scavenging abilities of superoxide anion and hydrogen peroxide, poor scavenging effects were also observed for PM1 (12.4%), PM2 (7.8%) and PM3 (17.4%) at low concentration (0.031 mg/ml). Following the rise of concentration from 0.031 to 2 mg/ml, the inhibition rate of various polysaccharides fractions showed a similar increase trend. At the same concentration of 2 mg/ml, PM3 showed the most effective scavenging activity (63.8%), followed by PM1 (57.9%) and PM2 (36.8%).

Reducing power

Reducing power is related to antioxidant activity. In this assay, it could be seen that the mixture color changed from yellow to various shades of blue associated with the reducing power of each polysaccharides. Figure 4 depicts

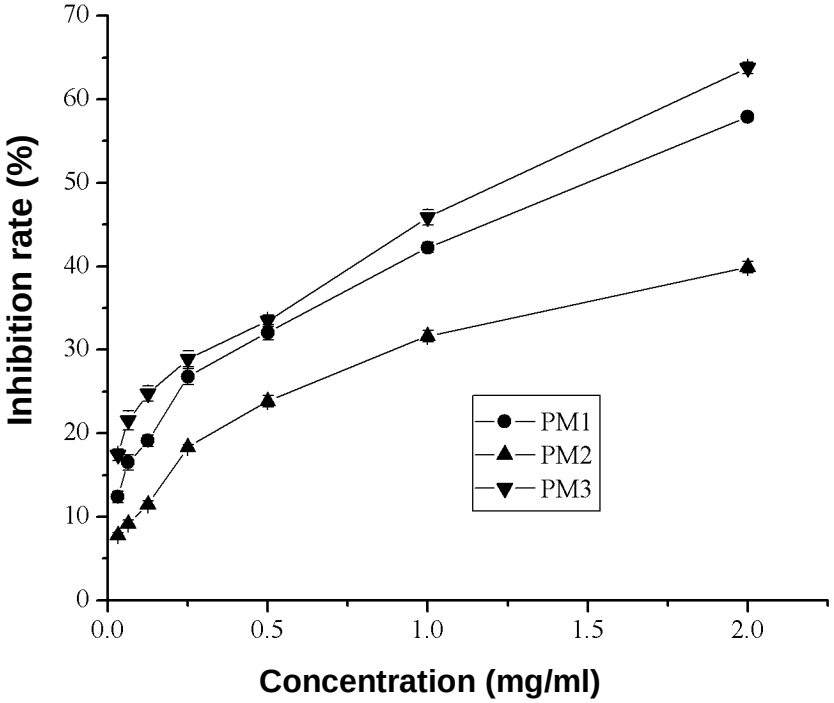

Figure 3. DPPH scavenging activities of different polysaccharides. Data are presented as mean ± SD (n = 3).

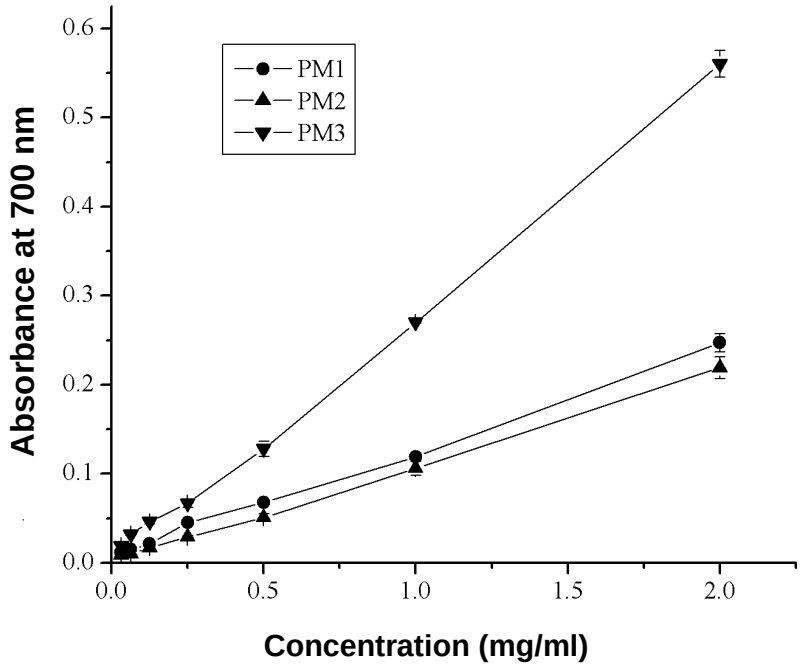

Figure 4. Reducing power of different polysaccharides. Data are presented as mean ± SD (n = 3).

the reducing power of three polysaccharides fractions. As shown, at low concentration (0.031 mg/ml), the polysaccharides fractions with various molecular weight all exhibited poor reducing power. When the

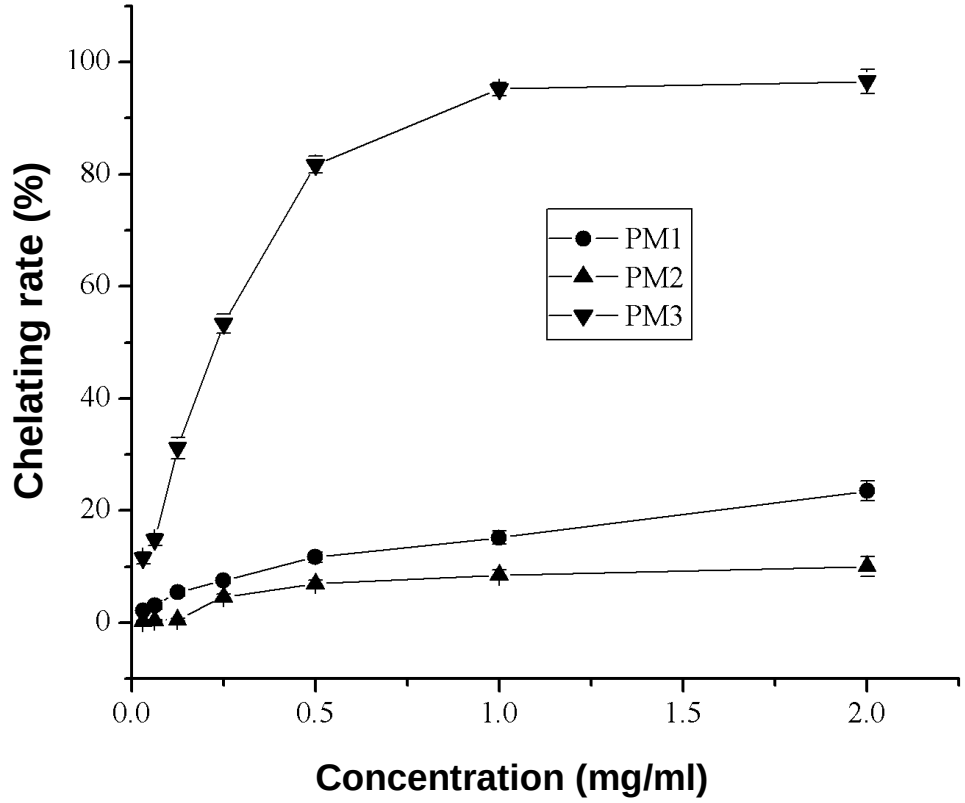

Figure 5. Chelating effect on metal ion of different polysaccharides. Data are presented as mean ± SD (n = 3).

concentration increased in the range from 0.031 to 2 mg/ml, the reducing power of various polysaccharides fractions increased. In comparison, reducing power of PM3 was 0.56 at a concentration of 2 mg/ml, which was much stronger than that of PM1 (0.24) and PM2 (0.22).

Chelating effect on metal ion

The chelating effect on metal ion is proven to be related to antioxidant activity. Because of high reactivity, transition metals iron is known as the most important lipid oxidation pro-oxidant (Liu et al., 2007). The high chelating effect on iron indicates high antioxidant activity. Ferrozine can form complex with ferrous ion quantitatively. In the present of chelating agents such as EDTA and polysaccharides, the complex formation is disrupted, and thus a decrease in the red color of the complex can be measured.

As shown in Figure 5, at low concentration (0.031 mg/ml), low chelating effects ranged from 0.24 to 11.6%. With the rise of the concentration, the chelating effect of three polysaccharides fractions all increased, especially for the PM3. Under the same concentration at 2 mg/ml, the value of chelating rate of PM3 achieved a peak value of 96.5%, which was 4.10 times of PM1 (23.5%) and 9.65 times of PM2 (10.0%).

Total antioxidant activity

This assay is based on the scavenging of relatively stable blue/green ABTS radical, converting it into colorless product (Arts et al., 2003). The equivalent antioxidant capacity (TEAC) values of polysaccharides were calculated by a Trolox standard curve. The standard curve of TEAC value was expressed as:

$Y = 68.00X + 1.984$ ($R^2 = 0.9978$)

where Y is inhibition rate, X is Trolox concentration. The abilities of scavenging ABTS radicals of PM1, PM2 and PM3 are shown in Figure 6. At low concentration, the TEAC value of each polysaccharides fraction was very low. And the TEAC value rose with the increase of polysaccharides concentration. Under the same concentration at 2 mg/ml, PM3 reached a peak TEAC value (1.12) followed by PM1 and PM2. The abilities of scavenging ABTS radicals of PMs were in accordance with those of polysaccharide fractions of rice bran (Zha et al. 2009). Furthermore, the TEAC value of PM3 was higher than that of rice bran polysaccharide fractions and

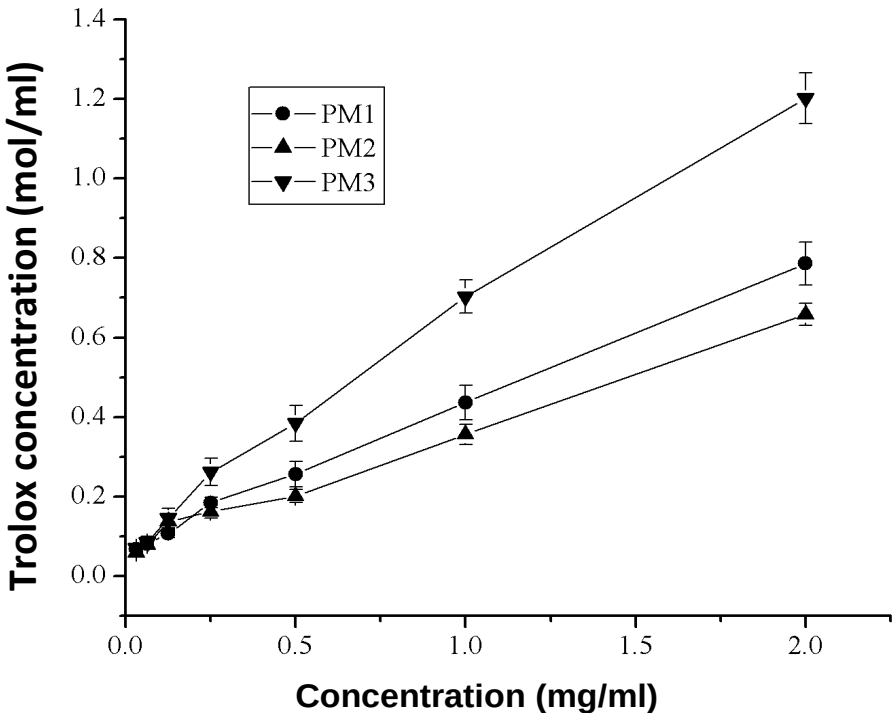

Figure 6. TEAC of different polysaccharides. Data are presented as mean ± SD (n = 3).

nearly equivalent to the value of ascorbic acid at the concentration of 1 mg/ml. Disease is often associated with free radical damage and immunodeficiency. Excess free radicals in the body can result in oxidative damage of the kidneys and liver, and hemorrhage of the heart and brain. It is well- known that there are various free radical in human body. And it is relative complicate to evaluate the antioxidant ability.

In this study, the generally used methods, including scavenging superoxide anion, hydrogen peroxide, DPPH, reducing power, chelating effect on metal ion and total antioxidant activity, are performed to evaluate the antioxidant activity of the polysaccharides extracted from *M. officinalis.* Data obtained here confirmed that the molecular weight was responsible for scavenging free radical ability of the polysaccharides of *M. officinalis*. And for the three fractions PMs, the PM3 with the lowest MW exhibited the highest free-radical scavenging capacities. This is consistent with the previous report that the smaller the MW of polysaccharides often led to the higher antioxidant activity (Qi et al., 2005; Asker et al., 2007). However, it is worthwhile to note that when the weight of the PM1 was compared with the highest molecular weight, the PM2 showed weak free-radical scavenging capacities. This phenomenon is somehow contradictory with the aforementioned reports (Qi et al., 2005; Asker et al., 2007). We think this might be related to the antioxidant mechanism and the functional groups of the PMs. Generally speaking, in the free radical theory, there are two antioxidant mechanisms, cleaning of the free radicals generated and suppression of the generation of free radicals. In the process of cleaning the free radicals mechanism, polysaccharides served as hydrogen-atom donors because of weak dissociation energy of O-H bond, and free radicals accepted donated electron to form stable products, so that the free radicals were quenched and chain reactions were interrupted (Yamashoji and Kajimoto, 1980; Yin et al., 2010). Under this premier, the more number of electron-withdrawing groups, such as hydroxyl and carboxyl groups in polysaccharides, the weaker dissociation energy of O-H bond and the higher antioxidant activity (Shimada et al., 1996; Yuan et al., 2005). Correlated the scavenge capacities of superoxide anion and DPPH radicals with the PMs, it can be hypothesized that the PM1 obtained might contain more electron-withdrawing groups than the PM2. This needs further investigation. Reducing power, chelating effect and scavenging hydrogen peroxide assays were considered to be based on the mechanism of suppression against free radicals generation. It is believed that the variety and amount of the reducing compounds in the polysaccharides often contribute to their reducing power (Lin et al., 2009). So we believe that the difference of the reducing power of the polysaccharides fractions of the *M. officinalis* might be determined by their composition. As for the metal ion chelating effect, it is often accepted that the PMs with particular spatial structure and functional group like

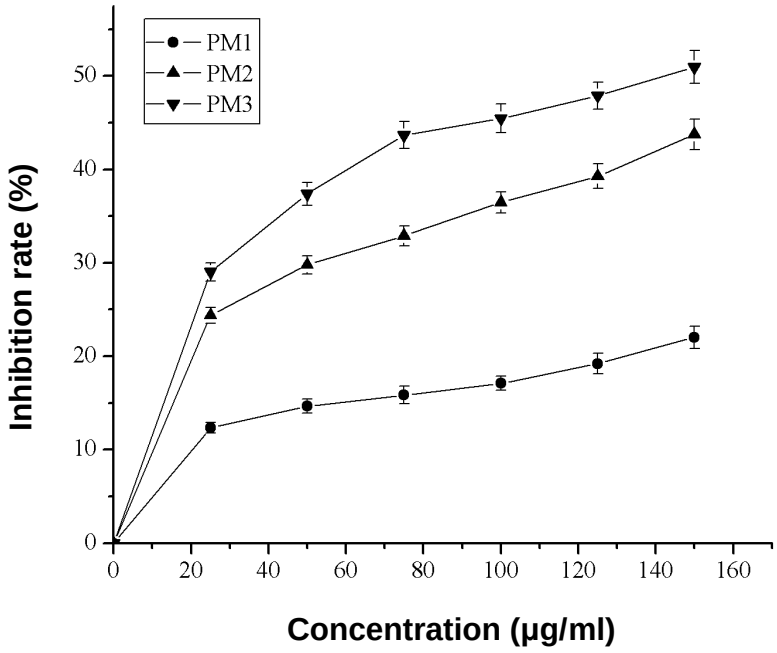

Figure 7. Cytotoxicity of different polysaccharides. Data are presented as mean ± SD (n = 3).

hydroxyl or carboxyl maybe possibly easy to chelate metal ion (Shimada et al., 1996; Yuan et al., 2005), which could interrupt the generation of free radicals. Based on these results, it can be inferred that besides the molecular weight, some other functional group in polysaccharides fractions, such as hydroxyl and carboxyl groups may also affect antioxidant activities.

Cytotoxicity of polysaccharides fractions on MCF-7 cell

In order to evaluate and compare the anti-tumor activity of the polysaccharides of *M. officinalis,* MCF-7 cells were treated with different concentrations of PM1, PM2 and PM3, cultured up to 48 h. Figure 7 shows cyctotoxic activity of various polysaccharides fractions. It can be seen that the growth of the MCF-7 cells was significantly inhibited by PM1, PM2 and PM3 and the inhibition rates all increased with the elevating of the concentration of the polysaccharides. Specifically, when the concentration increased from 25 to 150 μg/ml, the inhibition rate of various polysaccharides exhibited increase tendency ranging from 12.4 to 23.4% for PM1, 24.4 to 43.7% for PM2 and 29.0 to 51.0% for PM3. In contrast, PM3 exhibited the strongest inhibition rate and PM1 showed the weakest within the whole concentration range. This is in agreement with the report where they found that the polysaccharides with low molecular weight could be easy to invade the cell and produce a marked effect (Yuan et al., 2008).

Conclusion

In conclusion, three purified polysaccharides factions PM1, PM2, and PM3 with different molecular weights were extracted with boiling water, followed by precipitation with ethanol. They were analyzed for their composition and molecular weight. The result indicated that the molecular weight distributions of PM1, PM2 and PM3 ranged from 5.6 to 9.4 kDa, 4.7 to 5.4 kDa and 3.9 to 4.2 kDa, respectively. They were composed of Fuc, Xyl, Man, Glc, Gal and Ara, however, arabinose was detected only in PM3. The antioxidant and cytotocxicity of PM1, PM2 and PM3 were evaluated by several assays *in vitro*. PM3 with the lowest molecular weight presented higher antioxidant activity than others, moreover it had higher cytotoxicity than the others.

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