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Anti-oxidation and anti-microorganism activities of purification polysaccharide from *Lygodium japonicum* in vitro

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Abstract

In this study, we extracted polysaccharides from fern *Lygodium japonicum* and obtained purification polysaccharides by a DEAE-52 cellulose column (1.6 × 30 cm). The antioxidant activity of the purification polysaccharides was evaluated by various antioxidant assay, including $DPPH^-$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities, and inhibition against liposome peroxidation. Those various antioxidant activities were compared to standard antioxidants such as vitamin C. The results indicate that the purification polysaccharides showed strong antioxidant activity against liposome peroxidation, $DPPH^-$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities. This antioxidant property increase with increased amount of sample. In addition, the purification polysaccharides were still determined as anti-microorganism equivalents. The results obtained in the present study indicated that the purification polysaccharides is a potential source of natural broad-spectrum anti-microorganism. Although, the tests presented here show the usefulness of purification polysaccharides as in vitro antioxidants and antibacterial agent, it still needs to be that this purification polysaccharides show their activity in biological systems, health implications or dry foods, which will be implemented in our later work.

Keywords: *Lygodium japonicum*; Anti-oxidation; Anti-microorganism; Polysaccharides; Superoxide anion; Hydrogen peroxide

1. Introduction

The use of medicinal plants is a traditional form of providing relief from illness and can be traced back over five millennia in several civilizations (Hostettmann, Marston, Maillard, & Hamburger, 1995). Over the years, natural products have contributed enormously to the development of important therapeutic drugs used currently in modern medicine (Cragg, Newman, & Snader, 1997; De Smet, 1997; Shu, 1998). The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000 plant species existing world-wide, only a small percentage have been investigated phytochemically, and the fraction submitted to biological or pharmacological screening is even smaller (Hamburger & Hostettmann, 1991). Plants are naturally gifted at the synthesis of medicinal compounds. About 25% of all available modern drugs are derived directly or indirectly from higher plants (De Smet, 1997; Farnsworth & Bingel, 1997). The extraction and characterization of active compounds from medicinal plants and the search for these new pharmacologically active agents has led to the discovery of many clinically useful drugs that play a major role in the treatment of human disease (Colegate & Molyneux, 1993; Donehower & Rowinsky, 1993). A classic example is aspirin, which was initially discovered as salicylic acid in willow bark and leaves (Colegate & Molyneux, 1993); another noted example is taxol, recently proven to be effective against

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breast and ovarian cancers, which was initially discovered in bark of yew trees (Donehower & Rowinsky, 1993). This has renewed the interest of the major pharmaceutical companies in higher plant-derived secondary metabolites as part of the search for new clinically useful drugs, partly because of the realization that modern medicine is not capable of providing a "cure-all" solution against human diseases and that the presence of unwanted side-effects is almost unavoidable. Unlike modern drugs that invariably comprise a single active species, herb extracts and/or prescriptions contain multiple active constituents. Interestingly, natural compounds contained in these "herbal cocktails" can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side-effects (Kaufman, Csake, Warber, Duke, & Brielmann, 1999).

In recent years, the studies on "oxidative stress" and its adverse effects on human health have become a subject of considerable interest. It is a well-documented fact that exposure of organisms to exogenous and endogenous factors generates a wide range of reactive oxygen species (ROS), resulting in homeostatic imbalance (Bonnefont, Bastard, Jandon, & Delattre, 2000; Halliwell & Gutteridge, 1999; Sies, 1997; Thomas & Kalyanaraman, 1998). In addition, the increasing global incidence of food poisoning cases originating from food contaminated by pathogens has great social and economic costs and causes major concern, both to the general public and to the food industry (Bennett & Salmonella, 1997). The epidemiology of foodborne diseases is rapidly changing. The increased interest in biopreservation of food systems has recently led to the development of new natural antimicrobial compounds having different origins. Polysaccharides, as a important natural compound mainly from plenty of plants source, is causing of a great interest for an essential role in many molecular processes impacting eukaryotic biology and diseases and exhibit varied biological activities, such as antitumour, immunostimulant, anticancer, anticomplementary, anti-inflammatory, anticoagulant hypoglycaemic, antiviral, and immunological activities, which made them possible to be used in many fields including food, cosmetics, biomedicine, agriculture, environmental protection, and wastewater management (Abe & McKibbin, 1983; Majeti & Kumar, 2000; Shahidi & Synowiecki, 1991; Srivastava & Kulshreshtha, 1989).

Fern is sporous plant with fascicular, and now exists approximately 12,000 species around the globe, common in warm, darkness boondocks in torrid and subtorrid zone (Sun, Luo, Huang, Tian, & Wang, 2003). In china, there is plenty of fern source, about 61 families, 223 genus, 2600 species, in which about 300 species may be selected for the purpose of medicine usage. *Lygodium japonicum* (Thunb.), one of the most valued traditional Chinese medicines, is a member of fern Lygodiaceae Lygodium, and first year's growth liane, commonly found in hillside, forest, bosk, and lawn in the yangtse rive drainage area and many southern provinces in china.Whole plant or mature spore may be taken for medicine. It is commonly used in China for over 2000 years for the treatment of gorge gall, skin eczema, enteritis diarrhea, nephritis dropsy, urine calculus and infection, and so on (Mirna, Rožmanić, Peršić Branka Medjimurec, & Mira, 2002; Wynne et al., 1998). Modern medicine has revealed that polysaccharides in it are often associated with these pharmacological activities. Current works mainly focus on its clinic curative effect but less on the function mechanism of supporting its multiple bioactivity (Alfonso, Begonã Baamonde, Jesùs Santos, Astudillo, & Luis, 2004; Orellana & Claudia, 2001). In our work, we extracted crude polysaccharides from fern *L. japonicum* and further isolated, purified its polysaccharides component. In order to investigate tentative pharmacological function of the purification polysaccharides and to obtain sufficient material for more extensive biological and medicine studies, we in succession undertook the test of its anti-oxidation and anti-microorganism activity in vitro. The details are reported in this paper.

2. Materials and methods

2.1. Materials and chemicals

Ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween 20), 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{-}$), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), were purchased from Sigma Chemical Co. Vitamin C and ammonium thiocyanate were purchased from Nanjing JianCheng Biology Co. All others chemicals and reagents were analytical grade.

DEAE-cellulose-52 column (Whatman product); Sephadex G-200 column (Sigma product). TJ-170A automation collector (ZhengJiang Analysis Instrument Co.), 752 ultraviolet spectrophotometer and 200A precision electron balance (ShangHai 3th Analysis Instrument Co.).

Lygodium japonicums were purchased from a local herb market.

Microorganisms (Bacteria: *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*; actinomycetes: *Streptomyces griseus*, *Actinomyces propionici*, *Nocardia asteroids*; mildews; *Aspergillus oryzae*, *Penicillum chrysogenum*, *Mucor mucedo*; microzymes: *Saccharomyces cerevisiae*, *Hansenula anomala*, *Candida albicans*) were kindly provided by the Microorganism Culture Center in school.

In our laboratory, beef peptone culture medium, potato culture medium, were prepared.

2.2. Preparation of crude polysaccharide from L. japonicum

Lygodium japonicum freshly harvested was washed, dried at 60 °C and crushed up. The resulting sample was weighed, dipped in 20 volumes of double distilled

water at 100 °C for 3 h. The resulting suspension was centrifuged (2000*g* for 40 min) and the supernatant centrifuged again (20,000*g* for 20 min). The resulting supernatant was then membrane-filtered (0.45 μm, Millipore) The filtered aqueous solutions were pooled, and vacuum-concentrated at 50 °C, giving a final volume of about 100 ml. Three volumes of 96% ethanol were then added to this concentrate. The resulting mixture was left at 4 °C overnight. The resulting precipitate was separated by centrifugation, washed exhaustively with 96% alcohol, dissolved in deionized water, and dialyzed using cellulose sacks (Sigma). The non-dialyzed portion was lyophilized. The protein in the result extract was removed by method of Sevag (Matthaei, Oliver, Robert, & Marshall, 1962) and the result product was weighed, giving the crude polysaccharide extract.

2.3. Fractionation of the crude polysaccharide extracts by DEAE-52 cellulose column

One-hundred milligrams of crude polysaccharide extract was dissolved in distilled water and the solution was membrane-filtered (0.45 μm, Nucleopore) and applied to a DEAE-52 cellulose column (1.6 × 30 cm). The column was eluted first with water (500 ml) and then successively with 0.5 mol/L KCl (500 ml), at a flow rate of 30 ml/h, with collection of 6 ml outflow fractions for each tube. Polysaccharide content in each fraction was monitored by the phenol–sulfuric acid method (Dubois, Grilles, Hamilton, Rebers, & Smith, 1956). A fraction (purification polysaccharides) was obtained and then dialyzed and lyophilized.

2.4. Determination of physical character of the polysaccharides

Total sugar content in the purification polysaccharide was determined by the phenol-sulfuric acid method (Dubois et al., 1956), using D-glucose as standard. Protein contents were also determined, by the method of Bradford using a Bio-Rad Kit, with bovine serum albumin as standard. SO_4^{2+} content was determined by method of Lin, Chang, and Chang (1999).

2.5. Determination of the polysaccharides purification

The polysaccharide extract was separated by cellulose thin-layer chromatography, with butanol/water/ammonia (40:5:50) as solvent. For development, the cellulose plate was sprayed with a silver nitrate solution (0.1 ml of saturated $AgNO_3$ in 20 ml of acetone), dried, and then immediately washed with 96% ethanol after dyed with 0.5% methyl aniline blue solution. Ultraviolet detection: 0.1% the purification polysaccharides dissolved in water was detected between the wavelengths of 200 and 400 nm in UV detector.

Gel filtration: Two milligram sample were dissolved in 0.2 M NaCl and membrane filtered (0.45 μm, Nucleopore). The filtrate was then applied to a Sephadex G-200 column (1.6 × 30 cm) at 22 °C. Elution was done with 0.2 M NaCl at a flow rate of 1 ml/min, with collection of outflow fractions for each tube at 15-min interval. Polysaccharides were detected by the phenol–sulfuric acid method (Dubois et al., 1956). The column was previously calibrated with dextrans of known molecular weight. Elution curve was drawed by tube number as abscissa and absorbance as vertical coordinate.

2.6. Determination of free radical scavenging activity of the polysaccharides in vitro

2.6.1. Determination of superoxide anion scavenging activity of the purification polysaccharides

Measurement of superoxide anion scavenging activity of the purification polysaccharides was done based on the method described by Liu, Ooi, and Chang (1997) with slight modification. One milliliter of nitroblue tetrazolium (NBT) solution (156 mmol/L NBT in 100 mmol/L phosphate buffer, pH 7.4) 1 ml NADH solution (468 mmol/L in 100 mmol/L phosphate buffer, pH 7.4) and 0.1 ml of sample solution of the purification polysaccharides dissolved in water were mixed. The reaction started by adding 100 ml of phenazine methosulfate (PMS) solution (60 mmol/L PMS in 100 mmol/L phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the purification polysaccharides and standard (Ye, Wang, Liu, & Ng, 2000).

2.6.2. Determination of hydrogen peroxide scavenging activity of the purification polysaccharides

The ability of the purification polysaccharides to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng, and Klaunig (1989). A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 mol/L/cm. The purification polysaccharides (40–280 μg) in distilled water were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both purification polysaccharides and standard compounds

$$\% \text{ Scavenged } H_2O_2 = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of the purification polysaccharides and standard.

2.6.3. Determination of $DPPH^-$ radical scavenging activity of the purification polysaccharides

The free radical scavenging activity of the purification polysaccharides was measured by 1,1-diphenyl-2-picrylhydrazil ($DPPH^-$) using the method of Shimada, Fujikawa, Yahara, and Nakamura (1992). The 0.1 mmol/L solution of $DPPH^-$ in ethanol was prepared and 1 ml of this solution was added with 3 ml of the purification polysaccharides of different addition quantity (40–280 μg) in water. After 30 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The $DPPH^-$ concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.9035):

$$\text{Absorbance} = -2.9535 \times [\text{DPPH}^-]$$

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH}^- \text{ scavenging effect } \% = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of the purification polysaccharides.

2.6.4. Determination of antioxidative effect on liposome peroxidation of the purification polysaccharides

Liposome peroxidation was induced by Fe^{2+}-ascorbate and aquatinted by malondialdehyde-thiobarbituric acid (MDA-TBA) adduct according to the method described by Liao and Yin with slight modification (2000). Liposome (multi-lamellar vesicles) were prepared from 40 mg PC, 12 mg cholesterol, and 3.5 mg diacetyl phosphate at 4 °C. The solvents, chloroform and methanol, used for liposome preparation were removed and a lipid film was formed by rotary evaporation under N_2 flush. Then, the liposome was suspended with 10 ml of 50 mM sodium phosphate buffer (pH 7.2). A mixture containing 1 ml of liposome suspension, 0.6 ml of sodium phosphate buffer, 0.05 ml of 25 mM $FeCl_3$, 0.05 ml of 25 mM ascorbic acid, and the purification polysaccharides of varying addition quantity (40–280 μg) or vitamin C (40–280 μg) was incubated for 1 h at 37 °C. After incubation, the solution was mixed with TBA (0.4% in 0.2 M HCl) at a ratio of 1:2.7, and then heated at 100 °C for 30 min. After cooling the mixture, an equal volume of *n*-butanol was added to extract the chromogen in the mixture. The absorbance of the *n*-butanol layer was measured spectrophotometrically at 532 nm. The capability to inhibit MDA formation was calculated by the following equation:

$$\text{inhibition effect } (\%) = [1 - (\text{absorbance of sample at } 532\text{ nm}/\text{absorbance of control at } 532\text{ nm})] \times 100\%.$$

2.6.5. Determination of metal chelating activity of the purification polysaccharides

The chelating of ferrous ions by the purification polysaccharides was estimated by the method of Dinis, Madeira, and Almeida (1994). Briefly, the purification polysaccharide samples (40–280 μg) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was given below formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of the purification polysaccharides and standard.

2.7. Determination of anti-microorganism activity of the purification polysaccharides

Bacteria (*E. coli*, *Salmonella typhi*, *P. aeruginosa*), actinomycetes (*Streptomyces griseus*, *Actinomyces propionici*, and *Nocardia asteroides*), mildews (*A. oryzae*, *P. chrysogenum*, and *Mucor mucedo*), microzymes (*S. cerevisiae*, *Hansenula anomala*, and *C. albicans*) were selected as test organisms for this study. The microorganisms used for the assay were obtained from the Microbiology Laboratory of our institute. Antibacterial activity was evaluated by the filter disc diffusion plate method (Aruoma, 1998) and the agar dilution technique (Mitscher, Leu, Bathala, Wu, & Beal, 1972). The purification polysaccharides were dissolved in a minimum amount of DMSO (1% final concentration) and added to the nutrient medium. Nutrient agar was prepared in the usual fashion by autoclaving before the addition of the purification polysaccharides. A suspension of an overnight culture of each test organism containing 106 cells/ml was added to the medium. The result medium was then incubated in a incubator (36–37 °C; 48 h). The inhibition zones by the disc diffusion method were selected for the agar dilution test to determine the anti-microorganism activity more precisely. The procedures were carried out in asepsis condition. A blank plate containing only nutrient agar served as a control. Each test was replicated three times.

3. Results and discussion

3.1. Analysis of the crude polysaccharide extracts from L. japonicum

The crude polysaccharide extract from *L. japonicum* was obtained with yields of 4.7% (lyophilized weight). Only polysaccharides fraction (the purification polysaccharides) was obtained by a DEAE-52 cellulose column (1.6 × 30 cm) (Fig. 1). The results of chemical analyses

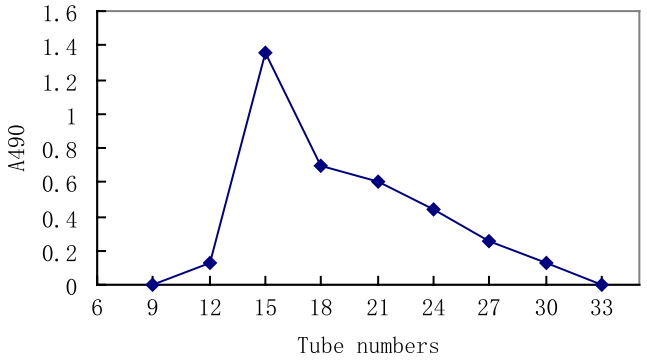

Fig. 1. Elution curve of the crude polysaccharides from *Lygodium japonicum* by DEAE-52 cellulose column.

of the crude polysaccharides and its extract are summarized as following: yellow powder, good water-solubility, and PH, content of sugar, SO_4^{2+} and protein in the extract solution being in turn 6.77, 24.1%, 13.2%, and 8.46%.

3.2. Analysis of the purity of the polysaccharide fractionation

Chromatography on a DEAE-52 cellulose column was carried out to obtain preliminary chemical information about the crude polysaccharide extracts evaluated in the later pharmacological assays. Cellulose thin-layer chromatography showed only spot from the purification polysaccharides under ultraviolet radiation. In addition, absorbance peaks of nucleic acid and protein were not be detected between the wavelengths of 200 and 400 nm by UV detector. All these confirm that the purification polysaccharides does not contain nucleic acid and protein impurity and is of high purity.

The elution profile for the polysaccharide extract by the Sephadex G-200 column showed symmetrical single peak, obtained with the concentrated solutions of KCl (0.2 M) as eluent (Fig. 2). This indicates that the purification polysaccharides, from the crude polysaccharide extracts from *L. japonicum*, is obtained as a homogeneous component.

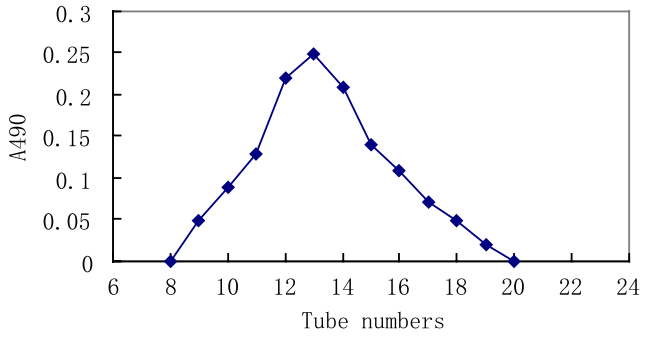

Fig. 2. Elution curve of the purification polysaccharides from *Lygodium japonicum* by Sephadex G-200 column.

3.3. Analysis of free radical scavenging activity of the polysaccharides in vitro

3.3.1. Scavenging activity of the purification polysaccharides to superoxide anion

Of the reactive oxygen species, superoxide anion radical is generated first. Although it is a relatively weak oxidant, it decomposes to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals (Dahl & Richardson, 1978). Further, superoxide anion radicals are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Meyer & Isaksen, 1995). So, its scavenging is extremely important to anti-oxidation work.

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Gülcn, S_at, Beydemir, Elmastas, & Küfrevioğlu, 2004; Oktay, Gülin, & Küfrevioğlu, 2003). Fig. 3 shows the % inhibition of superoxide radical generation of varying addition quantity (40, 80, 120, 160, 200, 240, and 280 μg) of purification polysaccharides at a amount-dependent manner (line regression equation $= -0.3246x + 14.8$; $R^2 = 0.9215$) and comparison with same doses of vitamin C. Result indicates that the purification polysaccharides have strong superoxide radical scavenging activity, which could bear comparison with that of vitamin C. The percentage inhibition of superoxide generation by 280 μg doses of the purification polysaccharides and vitamin C was found as 93.4% and 95.7%, respectively.

3.3.2. Scavenging activity of the purification polysaccharides to hydrogen peroxide

The ability of the purification polysaccharides to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). The scavenging ability of

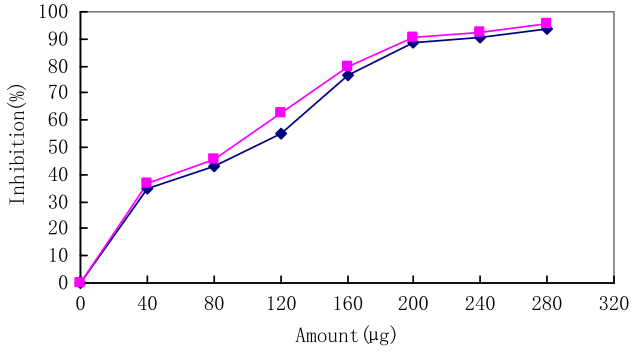

Fig. 3. Superoxide anion radical scavenging activity of the purification polysaccharides and vitamin C by the PMS/NADH-NBT method. Results are mean ± SD of five parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$ when compared to standard. –■– (purification polysaccharides); –■– (vitamin C).

purification polysaccharides on hydrogen peroxide is shown Fig. 4 and compared with vitamin C as standard. The purification polysaccharides was capable of scavenging hydrogen peroxide in an amount-dependent manner (line regression equation $= -0.1165x + 49.542$; $R^2 = 0.9942$). Two hundred eighty micrograms of purification polysaccharides exhibited 66.6% scavenging activity on hydrogen peroxide. On the other hand, at the same dose, vitamin C exhibited 83.2% hydrogen peroxide scavenging activity. The difference of scavenging activity between the purification polysaccharides and standard is significant ($p < 0.05$) at high addition quantity. These results showed that the purification polysaccharides had weaker hydrogen peroxide scavenging activity than vitamin C of same dose. Among the reactive oxygen species, the hydroxyl radical is the most active. Indeed, it is one of the most reactive chemical species known. The hydroxyl radical induces some oxidative damage to biomolecules, such as some proteins, DNA, PUFA, nucleic acid, and almost any biological molecule it touches, and this damage causes aging, cancer, and several diseases (Aruoma, 1998). Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

3.3.3. Scavenging activity of the purification polysaccharides to DPPH$^{\cdot}$ radical

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lai, Chou, & Chao, 2001; Lee, Hwang, Ha, Jeong, & Kim, 2003; Leong & Shui, 2002; Nagai, Inoue, Inoue, & Suzuki, 2003). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Fig. 5 illustrates a significant ($P < 0.05$) decrease the concentration of DPPH radical due to the scavenging ability of soluble solids in the both purification polysaccharides and standards (line regression equation $= -2.9525x + 815.67$; $R^2 = 0.9035$). Vitamin C was used as standard. The scavenging effect of

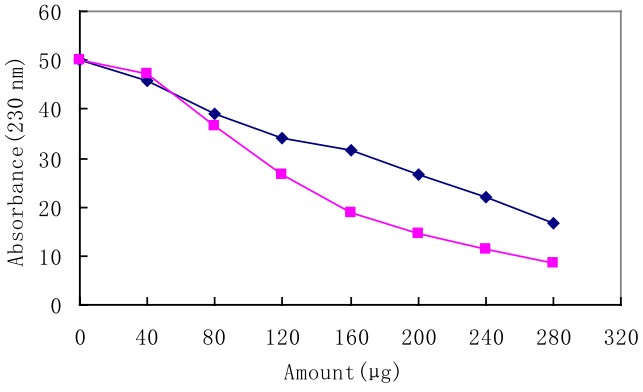

Fig. 4. Hydrogen peroxide scavenging activities of the purification polysaccharides and vitamin C. Results are mean ± SD of five parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$ when compared to standard. –■– (purification polysaccharides); –■– (vitamin C).

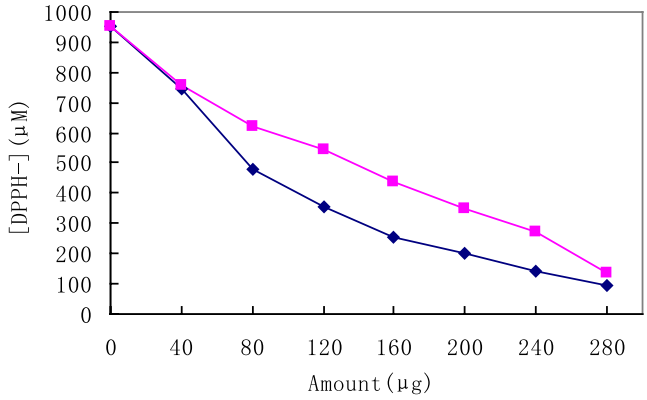

Fig. 5. Free radical scavenging activity of the purification polysaccharides and vitamin C by 1,1-diphenyl-2-picrylhydrazyl radicals. Results are mean ± SD of five parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$ when compared to standard. –■– (purification polysaccharides); –■– (vitamin C).

the purification polysaccharides and standard on the DPPH radical dose-dependently increased and were 92.5% and 85.4% at the dose of 280 μg, respectively. Both sustain approximately identical change trend of antioxidant activity. The difference of scavenging activity between the purification polysaccharides and standard is significant ($p < 0.05$). These results indicated that the purification polysaccharides have a noticeable effect on scavenging free radical, especially at high addition quantity.

3.3.4. Antioxidant effect of the purification polysaccharides on liposome peroxidation

Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. It has been suggested to be an important event in cellular damage, which is strongly associated with aging, carcinogenesis and other diseases (Halliwell, Gutteridge, & Cross, 1992). In addition, lipid peroxidation also plays an important role in the deterioration of foods during storage (Duthie, 1993). Liposomes, artificial biomembranes, have been used extensively as a model system for in vitro lipid peroxidation studies (Tsuda & Shiga, 1996; Yin & Cheng, 1998). The antioxidative activity of the purification polysaccharides and vitamin C on MDA formation in Fe^{2+}/ascorbate-mediated lipid peroxidation in the liposome system are shown in Fig. 6. It was also found that the inhibitory effects on the lipid peroxidation of the tested samples were concentration-dependent. Before the dose of 160 μg, the inhibitory effect of the purification polysaccharides is markedly stronger than vitamin C ($p < 0.05$) and after that, no longer obviously increase, whereas the inhibitory effect of vitamin C continues to increase and comes up with the purification polysaccharides at the addition quantity of 280 μg. The mechanism of the inhibitory effects by which the purification polysaccharides protect against a lipid peroxidation may involve radical-scavenging and reducing capability.

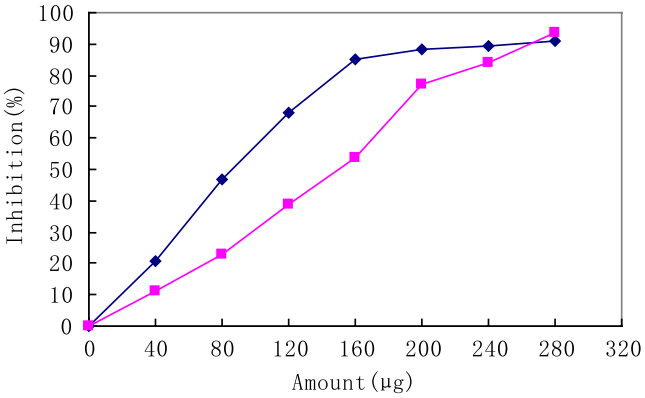

Fig. 6. Inhibition of the purification polysaccharides and vitamin C on Fe^{2+}/ascorbic acid-induced lipid peroxidation in a liposome model system. Results are mean ± SD of five parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$ when compared to standard. –■– (purification polysaccharides); –■– (vitamin C).

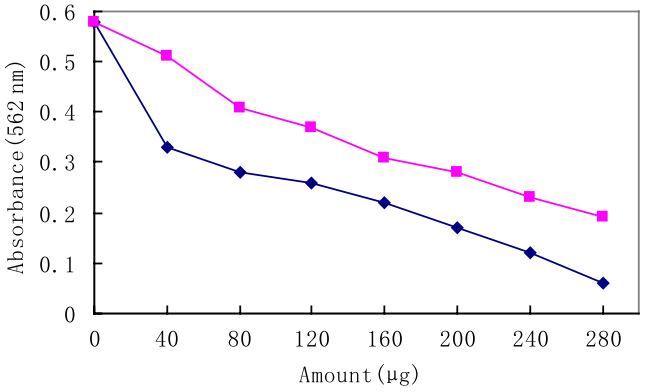

Fig. 7. Metal chelating effect of different amount of the purification polysaccharides and vitamin C on ferrous ions. Each value is expressed as mean ± SD of five parallel measurements and followed by the Student's *t*-test. Differences were considered to be statistically significant if $P < 0.05$ when compared to standard. –■– (purification polysaccharides); –■– (vitamin C).

3.3.5. Metal chelating activity of the purification polysaccharides

The chelating of ferrous ions by the extracts of the purification polysaccharides and standard was estimated by the method of Dinis et al. (1994). Fe^{2+} is able to generate free radicals from peroxides by Fenton reactions and may be involved in the progression of human cardiovascular disease. Thus, antioxidants capable of chelating with Fe^{2+} will minimize the ion's concentration and inhibit its capacity to catalyze free radical formation, which will results in protection against oxidative damage (Wu et al., 2006). In this assay the both purification polysaccharides and standard compounds interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. As shown in Fig. 7, the formation of the ferrozine–Fe^{2+} complex is not complete in the presence of the purification polysaccharides, indicating that the purification polysaccharides chelates the iron. The absorbance of Fe^{2+}–ferrozine complex was linearly decreased dose-dependently (from 40 to 280 μg) (line regression equation $= -0.0015x + 0.4633$; $R^2 = 0.8632$). The percentage of metal scavenging capacity of 280 μg of the purification polysaccharides and vitamin C were found as 89.7% and 67.2%, respectively. Results show that the purification polysaccharides has stronger metal chelating capacity than vitamin C ($P < 0.05$).

3.4. Antimicrobial activity of the polysaccharides in vitro

Antibacterial activity of the purification polysaccharides in vitro is showed in Table 1. Of all microorganisms tested, the purification polysaccharides was found to be the most effective against microzymes tested especially toward *H. anomala* and *C. albicans* with a zone of inhibition 36 and 33 mm, respectively, at a concentration of 20 mg/ml, following by the mildews. However, as shown in Table 1, the activity was lowest in the inhibition of bacteria and actinomycetes growth, especially toward *E. coli*, *S. typhi*, and *Nocardia asteroides* at a low concentration of

Table 1
Anti-microorganism activities (diameters of inhibition zone; mm) of purification polysaccharide from *Lygodium japonicum*

Microorganism	Concentration of purification polysaccharide (mg/ml)			
	5	10	15	20
Escherichia coli	n.t.	4 ± 0.98	8 ± 1.07	12 ± 2.23
Salmonella typhi	n.t.	–	10 ± 2.03	13 ± 1.45
Pseudomonas aeruginosa	–	7 ± 1.44	11 ± 1.58	14 ± 2.55
Streptomyces griseus	–	5 ± 1.23	12 ± 1.63	18 ± 1.76
Actinomyces propionici	4 ± 1.11	9 ± 1.83	15 ± 2.54	23 ± 2.68
Nocardia asteroides	n.t.	–	11 ± 1.05	15 ± 2.05
Aspergillus oryzae	–	11 ± 2.77	18 ± 2.65	24 ± 1.73
Penicillum chrysogenum	–	8 ± 1.35	17 ± 2.64	26 ± 1.87
Mucor mucedo	3 ± 0.87	7 ± 1.45	15 ± 1.61	21 ± 2.66
Saccharomyces cerevisiae	4 ± 1.76	12 ± 1.57	18 ± 1.62	27 ± 2.53
Hansenula anomala	4 ± 1.25	16 ± 2.22	27 ± 1.97	36 ± 2.06
Candida albicans	5 ± 1.57	15 ± 1.24	24 ± 2.03	33 ± 2.61

The data presented are means ± SD of three determinations.
–, not active; n.t., not tested.

5 mg/L, whose inhibition zone were not be observed. With the concentration increasing, the purification polysaccharides showed significant antibacterial activity against all selected microorganisms by a dose-dependently manner. This suggested that the purification polysaccharides may be employed as the broad-spectrum antibacterial agents.

4. Conclusions

Purification polysaccharide prepared in our work are confirmed of high purity. Anti-oxidation test in vitro shows that it possesses strong free radical scavenging activity, which may be comparable to vitamin C. In addition, the purification polysaccharides are also be confirmed a broad spectrum of antibacterial properties. We may rationally assume that *L. japonicum* playing its curative effect in traditional medicine partly by the mechanism of anti-oxidation and anti-microorganism of polysaccharides in it.

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