

Full Length Research Paper

Antioxidative potential of polysaccharide fractions produced from traditional Chinese medicinal macrofungus *Cordyceps jiangxiensis* *in vitro*

Jian-Hui Xiao^{1,2*}, Dai-Min Xiao¹, Zhong-Hua Sun¹, Yu Xiao¹ and Jian-Jiang Zhong^{2,3}

¹Division of Applied Mycology and Biochemical Pharmacy, Key Laboratory of Cell Engineering of Guizhou Province, Affiliated Hospital of Zunyi Medical College, Zunyi 563000, China.

²State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China.

³State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240, China.

Accepted 25 March, 2011

Cordyceps jiangxiensis, also called 'CaoMuWang', is a medicinal entomopathogenic macrofungus native to eastern China. Polysaccharide fractions from cultured *C. jiangxiensis* exhibited potent antitumor activity via the induction of cell cycle arrest and apoptotic pathway. Antioxidant pathway is also one action of mechanism of antitumor; thus, the antioxidant abilities of these polysaccharide fractions were overall evaluated by five *in vitro* assays such as the scavenging abilities on DPPH•, hydroxyl and superoxide anion radicals, the reducing power and the chelating ability on ferrous ions. Among these assays, the polysaccharide fractions presented more excellent scavenging abilities on superoxide anion radicals than that of the positive control. When compared with the positive control, the polysaccharide fractions from *C. jiangxiensis* only had moderate scavenging activities on both DPPH and hydroxyl free radicals, moderate reducing power and ferrous ion chelating activity. The antioxidant abilities of the different polysaccharide fractions had certain differences at all the tested doses and all had a dose-dependent manner. The results suggested that, polysaccharides are important antioxidant component in the medicinal *Cordyceps* fungi and have direct and potent antioxidant ability, and that *C. jiangxiensis* also is a promising potential source for the development of natural antioxidant.

Key words: *Cordyceps jiangxiensis*, polysaccharide, antioxidant activity.

INTRODUCTION

Free radicals, generally known as reactive oxygen and nitrogen species (ROS/RNS), are unavoidable consequences of aerobic life and play a dual role as both toxic and beneficial species. In general, free radicals, acting as secondary messengers in intracellular signaling cascades, exert beneficial effects on cellular responses and immune function at low or moderate levels while an excessive production of ROS/RNS outstripping antioxidant defense mechanisms through exogenous chemical and endogenous metabolic process in the

human body will generate a deleterious process termed as oxidative and/or nitrosative stress that can result in cell death and tissue damage by damaging lipids, protein and DNA. Furthermore, these adverse effects of oxidative stress on human health have become a serious medical issue, that is, oxidative stress was widely recognized for playing a major role in the development of chronic and degenerative ailments such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis, autoimmune disorders, cardiovascular and neurodegenerative diseases as well as in degenerative processes associated with ageing (Pham-Huy et al., 2008). However, antioxidant can quench the reactive free radicals and there is an inverse correlation between the dietary intake of antioxidant-rich

*Corresponding author. E-mail: jhxiao@yahoo.cn.

foods and the incidence of human degenerative diseases (Sies, 1993), hence, there are growing interests in the antioxidant substances that are supplied to human and animal organisms as food components or as specific pharmaceuticals. For example, some synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) have been widely used in the food and drug industry. Unfortunately, these synthetic antioxidants have been suspected of being responsible for the potential health hazard such as liver damage and carcinogenesis (Grice, 1988; Wichi, 1986). For this reason, the use of these synthetic antioxidants are under strict regulation in recent years, which results in an increasing interest on natural antioxidant substances worldwide (Krishnaiah et al., 2010).

Edible and medicinal macrofungi is one of important sources for the development of natural antioxidants. *Cordyceps*, a well-known and valued traditional Chinese medicinal macrofungus, has been commonly used in humans for centuries as the tonic for promoting vitality and longevity, and as the herbal medicine for treating various intractable diseases due to its diversified pharmacological actions in China (Liang et al., 2007). One of the known pharmacological actions is its antioxidant activity. Previous studies indicated that, the aqueous extract of natural and cultured *Cordyceps* species including *Cordyceps sinensis* and *Cordyceps militaris* possessed strong antioxidant activities such as scavenging free-radicals, inhibiting lipid peroxidation, chelating metal ion, etc. (Li et al., 2001; Yu et al., 2006; Dong and Yao, 2008). Interestingly, the antioxidant activities were increased to 10 to 30 folds in the partially purified polysaccharide fractions from the cultured *C. sinensis* mycelia (Li et al., 2001), which suggested that the polysaccharide of *Cordyceps* may be the major antioxidative substances. Recent investigations have also demonstrated that different polysaccharides from *Cordyceps* fungi showed potent antioxidant activities (Li et al., 2006; Yu, et al., 2009).

Cordyceps jiangxiensis, also called 'CaoMuWang' in China, is a medicinal entomopathogenic macrofungus native to eastern China, collected and denominated as a new species of the genus *Cordyceps* (Liang et al., 2007). It has been used in traditional Chinese medicine for centuries and its pharmacological activities have attracted much attention. The fermented conditions and chemical compositions of *C. jiangxiensis*, therefore, were thorough developed by our research group due to its medicinal values (Xiao et al., 2004a, b; 2006b; 2009a, b). Further investigations have demonstrated that, polysaccharide and chloroform extract of cultured *C. jiangxiensis* could significantly inhibit tumor cell proliferation via mitosis arrest and caspases-dependent apoptotic pathway (Xiao et al., 2006a; Xiao and Zhong, 2008). Recently, the potent antitumor cytotoxic compounds and other active metabolites in cultured *C. jiangxiensis* were also isolated using a bioassay-guided fractionation technique (Xiao

et al., 2010a, b). In this study, the antioxidant activities of the aqueous polysaccharide fractions from cultured *C. jiangxiensis* was developed and evaluated *in vitro* by chemical assay for the first time.

MATERIALS AND METHODS

Chemicals and reagents

2-Deoxy-D-ribose, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and butyl hydroxyanisole (BHA) were purchased from Sigma Inc. (St. Louis, MO, USA). Ethylenediamine tetraacetic acid (EDTA) and dimethyl sulphoxide (DMSO) were purchased from Invitrogen Inc. (Carlsbad, CA, USA). Trichloroacetic acid (TCA), ferrozine, potassium ferricyanide, ferrous chloride and ascorbic acid were from Advanced Technology and Industrial Co. Ltd (Hongkong, China). Ortho-phenanthroline (1,10-phenanthroline) was from Damao Chemical Reagents Factory (Tianjin, China) and Thiourea (TU) was from Kexing biotechnology Co. Ltd. (Shanghai, China). All chemicals used were of analytical grade.

Medicinal fungus, media and sample preparation

The strain JXPJ0109 of *C. jiangxiensis* was obtained from Jinggang Mountain region, Jiangxi Province, China. *C. jiangxiensis* was maintained on Sabouraud's agar (glucose 4 g, peptone 1 g, agar 1.8 g and distilled water 1 L at initial of pH 7.0) slant at 4°C and was transferred to a new slant once every three months. The stock culture was incubated at 26°C for 15 days and then stored in a refrigerator (about 4°C) before use. As described previously (Xiao et al., 2004a, 2006b), the seed culture was grown in a 250 ml flask containing 100 ml of basal medium (sucrose 3 g, peptone 0.5 g, yeast extract 0.5 g, KH₂PO₄ 0.1 g, MgSO₄ 0.05 g, CaCl₂ 0.05 g and distilled water 1 L and initial of pH 7.0) at 26°C on a rotary shaker incubator at 150 r/min for 5 days. The fermentation medium (sucrose 2 g, glycerol 1 g, soybean extract 0.5 g, yeast extract 0.5 g, KH₂PO₄ 0.1 g, MgSO₄ 0.05 g, distilled water 1 L, at initial pH 6.5) was inoculated with 5% (ϕ) of the seed culture and then was cultivated in a 500 ml flask containing 200 ml of the medium. The fermentations were carried out in a shake flask at 28.5°C and at an agitation rate of 180 r/min. The mycelial biomass were filtered in vacuum, then the mycelia were washed three times with distilled water, transferred to a loft drier in vacuum and dried to a constant weight at 60°C.

Isolation and analysis of polysaccharide

The isolation of the mycelial polysaccharide was carried out according to previous report (Xiao and Zhong, 2008) and the polysaccharide-enriched fractions of *C. jiangxiensis* were obtained. 1 Kg of dry mycelial powder (200 mesh) of *C. jiangxiensis* was extracted three times with 80% methanol water solution at 65°C for 8 h, of which supernatants were collected, combined, concentrated to a suitable volume and were extracted successively three times at room temperature for 12 h with petroleum ether, chloroform, acetic ether and *n*-butanol, respectively. The extraction by all the solvents was performed in Erlenmeyer flasks shaken on a rotary water-bath shaker at 120 rpm and at the earlier specific temperatures. Simultaneously, the earlier mycelial residues, collected by centrifugation (4500×g for 15 min) and dried at room temperature, were extracted three times with distilled water at 70°C for 2 h (residue/distilled water ratio: 1:10), and then was centrifuged at 4500×g for 15 min. Then, the water extracts and the above methanol residual extracts were further combined and concentrated

to 1/5 of the total volume at 70°C. Ethanol was added to a final concentration of 80%. Precipitation of the polysaccharides proceeded at 4°C for 12 h and the precipitate was collected by centrifugation at 4500×g for 15 min. The polysaccharide precipitates were washed three times with 70% ethanol, freeze-dried to a constant weight in a lyophilizer and mycelial polysaccharide of *C. jiangxiensis* (MPCJ) obtained was determined. Finally, MPCJ was re-dissolved with distilled water, depigmented by H₂O₂ method, deproteinized by Sevag method and dialyzed against distilled water for 3 days. The non-dialyzable solution re-concentrated was then precipitated fractionally using acetone solution at various final concentration of 30, 50, 70, 90 and 95% and lyophilized to a constant weight, where different polysaccharide fractions including MPCJ1, MPCJ2, MPCJ3, MPCJ4 and MPCJ5 were obtained, respectively. The contents of polysaccharide for these polysaccharide-enriched fractions isolated were also estimated by phenol-sulfuric acid colorimetric assay with glucose as a standard (Dubois et al., 1956). The sample of polysaccharide was dissolved in PBS buffer and stored at -20°C before use.

Evaluation of reducing ability

For the total antioxidant activity of the polysaccharide fractions from *C. jiangxiensis*, a modified Prussian blue method (Gülçin et al., 2007) was employed to assess the Fe³⁺-Fe²⁺ transformation ability in the presence of the samples. 1 ml of these samples were tested at different concentrations and 2.5 ml of pH 6.6 phosphate buffer and 2.5 ml of 1% (m/V) K₃Fe(CN)₆ were added and incubated at 50°C for 20 min in a glass tube. Then, 2.5 ml of 10% (m/V) TCA was added to the mixture followed by centrifugation at 1000×g for 10 min. 2.5 ml of the supernatant was diluted with 2.5 ml of ultrapure water and then, was reacted for 10 min by the addition of 2.5 ml of 0.1% fresh ferric chloride. Later, the reaction mixture was cooled to room temperature and its absorbance was measured at 700 nm. The antioxidants thiourea (TU) and BHT were also assayed at the same concentration for comparison. The reference solution was prepared as stated earlier and was used as the blank, but contained ultrapure water instead of the samples or antioxidants. All the tests were performed in triplicate. The total antioxidant ability was expressed as the absorbance value at 700 nm, and higher absorbance value of the reaction mixture indicates greater antioxidant activity. The total antioxidant ability was calculated as follows:

$$A=As-Ab, \quad (1)$$

Where, Ab is the absorbance of the blank and As is the absorbance of the polysaccharide fractions of the sample or antioxidants at 700 nm.

Assay of DPPH free radical scavenging

To assess the scavenging ability on lipid-soluble DPPH free radical, a modified method was applied in this study according to the previous method described by Blois (1958). 2 ml of pH 6.86 phosphate buffer, 2 ml of 0.5 mM fresh DPPH• in ethanol solution and 0.5 ml of the samples tested at different concentrations were added to a glass tube in sequence. Then, the reaction mixture was shaken vigorously and allowed to stand at room temperature for 30 min under dark condition. The ability of samples to scavenge the DPPH radical, which results in the bleaching of the purple color exhibited by the stable DPPH radical, was monitored at an absorbance of 520 nm. Synthetic antioxidants such as BHA and thiourea (TU) were used as positive controls and the sample solution without DPPH• was used as the sample blank. The ultrapure water was used as the blank control; without samples or

antioxidant. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging ability (\%)}=(1-(As-Asb)/Ab) \times 100 \quad (2)$$

Where, As, Asb and Ab are the absorbance at 520 nm for the reaction mixture of the sample or antioxidants, sample blank and blank, respectively. Three replicates were carried out.

Assay of hydroxyl free radical scavenging

The scavenging activity for hydroxyl radical produced by Fenton reaction was measured using ortho-phenanthroline method (Nagulendran et al., 2007) with some modification. In this experiment, 4 ml of pH 7.4 phosphate buffer, 1.5 ml of 5 mM ortho-phenanthroline in ethanol and 1 ml of 7.5 mM FeSO₄ were mixed immediately. Then 1 ml of the samples at different concentrations, 1.5 ml ultrapure water, and 1 ml of 1% (ϕ) hydrogen peroxide were added to the mixture solution in sequence. After incubating at 37°C for 60 min, the change of reaction mixture in absorbance caused by the color change of iron-ortho-phenanthroline was measured at 510 nm. All the tests were performed in triplicate. The antioxidant TU was used as the positive control, the ultrapure water in place of sample and antioxidant was used as damage control (the control in the hydroxyl radicals generation system) and the ultrapure water was used as the blank without sample, antioxidant and hydrogen peroxide. The hydroxyl radical scavenging activity was calculated using the following equation:

$$\text{Scavenging ability (\%)}=[(As-Ao)/(Ab-Ao)] \times 100 \quad (3)$$

Where, As, Ao and Ab are the absorbance at 510 nm of reaction mixture of the sample or antioxidants, damage control and blank, respectively.

Assay of superoxide anion free radical scavenging

Superoxide anion radical scavenging activity was determined by 2,4-iodophenyl-3,4- nitrophenyl-5-phenyltetrazolium chloride (NBT) method. An antioxidant kit was used in this experiment as reported by Dong and Yao (2008). The superoxide anion free radicals generated by the xanthine-xanthine oxidase system reacted with the NBT to form amethyst formazan and then, the absorbance of formazan in the reaction system was measured at 550 nm, which was used to indirectly reflect the superoxide anion free radicals. The production of formazan is inversely related to the superoxide anion radical scavenging activity of the samples tested. The final results were expressed as the inhibition degree of formazan production. BHT was used as the positive control and ultrapure water was used in the place of samples or antioxidant as blank. The percentage inhibition of the superoxide anion radicals was calculated as:

$$(1-As/Ab) \times 100 \quad (4)$$

Where As, and Ab are the absorbance of the reaction mixture of the sample or antioxidants, and blank at 550 nm, respectively.

Assay of metal chelating activity

The chelating activity of the polysaccharide fractions from *C. jiangxiensis* on ferrous ion Fe²⁺ was measured as reported by Decker and Welch (1990). 0.5 ml of the sample tested at various concentrations was mixed with 1.8 ml of ultrapure water and then,

Table 1. Scavenging effect of polysaccharide from cultured *C. jiangxiensis* on DPPH radicals.

Dose (mg/ml)	Scavenging effect (%)					
	MPCJ1	MPCJ2	MPCJ3	MPCJ4	MPCJ5	BHA
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	9.24±1.39	26.60±1.50	0.00	0.81±0.00	0.00	96.22±0.62
2	16.19±1.52	52.82±0.39	0.00	7.85±0.53	11.61±1.18	98.74±1.34
8	29.57±2.36	77.92±3.24	29.31±2.00	44.69±0.64	18.87±1.20	99.62±1.87

Results are mean ± S.D. of at least three determinations. MPCJ1 to 5 represent the fractions of mycelial polysaccharides of *Cordyceps jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively. BHA, Butyl hydroxyanisole.

0.05 ml of 2 mM FeCl₂ and 0.1 ml of 5 mM ferrozine were added to the mixture solution. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 20 min at room temperature, the absorbance of the Fe²⁺-Ferrozine complex in the reaction mixture was measured at 562 nm. EDTA was used as positive control and ultrapure water was used in place of the sample or EDTA as blank. The chelating activity of the polysaccharide fraction on Fe²⁺ was calculated as:

$$\text{Chelating rate (\%)} = (\text{Ab}-\text{As})/\text{Ab} \times 100 \quad (5)$$

Where, Ab is the absorbance of the blank without sample or EDTA, and As is the absorbance in the presence of the sample or EDTA.

Statistical analysis

The experimental results were processed by SPSS 11.0 (SPSS Inc.). The data were analyzed by one-way analysis of variance (ANOVA), and were expressed as mean ± standard deviation (SD) of triple determinations. Dunnett's *t*-test was used to compare the differences between the treated groups and control groups and differences were regarded as significant at *p* < 0.05.

RESULTS

DPPH radical scavenging activity

The DPPH radical scavenging effects of all the polysaccharide fractions from the cultured *C. jiangxiensis* were evident at most of the tested doses, but were lower than that of the positive control BHA (Table 1). The scavenging effect increased with the increased doses ranging from 0.5 to 8.0 mg/ml and also was exhibited in a dose-dependent manner. Among the tested polysaccharide fractions, MPCJ2 showed stronger activity than those of the other fractions at the same doses.

Scavenging effect on hydroxyl radicals

The scavenging effect of different polysaccharide fractions from the cultured *C. jiangxiensis* on hydroxyl radicals is shown in Figure 1. The different polysaccharide fractions from the cultured *C. jiangxiensis* all exhibited dose-dependence at the doses from 0.5 to 8.0 mg/ml, except for MPCJ5. Among the polysaccharide

fractions tested, MPCJ2 showed more significant scavenging activity toward the hydroxyl radicals than those of the other polysaccharide fractions at all the tested doses, except for MPCJ1 at a high dose of 8.0 mg/ml.

Superoxide anion scavenging activity

The superoxide anion scavenging activities of the different polysaccharide fractions from the cultured *C. jiangxiensis* are given in Figure 2. All the polysaccharide fractions tested exhibited dose-dependence manner towards superoxide anion scavenging activities, of which MPCJ2, MPCJ4 and MPCJ5 showed more excellent superoxide anion scavenging effects compared with the commercial synthetic antioxidant TU at all the tested doses. Also, MPCJ3 was significantly better than that of TU at a dose of 8.0 mg/ml. Among the polysaccharide fractions tested, MPCJ2 had the highest superoxide anion scavenging activity at all the tested doses, except at 8.0 mg/ml.

Reducing power

For the measurements of the reducing ability, the Fe³⁺-Fe²⁺ transformation was investigated in the presence of polysaccharide fractions from *C. jiangxiensis* by Prussian blue method. In this assay, the presence of reductant in the antioxidant sample causes the reduction of the Fe³⁺/ferricyanide complex to the Fe²⁺/ferrous form, so the reducing power of the sample can be monitored by measuring the formation of Prussian blue at 700 nm. As shown in Table 2, the reductive potential of the different polysaccharide fractions exhibited a dose-dependent activity within a concentration range of 0 to 8.0 mg/ml. However, their reducing powers were relatively lower than that of the positive control (TU).

Ferrous ion chelating activity

The ferrous ion (Fe²⁺) chelating activities of the different polysaccharide fractions from the cultured *C. jiangxiensis*

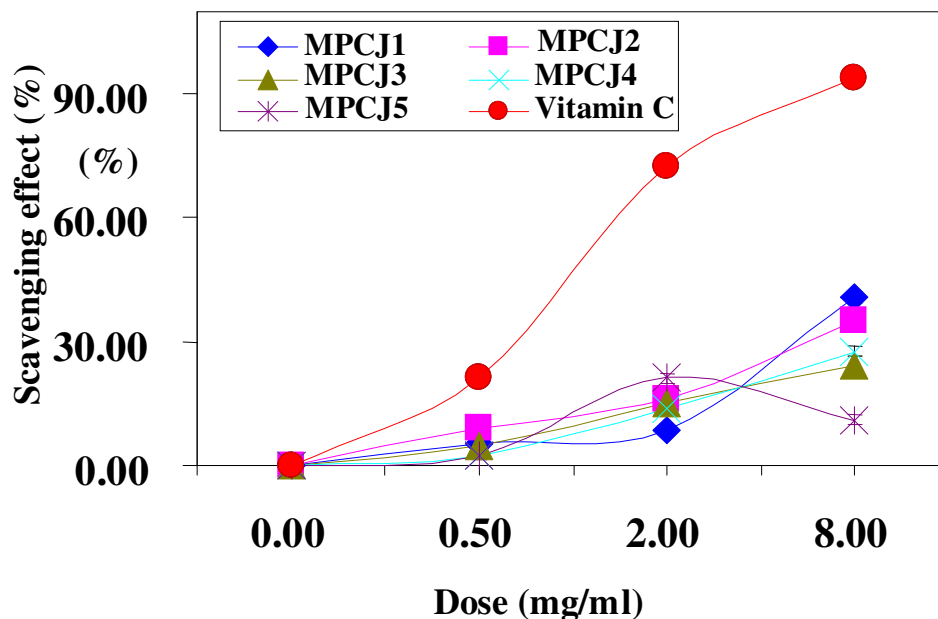


Figure 1. Scavenging effect of the polysaccharide from cultured *C. jiangxiensis* on hydroxyl free radicals. Symbols MPCJ1 to 5 represent the fractions of mycelial polysaccharides of *C. jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively.

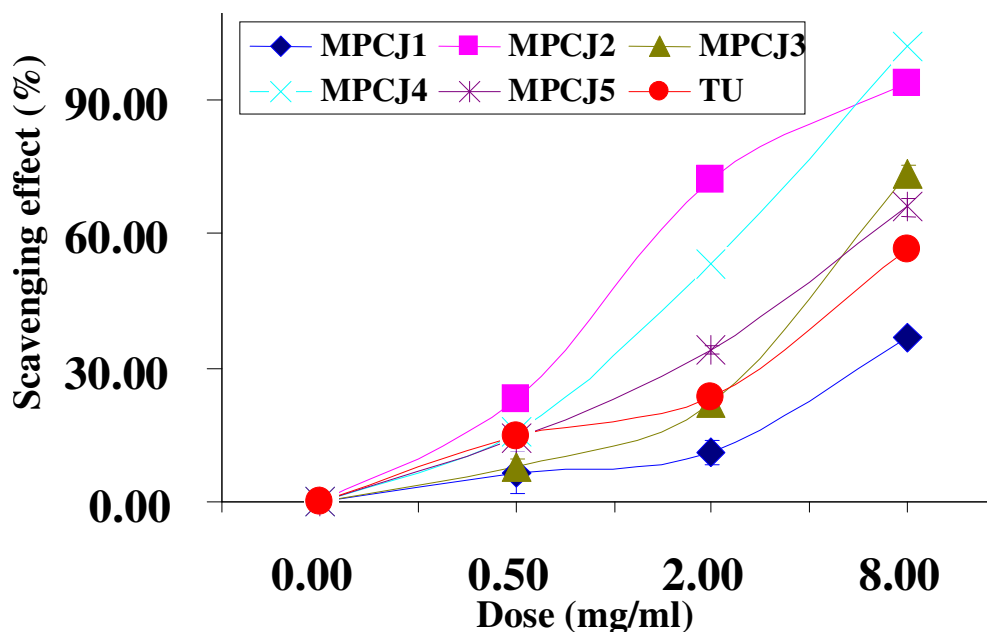


Figure 2. Scavenging effect of the polysaccharide from cultured *C. jiangxiensis* on superoxide anion free radicals. Symbols MPCJ1 to 5 represent fractions of the mycelial polysaccharides of *Cordyceps jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively. TU, Thiourea.

are listed in Table 3. Like the other antioxidant activity, all the tested polysaccharide fractions from *C. jiangxiensis* exhibited dose-dependency on the chelating abilities at all the tested doses of 0.5 to 8.0 mg/ml, of which MPCJ1,

MPCJ3, and MPCJ4 showed a moderate ferrous ion chelating ability and reached a range from 41.59 to 53.43% at a dose of 8.0 mg/ml. However, the positive control (EDTA) gave more effective ferrous ion chelating ability at

Table 2. Reducing ability of polysaccharide from cultured *C. jiangxiensis*.

Dose (mg/ml)	Reducing ability (%)					
	MPCJ1	MPCJ2	MPCJ3	MPCJ4	MPCJ5	TU
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.04±0.00	0.08±0.00	0.06±0.00	0.07±0.00	0.04±0.00	0.85±0.00
2	0.08±0.00	0.22±0.00	0.13±0.00	0.16±0.00	0.07±0.00	1.43±0.06
8	0.22±0.00	0.69±0.02	0.33±0.01	0.44±0.00	0.17±0.00	2.46±0.00

Results are mean ± S.D. of at least three determinations. MPCJ1 to 5 represent the fractions of mycelial polysaccharides of *Cordyceps jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively. TU, Thiourea.

Table 3. Fe²⁺ chelating activities of polysaccharide from cultured *C. jiangxiensis*.

Dose (mg/ml)	Fe ²⁺ chelating activity (%)					
	MPCJ1	MPCJ2	MPCJ3	MPCJ4	MPCJ5	EDTA
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	6.26±0.95	2.30±0.42	4.15±0.32	5.98±0.44	1.89±0.79	47.29±0.19
1	7.32±0.80	5.40±0.23	14.67±1.03	13.49±0.46	2.62±0.68	78.88±2.83
2	14.56±0.44	8.91±0.52	12.66±1.04	18.73±0.60	2.53±0.72	84.67±7.52
4	18.21±0.27	15.61±1.60	27.14±1.56	29.27±0.75	3.39±0.23	96.67±0.70
8	41.59±1.04	27.13±1.49	53.43±1.13	46.91±1.04	3.68±0.42	98.03±0.05

Results are mean ± S.D. of at least three determinations. MPCJ1 to 5 represent the fractions of mycelial polysaccharides of *Cordyceps jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively. EDTA, Ethlenediamine tetraacetic acid.

all the tested doses.

EC₅₀ value of antioxidant properties

The antioxidant properties assayed in this study are summarized in Table 4, which were normalized and expressed as EC₅₀ value (mg/ml) for comparison. Among the polysaccharide fractions tested, MPCJ2 showed a relatively lower EC₅₀ value of all tested antioxidation indexes, except for Fe²⁺ chelating assay. In addition, most of the polysaccharide fractions exhibited stronger scavenging superoxide anion radicals abilities with relative lower EC₅₀ values compared with that of the positive controls.

DISCUSSION

Oxidation phenomena have been implicated in many degenerative illnesses such as cancer, rheumatoid arthritis, cirrhosis, arteriosclerosis, autoimmune disorders, cardiovascular and neurodegenerative diseases. For example, superabundant free radical molecules can induce the initiation, promotion and progression of cancer at a certain extent via attacking membrane phospholipids and ultimately causing damage to DNA and proteins. Hence, antioxidant activity has become one of the

focuses of novel drug screening. It is obvious that no single method is capable of presenting a comprehensive antioxidant profile of a tested sample due to the complexity of the oxidation-antioxidation processes. For these reasons, there are numerous antioxidant methods and modifications for the evaluation of antioxidant activity (Huang et al., 2005). Additionally, a report presented that the polysaccharide was the key component exhibiting the antioxidation activity in the cultured *Cordyceps* species (Li et al., 2001). In this study, therefore, the antioxidant properties of the different polysaccharide fractions from cultured *C. jiangxiensis* were demonstrated by using an overall testing system *in vitro*. Our results suggested that, polysaccharide fractions from cultured *C. jiangxiensis* had direct and potent antioxidant activities. Different polysaccharide fractions, however, showed evident antioxidant differences. For example, most of the polysaccharide fractions, especially MPCJ2, had more effective antioxidant activities in different systems when compared with MPCJ1 and MPCJ5 as shown by the EC₅₀ values in Table 4. As earlier mentioned, the polysaccharide fractions MPCJ1 to MPCJ5 were acetone precipitates of mycelial polysaccharides of *C. jiangxiensis* at various final concentrations of 30, 50, 70, 90 and 95%, respectively; therefore, they had molecular weight differences among the polysaccharide fractions. Similar results by Ohmori et al. (1989) showed that different molecular weight of polysaccharides may affect their biological

Table 4. EC₅₀ of antioxidative properties of polysaccharide fractions from cultured *C. jiangxiensis*.

Sample	EC ₅₀ value (mg/ml)				
	Reducing power activity	Scavenging DPPH radical	Scavenging hydroxyl radical	Scavenging superoxide anion radical	Fe ²⁺ chelating effect
MPCJ1	18.47	12.68	9.85	10.86	9.73
MPCJ2	5.63	3.66	11.00	2.84	14.50
MPCJ3	11.95	13.06	14.81	5.32	7.36
MPCJ4	8.97	8.98	13.55	3.20	7.83
MPCJ5	23.46	18.06	10.64	5.29	91.01
Positive control	-	-	-	4.60	-
BHA	0.37	4.11	-	-	-
TU	-	-	-	-	1.04
EDTA	-	-	2.85	-	-
Vitamin C	-	-	-	-	-

- = not tested. MPCJ1 to 5 represent the fractions of mycelial polysaccharides of *C. jiangxiensis* by fractional precipitation with acetone at various final concentrations of 30, 50, 70, 90 and 95%, respectively. BHA, Butyl hydroxyanisole; TU, thiourea; EDTA, ethlenediamine tetraacetic acid.

activities. So far, the antioxidant mechanism of polysaccharides remained to be solved. The antioxidant mechanism of polysaccharide from *C. jiangxiensis* also needs further investigations. Recent literature presented that, a possible antioxidant mechanism of polysaccharides in *C. sinensis* may be through the donation of hydrogen to break chain-reactions or scavenging free radicals and speculated that, the abstraction of anomeric hydrogen from monosaccharides was the reason for the free radical scavenging ability (Wang et al., 2005). Previous studies still demonstrated that, polysaccharide fractions from cultured *C. jiangxiensis* had potent antitumor activity through cell cycle arrest and induction of cell apoptosis (Xiao and Zhong, 2008). Wang et al. (2005) demonstrated that, the scavenging free radicals and anti-tumor capacities of cultured *C. sinensis* were correlated closely with the polysaccharides content, but not cordycepin concentration. Thus, the antitumor action of the polysaccharide fractions from cultured *C. jiangxiensis* seems to be at least partially associated with its antioxidant activity. Of course, it still needs further evidences.

DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to quickly evaluate antioxidant activity (Blois, 1958). The different polysaccharide fractions exhibited large differences at the various doses tested in this study, which are possibly associated with the molecular weight of the polysaccharide (data not shown). MPCJ2 showed a relative lower scavenging DPPH• abilities at the all doses tested compared with a well-known synthetic antioxidant (BHA), but scavenging ability of approximately 80% at a dose of 8 mg/ml suggested that, *C. jiangxiensis* is a potential resource for the development and discovery of natural antioxidant.

Previous studies found that, some antioxidative compounds such as phenolics and tocopherol reduce the DPPH radicals by their hydrogen donating ability (Li et al., 2009), while the scavenging mechanism of DPPH• for polysaccharide need further investigation.

Hydroxyl free radicals, superoxide anions and hydrogen peroxides as reactive oxygen species (ROS), are related to the pathogenesis of various diseases. It is well-known that hydroxyl radicals are the most extremely reactive chemical species among the oxygen radicals that can induce severe damage to any adjacent biomolecules and their damaging actions are also the strongest among the free radical species. Hydroxyl radicals are generally formed from the reaction of various hydroperoxides with transition metal ions such as Fenton reaction and/or iron-catalyzed Haber–Weiss reaction (Erel, 2004). In this study, *in vitro* antioxidant activity was evaluated using the hydroxyl radical system generated by the Fenton reaction. All the polysaccharide fractions tested showed slightly lower scavenging effects on the hydroxyl radicals compared with the positive control (vitamin C). Similar results by Dong and Yao (2008) showed that, extracts from both natural and cultured *C. sinensis* also had relatively lower scavenging activities than that of the positive control.

Superoxide anions radicals are another main free radical which can induce many oxidations directly. For the scavenging superoxide anion, most of the polysaccharide fractions from the cultured *C. jiangxiensis* exhibited potent scavenging abilities when compared with other *Cordyceps* species and synthetic antioxidant (thiourea) (Dong and Yao, 2008). For example, the scavenging effect of MPCJ2 was 2 to 3 folds stronger than that of thiourea. Generally, superoxide anion, the one-electron reduced form of molecular oxygen, is a precursor of other

reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen. Although, the superoxide anion is relatively a weak oxidant, it may combine with other reactive species such as nitric oxide, produced by macrophages, to give more reactive species (Ardestani and Yazdanparast, 2007). *C. jiangxiensis*, therefore, is a promising resource for natural antioxidants. At the same time, these results suggested that the antioxidative abilities of antioxidants exist a certain extent difference for scavenging different free radicals and it is very necessary that the true abilities of the antioxidant need a relative comprehensive antioxidative evaluation system.

The reductive ability, a significant indicator for its potential antioxidant activity might be because of a hydrogen-donating ability and is generally associated with the presence of reductones (Jiang et al., 2005). For the measurement of reducing power, the potassium ferricyanide reduction method was employed to detect the reductive activities of the different polysaccharide fractions from *C. jiangxiensis*. Like previous report on the extract of *C. sinensis* (Dong and Yao, 2008), most of the polysaccharide fractions from *C. jiangxiensis* had moderate reducing power abilities on Fe^{3+} - Fe^{2+} transformation.

As we known, transition metal ions play a vital role for the generation of oxygen free radicals in living organisms. Therefore, the Fe^{2+} chelating assay may be assigned as an important index for the antioxidation effect of antioxidants. In general, iron exists in two distinct oxidation states that is, active ferrous ion (Fe^{2+}) and inactive ferric ion (Fe^{3+}). Unfortunately, Fe^{3+} can be easily reduced to the Fe^{2+} via Fenton reactions which produce hydroxyl radicals, and/or Haber-Weiss reactions which produce superoxide anions radicals (Kehrer, 2000). The production of these radicals can lead to lipid peroxidation, protein modification and DNA damage. Chelating agents therefore, may inactivate metal ions and indirectly inhibit the generation of metal-dependent free radicals. In this study, Fe^{2+} chelating activities of polysaccharide fractions was lower than that of EDTA, but which was slightly higher than that of the extracts of both the cultured and natural *C. sinensis* at all the tested doses (Dong and Yao, 2008).

ACKNOWLEDGEMENTS

The authors are grateful to the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, for their precious technological supports. Financial supports from the National Natural Science Foundation of China (Nos.20762017, 81060260), Program for Excellent Young Talents of S&T of Guizhou Province (No.QKH-R-200710), the Key Program for Social Development and Science and Technology of Guizhou Province during the 11th Five-Year Plan Period (No. QKH-SZ-20093048) "15851

Talents Project" Foundation of Zunyi City (No 201013) etc. are gratefully acknowledged.

REFERENCES

- Ardestani A, Yazdanparast R (2007). Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. Food Chem. 104: 21-29.
- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature, 26: 1199-1200.
- Decker EA, Welch B (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem. 38: 674-677.
- Dong CH, Yao YJ (2008). *In vitro* evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. LWT-Food Sci. Technol. 41: 669-677.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugar and related substance. Anal. Chem. 28: 350-356.
- Erel O (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. Clin. Biochem. 37: 112-119.
- Grice HP (1988). Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chem. Toxicol. 26: 717-723.
- Gülçin İ, Elias R, Gepdiremen A, Boyer L, Köksal E (2007). A comparative study on the antioxidant activity of fringe tree (*Chionanthus virginicus* L.) extracts. Afr. J. Biotechnol. 6: 410-418.
- Huang DJ, Ou BX, Prior RL (2005). The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53: 1841-1856.
- Jiang YH, Jiang XL, Wang P, Hu XK (2005). *In vitro* antioxidant activities of water-soluble polysaccharides extracted from *Isaria farinose* B05. J. Food Biochem. 29: 323-335.
- Kehrer JP (2000). The Haber-Weiss reaction and mechanisms of toxicity. Toxicol. 149: 43-50.
- Krishnaiah D, Sarbatly R, Nithyanandam R (2010). A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process doi:10.1016/j.fbp.2010.04.008.
- Li H, Wang X, Li Y, Li P, Wang H (2009). Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem. 112: 454-460.
- Li SP, Li P, Dong TTX, Tsim KWK (2001). Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. Phytomed. 8: 207-212.
- Li SP, Zhang GH, Zeng Q, Huang ZG, Wang YT, Dong TT, Tsim KW (2006). Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia. Phytomed. 13: 428-433.
- Liang ZQ, Liu AY, Liu ZY (2007). Flora fungorum sinicorum *Cordyceps*. Science Press, Beijing, China. Vol. 32.
- Nagulendran KR, Velavan S, Mahesh R, Hazeena Begum V (2007). *In vitro* antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. Eur. J. Chem. 4: 440-449.
- Ohmori T, Tamura K, Ohgane N, Nakamura T, Kawanishi G, Yamada H, Nomoto K (1989). The correlation between molecular weight and antitumor activity of galactosaminoglycan (CO-N) from *Cordyceps ophioglossoides*. Chem. Pharm. Bull. 37: 1337-1340.
- Pham-Huy LA, He H, Pham-Huy C (2008). Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4: 89-96.
- Sies H (1993). Strategies of antioxidant defense. Eur. J. Biochem. 215: 213-219.
- Wang BJ, Won SJ, Yu ZR, Su CL (2005). Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. Food Chem. Toxicol. 43: 543-552.
- Wichi HC (1986). Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem. Toxicol. 24: 1127-1130.
- Xiao JH, Chen DX, Fang N, Liu ZL, Zhang T (2006a). Growth arrest of human gastric adenocarcinoma cells by bioactive compounds of *Cordyceps jiangxiensis* (CaomuWang) through induction of apoptosis. J. Food Agric. Environ. 4(3-4): 66-73.

- Xiao JH, Chen DX, Liu JW, Liu ZL, Wan WH, Fang N, Xiao Y, Qi Y, Liang ZQ (2004a). Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by *Cordyceps jiangxiensis* JXPJ 0109. J. Appl. Microbiol. 96: 1105-1116.
- Xiao JH, Chen DX, Wan WH, Hu XJ, Qi Y, Liang ZQ (2006b). Enhanced simultaneous production of mycelia and intracellular polysaccharide in submerged cultivation of *Cordyceps jiangxiensis* using desirability functions. Process Biochem. 41: 1887-1893.
- Xiao JH, Liu JW, Liu ZL, Fang N, Xiao Y, Qi Y, Wan WH, Chen DX, Liang ZQ (2004b). Optimization of submerged culture conditions for the mycelial growth of *Cordyceps jiangxiensis*. Acta. Edulis Fungi (in Chinese), 11: 26-31.
- Xiao JH, Xiao DM, Sun ZH, Xiong Q, Liang ZQ, Zhong JJ (2009a). Chemical compositions and antimicrobial property of three edible and medicinal *Cordyceps* species. J. Food Agric. Environ. 7(3-4): 91-100.
- Xiao JH, Xiao DM, Xiong Q, Liang ZQ, Zhong JJ (2009b). Optimum extraction and high-throughput detection of cordycepic acid from medicinal macrofungi *Cordyceps jiangxiensis*, *Cordyceps taii* and *Cordyceps gunnii*. J. Food Agric. Environ. 7(3-4): 328-333.
- Xiao JH, Sun ZH, Pan WD, Chen C, Zhong JJ (2010a). Chemical compounds of medicinal macrofungus *Cordyceps jiangxiensis* mycelium. In: Proceedings of 2010 International Conference of Natural Product and Traditional Medicine. USA: Scientific Technical Development Press, pp.199-202.
- Xiao JH, Sun ZH, Pan WD, Liang ZQ, Zhong JJ (2010b). Steroids isolated from cultured *Cordyceps jiangxiensis* with potent antitumor activity. In: Proceedings of 2010 International Conference of Natural Product and Traditional Medicine. USA: Scientific Technical Development Press, pp.101-104.
- Xiao JH, Zhong JJ (2008). Inhibitory effect of polysaccharides produced by medicinal macrofungus *Cordyceps jiangxiensis* on cancer cells via apoptotic pathway and cell cycle arrest. J. Food Agric. Environ. 6(2): 61-67.
- Yu HM, Wang BS, Huang SC, Duh PD (2006). Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. J. Agric. Food Chem. 54: 3132-3138.
- Yu R, Yin Y, Yang W, Ma W, Yang L, Chen X, Zhang Z, Ye B, Song L (2009). Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured *Cordyceps militaris*. Carbohydr. Polymer. 75: 166-171.