Journal of Integrative Plant Biology 2006, **48** (11): 1365–1370

Antioxidant Activities of Aqueous Extract from Cultivated Fruit-bodies of *Cordyceps militaris* (L.) Link *In Vitro*

Yu Zhan[1, 2], Cai-Hong Dong [1, 2] and Yi-Jian Yao [1*]

(1. *Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, the Chinese Academy of Sciences,* Beijing 100080, China;
2. *Graduate University of the Chinese Academy of Sciences,* Beijing 100049, China)

Abstract

Biological antioxidants extracted from plants and fungi have potential abilities to scavenge free radicals and inhibit lipid peroxidation, playing important roles in preventing diseases, for example, cancer, and aging induced by reactive oxygen species, which may cause oxidative damage to DNA, proteins and other macromolecules. The antioxidant potency of cultivated fruit-bodies of *Cordyceps militaris* (L.) Link was investigated in this study. Five established *in vitro* systems were employed, including the 1,1-diphenyl-2-picryldrazyl (DPPH) free radical scavenging, hydroxyl radical eliminating, iron chelating, inhibition of linoleic acid lipid peroxidation and reducing power. The aqueous extract from cultivated fruit-bodies was subjected to the test of amino acid, polysaccharide and mannitol. Ascorbic acid (Vc), butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as positive controls for comparisons. Among the assays, the aqueous extract of *C. militaris* fruit-bodies shows a significant scavenging effect on DPPH, eliminating the capability on hydroxyl radicals and the chelating effect on ferrous iron. The extract also shows positive results of inhibiting linoleic acid lipid peroxidation and reducing power.

Key words: antioxidation; aqueous extract; *Cordyceps militaris*; free radicals; *in vitro.*

Zhan Y, Dong CH, Yao YJ (2006). Antioxidant activities of aqueous extract from cultivated fruit-bodies of *Cordyceps militaris* (L.) Link *in vitro*. *J Integr Plant Biol* **48**(11), 1365–1370.

doi: 10.1111/j.1672-9072.2006.00345.x, available online at www.blackwell-synergy.com; www.jipb.net

Cordyceps militaris (L.) Link, an edible and medicinal fungus, was reported to have various pharmacological activities in recent years (Nan et al. 2001; Yoo et al. 2004; Won and Park 2005). Recent studies have demonstrated that *C. militaris* can be used to treat a wide range of conditions, including liver disorders (Nan et al. 2001), tumors (Yoo et al. 2004), and aging and inflammation (Won and Park 2005). Various metabolites extracted from *C. militaris*, for example, cordycepin, cordycepic acid, superoxide dismutase (SOD) and polysaccharides (Zhang et al. 2003), have been proven to have pronounced biological effects on the human body (Wasser 2002; Yoo et al. 2004).

Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (ROS) (Willcox et al. 2004), which result in oxidative damage to DNA, proteins and other macromolecules and are associated with degenerative or pathological events, such as aging, asthma, and cancer (Klaunig and Kamendulis 2004; Balaban et al. 2005). However, there are serious concerns about the carcinogenic potential of synthetic antioxidants widely used in the food industry, for example, butylated hydroxyanisole (BHA) (Thompson and Moldeus 1988) and butylated hydroxytoluene (BHT) (Witschi 1986; Thompson and Moldeus 1988). Therefore, intensive research has been carried out to develop natural alternatives, which may serve as potent candidates in combating carcinogenesis and aging processes. One of the possible natural sources may be derived from the edible and medicinal fungi.

The antioxidant activity of *C. militaris* has attracted many research interests. The exo-polysaccharides from culture

Received 29 Apr. 2006 Accepted 7 Jul. 2006

Supported by the National Natural Science Foundation of China (30025002), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-101C), and the Scheme of Introduction of Overseas Outstanding Talents, the Chinese Academy of Sciences.

*Author for correspondence. Tel (Fax): +86 (0)10 6264 1794; E-mail: <yaoyj@sun.im.ac.cn>.

liquid of *C. militaris* were reported to have moderate anti-oxidative effects for lard (Li and Xu 1997). The antioxidant activity of aqueous extract from cultivated *C. militaris* has also been found to scavenge hydroxyl and superoxide anion radicals (Shen and Shen 2001). There has been no report on the scavenging effect on DPPH, metal chelating and reducing power of the extract from *C. militaris* so far. However, a direct correlation between antioxidant activity and metal chelating and also reducing power has been reported for extracts from some plants and fungi, for example, *Coleus aromaticus* Benth. (Kumaran and Joel Karunakaran, 2006) and *Antrodia camphorata* (M. Zhang et C.H. Su) Sheng H. Wu, Ryvarden et T. T. Chang (Mau et al. 2004). Due to the increasing interest in using natural antioxidants to scavenge the ROS, there is a need to obtain an overall assessment of the antioxidant activities of the extract from fruit-bodies of *C. militairs*, which can now be cultivated in large scales.

In the present study, the antioxidant activities of the hot water extract from cultivated fruit-bodies of *C. militaris* were investigated *in vitro* and evaluated by the scavenging abilities on DPPH and hydryoxyl radicals, the chelating ability on ferrous ions, the reducing power and the inhibition of linoleic acid peroxidation.

Results

Contents of amino acids in the raw powder, and polysaccharides and mannitol of hot water extracts from cultivated fruit-bodies of *C. militaris*

Seventeen amino acids with the total percentage of 29.89% were found in the raw power of *C. militaris* fruit-bodies, in which all kinds of essential amino acids occurred except tryptophan (Table 1). The extract with 5.14 mg/mL polysaccharides and 1.67 mg/mL mannitol, i.e. 12.86% polysaccharides and 4.18% mannitol in the raw powder, was used in further study to determine the antioxidant activities.

Table 1. Contents of amino acids from cultivated fruit-bodies of *Cordyceps militaris*

Amino acid	Content (mg/g)[a]
Threonine	20.91
Valine	9.68
Methionine	2.63
Isoleucine	49.65
Leucine	11.30
Phenylalanine	10.07
Lysine	16.81
Histidine	7.68
Tryptophan	ND
Hydroxyproline	ND
Tyrosine	11.82
Taurine	0
Aspartic acid	26.48
Serine	16.39
Glutamic acid	30.25
Glycine	12.07
Alanine	19.58
γ-Aminobutyric acid	10.22
NH_3	0
Arginine	26.53
Proline	16.84
Cysteine	0
Total	298.91

[a]The weight (mg) of amino acid in every gram of raw powder. ND, not detectable.

Scavenging effect on DPPH radicals

DPPH radical scavenging activity of the extract was evident at all of the tested concentrations (Figure 1). The scavenging effect increased with the increasing concentrations from 0.5–5.0 mg/mL and at concentrations of 5.0–9.0 mg/mL, it was found to be over 80%, significantly higher than that of ascorbic acid at the same concentration.

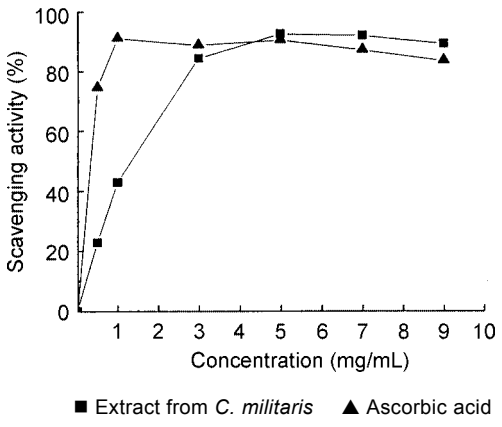

Figure 1. The scavenging ability on 1,1-diphenyl-2-picryldrazyl (DPPH) radicals of the extract from cultivated fruit-bodies of *Cordyceps militaris*.

Scavenging effect on hydroxyl radicals

As shown in Figure 2, the extract from fruit-bodies of *C. militaris* had scavenging activity toward hydroxyl radicals in a dose-dependent manner. The scavenging activity was relatively lower than that of BHT within a concentration range of 0.1–8.0 mg/mL. However, it exceeded BHT at a higher concentration (10.0 mg/mL) where the scavenging activity of BHT

decreased.

Fe^{2+} chelating activity

The extract showed a moderate ability of ferrous ion chelating and reached 66% at a concentration of 8.0 mg/mL (Figure 3) and slightly decreased as the concentration increased. EDTA showed a better chelating ability at all the tested concentrations.

Reducing power

The reducing power of the extract from *C. militaris* was shown in Figure 4. Like the other antioxidant activities, the reductive potential of the extract exhibited a dose-dependent activity within a concentration range of 1.0–8.0 mg/mL and a slight decrease at the concentration of 10.0 mg/mL.

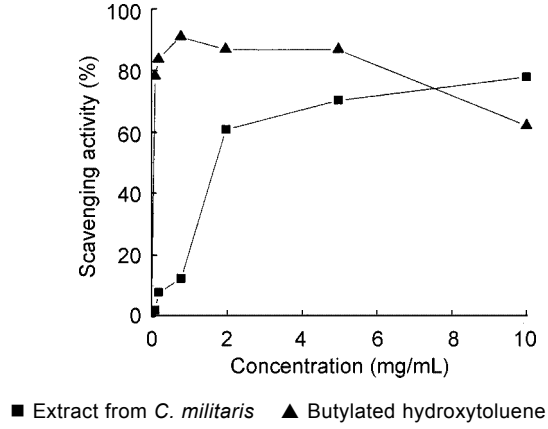

Figure 2. The scavenging ability on hydroxyl radicals of the extract from cultivated fruit-bodies of *Cordyceps militaris*.

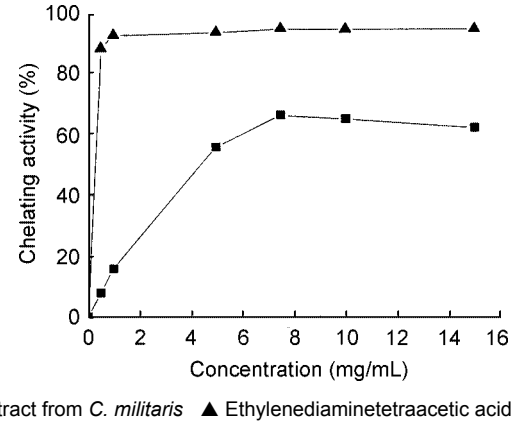

Figure 3. The Fe^{2+} chelating activity of the extract from fruit-bodies of *Cordyceps militaris*.

Antioxidant activity on linoleic acid peroxidation

The inhibition of peroxidation of *C. militaris* in the linoleic acid system was moderate at all of the tested concentrations but lower than that of ascorbic acid (Figure 5). The scavenging effect increased with the increasing concentration up to a certain extent (7.0 mg/mL) and then decreased with further concentration increase.

Discussion

There are numerous methods and modifications for evaluation of antioxidant activity of a compound. A single method may not provide a comprehensive picture of the antioxidant profile of a studied sample. As in Dong and Yao (2006, unpublished data), the antioxidant properties of hot water extract from fruit-bodies

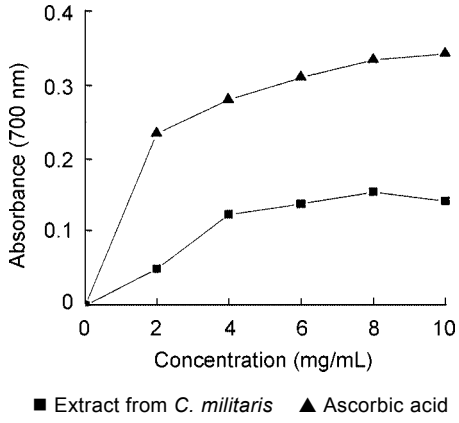

Figure 4. The reducing power of the extract from cultivated fruit-bodies of *Cordyceps militaris*.

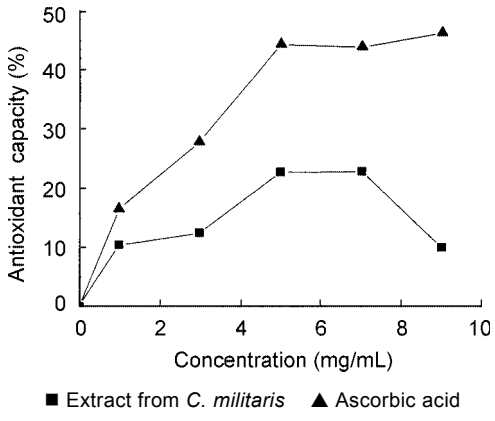

Figure 5. Effects of the extract from cultivated fruit-bodies of *Cordyceps militaris* on linoleic acid peroxidation.

of *C. militaris* was evaluated by using a range of testing systems in the present study and the results suggested that the extract had direct and potent antioxidant activities.

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index (Mokbel and Hashinaga 2006). The hot water extract from cultivated fruit-bodies of *C. militaris* showed stronger DPPH scavenging ability at the concentration over 5.0 mg/mL than that of ascorbic acid, a well-known antioxidant (Figure 1). Further, the scavenging ability of the extract reached more than 80% from the concentration of 3.0 mg/mL. Apparently, it is a promising resource for natural antioxidants.

The hot water extract of cultivated fruit-bodies of *C. militaris* was also reported to be effective on the scavenging hydroxyl radicals (Shen and Shen 2001). The extraction in hot water of 95 °C for 30 min was adopted by Shen and Shen (2001), and their results showed 76% of the highest scavenging ability of hydroxyl radicals at the final concentration of 3.67 g/L (sample/water). However, the extract method in the present study was 95–100 °C for 2 h, repeated three times, and the scavenging ability of the extract reached 77% (Figure 2) at the concentration of 0.1 mL of 10.0 mg/mL (sample/water) sample in the 3 mL reaction system (equivalent to a final concentration of 0.33 g/L). The longer extraction period, coupled with repetition, is apparently more effective than the shorter and single extraction for aqueous extraction of antioxidant active compounds from fruit-bodies of *C. militaris*.

For the iron chelating, the reducing power and the inhibition of linoleic peroxidation, the extract from cultivated fruit-bodies of *C. militaris* showed moderate abilities (Figures 3–5), although comparatively lower than that of the commercial antioxidants at the same concentrations. However, in considering the overall antioxidant activity, the extract can still be a useful bioactive material and purification of the extract may lead to better abilities.

The antioxidant activities of the extract from cultivated fruit-bodies of *C. militaris* in the assays performed in this study prompt the possible uses of the cultivated *C. militaris* to meet the needs of the natural antioxidant sources for human health. The fruit-bodies of the fungus are now readily available in quantity as the species can now be cultivated in large scales, providing sufficient raw material.

Materials and Methods

Cultivation of fruit-bodies of *Cordyceps militaris* (L.) Link

The strain of *C. militaris* was originally isolated by the first author in 2004 and maintained on potato dextrose agar (PDA) at 4 °C. For the strain identification, the Internal Transcribed Spacer (ITS1-5.8S-ITS2) of nuclear rDNA was amplified and 510 bp of the fragment were obtained from DNA sequencing. The sequence was compared with a data set for identification of *Cordyceps* species generated in this laboratory and the identity of the strain was verified both morphologically and molecularly.

For cultivation, the strain of *C. militaris* was incubated on PDA medium at 20 °C for 10 d in a Petri dish, and then transferred to the seed culture medium, potato dextrose liquid medium, by punching out 5 mm diameter of the agar plate culture with a sterilized cutter. The seed cultures were grown in a 500 mL flask containing 150 mL of potato dextrose liquid medium at 20 °C on a rotary shaker incubator at 150 r/min for 3 d. The cultivation was performed in 250-mL glass bottle each with solid culture medium. The fruit-bodies of *C. militaris* were harvested after 70 d of cultivation and dried in an oven at 45 ºC to constant weight.

Chemicals

Ferrozine, 2-deoxyribose, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and linoleic acid (Analytical grade) were purchased from Sigma-Aldrich (Steinheim, Germany); butylated hydroxytoluene (BHT, Chemical grade) from China National Pharmaceutical Group Shanghai Chemical Reagents Company (Shanghai, China); ethylenediamine tetra-acetic acid (EDTA, Purity > 99.0%) from Amresco (Ohio, USA); sodium acetate trihydrate (Analytical grade), acetic acid (Analytical grade), hydrogen peroxide (H_2O_2), trichloroacetic acid (TCA, Analytical grade) and ascorbic acid (Analytical grade) from Beijing Chemical Reagents Company (Beijing, China); and thiobarbituric acid (TBA, Purity > 98.0%) from Acros Organics (Geel, Belgium); Mannitol (Analytical grade) from Shanghai Zhengxiang Science and Technology Company (Shanghai, China).

Preparation of the extract from cultivated fruit-bodies of *C. militaris*

After the fruit-bodies of *C. militaris* were dried to consistent weight at 45 °C, they were weighed and ground into a fine powder. Raw powder (10.0 g) was extracted with 200 mL distilled water at 95–100 °C for 2 h and repeated three times. The filtrate was collected after vacuum filtration and adjusted to a final volume of 250.0 mL. The extract at the concentration of 40.0 mg/mL (sample/distilled water) was stored in the dark at 4 °C before use.

Determination of amino acid, polysaccharide and mannitol contents of cultivated fruit-bodies of *C. militaris*

As a quality control of the fungal material used in the assays, the contents of amino acids in the raw powder, and the polysaccharide and mannitol contents in raw extract were determined.

Determination of amino acids in the raw power of fruit-bodies of *C. militaris* was conducted using a NEC 835–50 Automatic Amino Acid Analyser (NEC Japan) at the Medical College of Peking University (Beijing, China). The polysaccharide contents in extracts were determined using the phenol-sulfuric acid method (Dubois et al. 1956), and the mannitol contents by the colorimetric method (Li et al. 1999) with some modifications. In brief, for the contents of mannitol, 1 mL of the solution containing 0.04 mL of extract and 1 mL of sodium periodate (0.015 mol/L) were mixed. After 10 min, 2 mL of rhamnose (0.1%) and 4 mL of fresh Nash reagent (2 mol/L ammonium acetate mixed with 2 mL acetic acid and 2 mL acetyl acetone) were added to the mixture, which was placed in a water bath at 53 °C for 15 min. The absorbance was measured at a wavelength of 412 nm on a Unico-2100 spectrophotometer (Shanghai, China). A blank test was prepared by substituting distilled water for the extract solution. A standard curve was prepared using a mannitol standard. One millilitre of solution containing up to 50 μg/mL of mannitol was determined by the above method and the mannitol content of samples was calculated by the linear regression equation from the standard curve.

Scavenging effect on DPPH radicals

The scavenging effect of aqueous extract from *C. militaris* on DPPH radicals was determined by the method of Blois (1958) with some modifications. An acetate buffer (0.05 mol/L, pH 5.5) and solution of DPPH in ethanol (0.50 mmol/L) were prepared. The extract (0.5–9.0 mg/mL) of 1.0 mL was mixed with 2.0 mL of acetate buffer, 1.0 mL of absolute ethanol and 1.0 mL of DPPH solution. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance at 517 nm was then measured using a spectrophotometer (UNICO 2100, Shanghai China). Ascorbic acid was used as a positive control, distilled water in place of extract or ascorbic acid was used as a blank, and the sample solution without adding DPPH was used as a sample blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation, with Ab, As and Asb as the absorbance at 517 nm of DPPH of the blank, extract or control, and sample blank respectively.

$$\text{Scavenging activity (\%)} = \frac{Ab-(As-Asb)}{Ab} \times 100 \qquad (1)$$

Scavenging effect on hydroxyl radicals

Scavenging effect on hydroxyl radicals was studied following the method of Halliwell et al. (1987). The 1.0 mL reaction mixture containing 0.4 mL of sodium phosphate buffer (20.00 mmol/L, pH 7.4), 0.1 mL of extract (at concentrations of 0.1–10.0 mg/mL), 0.1 mL of deoxyribose (60.00 mmol/L), 0.1 mL of hydrogen peroxide (10.00 mmol/L), 0.1 mL of ferric chloride (1.00 mmol/L), 0.1 mL of EDTA (1.04 mmol/L) and 0.1 mL of ascorbic acid (2.00 mmol/L), was incubated at 37 °C for 1 h. Solutions of ferric chloride and ascorbic acid were made up immediately before use in deaerated water. The reaction was stopped by adding 1.0 mL of TBA (1%) and 1.0 mL of TCA (2.8%). The mixture was then boiled for 15 min, cooled in ice, and then measured for the absorbance at 532 nm. BHT was used as a positive control. The radical scavenging activity was calculated using the equation mentioned in the above section on scavenging effect on DPPH radicals, where Ab, As and Asb are the absorbances at 532 nm of the blank, extract or BHT, and sample blank respectively.

Ferrous ion chelating activity assay

The chelating activity of the extract on ferrous ion was carried out following the method described by Decker and Welch (1990). The 1.0 mL extract (0.1–15.0 mg/mL) was mixed with 3.7 mL of deionised water and then the mixture was reacted with 0.1 mL of ferrous chloride (2.00 mmol/L) and 0.2 mL of ferrozine (5.00 mmol/L) for 20 min. The absorbance at 562 nm was determined. EDTA was used as positive control and chelating activity on ferrous ion was calculated as the following equation with Ab as the absorbance of the blank without extract or EDTA and As as the absorbance in the presence of the extract or EDTA.

$$\text{Inhibition (\%)} = \frac{Ab-As}{Ab} \times 100 \qquad (2)$$

Measurement of reducing power

The reducing power of the extract was measured as reported by Yen and Chen (1995) with some modification. The extract (2.0–10.0 mg/mL) of 1.0 mL in 2.5 mL of phosphate buffer (0.20 mol/L, pH 6.6) was added to 2.5 mL of potassium ferricyanide (10 mg/mL), and the mixture was incubated at 50 °C for 20 min. After 2.5 mL TCA (10.0 mg/mL) was added, the mixture was centrifuged at 1 160*g* for 10 min, and then 2.5 mL of the supernatant was mixed with 2.5 mL of deionized water and 0.5 mL of ferric chloride (1.0 mg/mL). Ascorbic acid was used as a control. The absorbance was then measured at 700 nm against a blank in the spectrophotometer. A higher absorbance of the reaction mixture indicates a higher reducing power.

Antioxidant activity in a linoleic acid system

The thiocyanate method was used to determine the antioxidant capacity of the extract on inhibition of linoleic acid peroxidation (Chang et al. 2002). Linoleic acid emulsion was prepared with linoleic acid (0.280 4 g) and Tween 20 (0.280 4 g) in 50 mL of phosphate buffer (0.20 mol/L, pH 7.0). A reaction solution,

containing 0.5 mL of extract (1.0–9.0 mg/mL), 2.5 mL of linoleic acid emulsion and 2.0 mL of phosphate buffer (0.20 mol/L, pH 7.0) were mixed with a homogeniser. The reaction mixture was incubated at 60 °C in the dark to accelerate the oxidation process for 8 h. To 4.5 mL of ethanol (75%), 0.2 mL of ammonium thiocyanate solution (300 g/L), 0.1 mL of the sample solution and 0.2 mL of the ferrous chloride solution (20.0 mmol/L in hydrochloric acid) were added in sequence. The solution was stirred for 3 min and its absorption value at 500 nm was taken as the peroxide value. The inhibition of ascorbic acid on linoleic acid peroxidation was also assayed at the same concentration for comparison. The solution without adding extract or ascorbic acid was used as blank. All the tests were performed in triplicate.

Inhibition effect in the linoleic acid system was calculated using the same equation given in the section on ferrous ion chelating activity assay above, where Ab is the absorbance of the blank and As is the absorbance of the aqueous extract or ascorbic acid at 500 nm.

References

Balaban RS, Nemoto S, Finkel T (2005). Mitochondria, oxidants and aging. *Cell* **120**, 483–495.

Blois MS (1958). Antioxidants determination by the use of a stable free radical. *Nature* **181**, 1199–1120.

Chang LW, Yen WJ, Huang SC, Duh PD (2002). Antioxidant activity of sesame coat. *Food Chem* **78**, 347–354.

Decker EA, Welch B (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J Agr Food Chem* **38**, 674–677.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Anal Chem* **28**, 350–356.

Halliwell B, Gutteridge JMC, Arugma OI (1987). The deoxyribose method: A simple "Test-Tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* **165**, 215–219.

Klaunig JE, Kamendulis LM (2004). The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol* **44**, 239–267.

Kumaran A, Joel Karunakaran R (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem* **97**, 109–114.

Li XQ, Bao TT, Wang Y (1999). Determination of mannitol in *Cordyceps sinensis* by colorimetric method. *Chin Tradit Herb Drugs* **30**, 19–21 (in Chinese with an English abstract).

Li X, Xu L (1997). Studies on exopolysaccharide (EPS) fermentation by *Cordyceps militaris*, and its physical and chemical properties and antioxidation. *J Microbiol* **17**, 13–17 (in Chinese with an English abstract).

Mau JL, Huang PN, Huang SJ, Chen CC (2004). Antioxidant properties of methanolix extracts from two kinds of *Antrodia camphorata* mycelia. *Food Chem* **86**, 25–31.

Mokbel MS, Hashinaga F (2006). Evaluation of the antioxidant activity of extracts from buntan (*Citrus grandis* Osbeck) fruit tissues. *Food Chem* **94**, 529–534.

Nan JX, Park EJ, Yang BK, Song CH, Ko G, Sohn DH (2001). Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. *Arch Pharm Res* **24**, 327–332.

Shen QY, Shen QY (2001). Study on the resisting oxygen free radical and hydroxyl free radical effect of *Cordyceps militaris*. *Guihaia* **21**, 252–254 (in Chinese with an English abstract).

Thompson D, Moldeus P (1988). Cytotoxicity of butylated hydroxyanisole and butylated hydroxytoluene in isolated rat hepatocytes. *Biochem Pharmacol* **37**, 2201–2207.

Wasser SP (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl Microbiol Biotechnol* **60**, 258–274.

Willcox JK, Ash SL, Catignani GL (2004). Antioxidants and prevention of chronic disease. *Crit Rev Food Sci* **44**, 275–295.

Witschi HP (1986). Enhanced tumour development by butylated hydroxytoluene (BHT) in the liver, lung and gastro-intestinal tract. *Food Chem Toxicol* **24**, 1127–1130.

Won SY, Park EH (2005). Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. *J Ethnopharmacol* **96**, 555–561.

Yen GC, Chen HY (1995). Antioxidant activity of various tea extract in relation to their antimutagenicity. *J Agr Food Chem* **43**, 27–32.

Yoo HS, Shin JW, Cho JH, Son CG, Lee YW, Park SY et al. (2004). Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. *Acta Pharmacol Sin* **25**, 657–665.

Zhang P, Zhu SJ, Qian DS, Jiang N, Li JJ (2003). Functional components and the analysis of health function of *Cordyceps militaris*. *Jiangsu Agr Sci* (6), 105–107 (in Chinese with an English abstract).

(Managing editor: Wei Wang)