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Food Chemistry 98 (2006) 670–677
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant properties of hot water extracts from *Agrocybe cylindracea*

Shu-Yao Tsai, Shih-Jeng Huang, Jeng-Leun Mau *

Department of Food Science and Biotechnology, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC

Received 10 January 2005; accepted 5 July 2005

Abstract

Agrocybe cylindracea (DC: Fr.) Mre. was available in the form of fruit bodies, mycelia and fermentation filtrate. From these three forms, hot-water extracts were prepared and their antioxidant properties were studied. Antioxidant activities of hot-water extracts from fruit bodies, mycelia and filtrate were 63.6%, 81.6% and 56.8% at 20 mg ml^{-1}, respectively. EC_{50} values in reducing power were 2.72, 3.97 and 3.09 mg ml^{-1} whereas those in scavenging abilities of 1,1-diphenyl-2-picrylhydrazyl radicals were 0.62, 1.66 and 0.82 mg ml^{-1} for fruit bodies, mycelia and filtrate, respectively. At 20 mg ml^{-1}, the scavenging abilities of hydroxyl radicals were 80.1%, 57.0% and 54.3% for fruit bodies, mycelia and filtrate, respectively. With regard to EC_{50} values in chelating abilities on ferrous ions, the hot-water extract from filtrate was better than that from mycelia. Total phenols were the major naturally occurring antioxidant components found in hot-water extracts and in the range of 23.74–30.16 mg g^{-1}. From EC_{50} values obtained, it can be concluded that hot-water extracts from three forms of *A. cylindracea* were good in antioxidant properties.

Keywords: *Agrocybe cylindracea*; Antioxidant activity; Reducing power; Scavenging ability; Chelating ability; Antioxidant components

1. Introduction

Agrocybe cylindracea (DC: Fr.) Mre. [syn. *Agrocybe aegerita* (Briganti) Singer], also called black poplar mushroom, is one of cultivated mushrooms in Taiwan (Leu, 1992) and has become increasingly popular recently due to its delicious taste and unique texture. Generally, commercial products of *A. cylindracea* have long stipes and closed caps. Also, the mushrooms are thought to have better bite and chew texture than oyster mushrooms (*Pleurotus* spp.) and are tastier than shiitake (*Lentinula edodes*).

Like other mushrooms, *A. cylindracea* is also highly valued as functional food for its antitumor and other physiological benefits. Recently, fruit bodies of this mushroom are found to be medically active in several therapeutic effects such as antitumor, antifungal, nerve tonic, hypercholesterolemia and hyperlipidemia (Wasser & Weis, 1999). Extracts from *A. cylindracea* possessed antimutagenic activities and might play a role in the prevention of cancer (Shon & Nam, 2001a). Furthermore, two new indole derivatives were isolated from methanolic extract of *A. cylindracea* and inhibited lipid peroxidation in rat liver microsomes (Kim et al., 1997). Soybean fermented with *A. cylindracea* also formed polysaccharides with cancer chemopreventive activity (Shon & Nam, 2001b).

Most recent studies have placed major emphases on the identification of pharmaceutical components in *A. cylindracea* and their mechanism of action in human bodies. In addition, the nutritional values and taste components of three strains of *A. cylindracea* were clearly studied (Mau & Tseng, 1998). However, the antioxidant properties of this mushroom are not available.

* Corresponding author. Tel.: +886 4 2285 4313; fax: +886 4 2287 6211.
E-mail address: jlmau@dragon.nchu.edu.tw (J.-L. Mau).

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doi:10.1016/j.foodchem.2005.07.003

Our objective was to study and compare the antioxidant properties of hot-water extracts from *A. cylindracea* in the form of fruit bodies, mycelia and fermentation filtrate from the submerged culture. Antioxidant properties were assayed in terms of antioxidant activity by the conjugated diene method, reducing power, scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals and chelating abilities on ferrous and cupric ions. The contents of potential antioxidant components of hot-water extracts were also determined.

2. Materials and methods

2.1. Mushroom fruit bodies, mycelia and fermentation filtrate

Mycelia and fermentation filtrate of *A. cylindracea* strain M were obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan whereas fruit bodies were cultivated at Lung-Kuo Mushroom Farm, Taichung Country, Taiwan. Three forms of *A. cylindracea* samples were lyophilized. For each of fruit bodies, mycelia and fermentation filtrate, three lyophilized samples (~50 g each) were randomly selected and prepared for analyses.

After a fine powder (20 mesh) was obtained using a mill (Retsch ultra centrifugal mill and sieving machine, Haan, Germany), a subsample (10 g) was heated with 200 ml deionised water at reflux for 3 h. The mixture was cooled to room temperature and filtered through Whatman No. 4 filter paper. The residue was then refluxed with two additional 100 ml portions of deionised water as described above. The combined hot water extracts were lyophilized and the dried extract thus obtained was used directly for analyses of antioxidant components or redissolved in water to a concentration of 50 mg ml^{-1} and stored at 4 °C for further uses.

2.2. Antioxidant activity

The antioxidant activity was determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). Each extract (1–20 mg ml^{-1}) in deionised water (100 μl) was mixed with 2 ml of 10 mM linoleic acid emulsion (pH 6.5) in test tubes and placed in darkness at 37 °C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol (Mallinckrodt Baker, Paris, KY) in deionised water was added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows: AOA (%) = $[(\Delta A_{234}$ of control − ΔA_{234} of sample)/ΔA_{234} of control] × 100%. An AOA value of 100% indicates the strongest antioxidant activity. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Ascorbic acid, (Sigma Chemical Co., St. Louis, MO), butylated hydroxyanisole (BHA, Sigma) and α-tocopherol (Sigma) were used as controls.

2.3. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (1–20 mg ml^{-1}) in deionised water (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6, Wako Pure Chemical Co., Osaka, Japan) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v, Wako) were added, the mixture was centrifuged at 200*g* for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm against a blank in a Hitachi U-2001 spectrophotometer. A higher absorbance indicates a higher reducing power. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHA and α-tocopherol were used as controls.

2.4. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

Each extract (1–20 mg ml^{-1}) in deionised water (4 ml) was mixed with 1 ml of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank (Shimada, Fujikawa, Yahara, & Nakamura, 1992). EC_{50} value (mg extract ml^{-1}) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. Ascorbic acid, BHA and α-tocopherol were used as controls.

2.5. Scavenging ability on hydroxyl radicals

The hydroxyl radicals reacted with the nitrone spin trap 5,5-dimethyl pyrroline-N-oxide (DMPO, Sigma) and the resultant DMPO-OH adducts were detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing each extract (1–10 mg ml^{-1}) in deionised water (200 μl) with 200 μl of 10 mM H_2O_2 (Merck, Darmstadt, Germany), 200 μl of 10 mM Fe^{2+} (Sigma) and 200 μl of 10 mM DMPO using a Bruker EMX-10 EPR spectrometer at the following settings: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant, and 200 s scan period (Shi, Dalal, & Jain, 1991). BHA was used as a control.

2.6. Chelating abilities on ferrous ions

Chelating ability was determined according to the method of Shimada et al. (1992). To 2 ml of the mixture consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma) and 9 mM ferrous sulphate (Union Chemical Works, Hsinchu, Taiwan) were added each extract (1–20 mg ml^{-1}) in deionised water (2 ml) and 200 μl of 1 mM tetramethyl murexide (TMM, Sigma). After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm against a blank. A lower absorbance indicates a higher chelating power. EC_{50} value (mg extract ml^{-1}) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. Citric acid (Sigma) and ethylenediaminetetraacetic acid (EDTA, Sigma) were used as controls.

2.7. Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each extract (20 mg) was extracted with 10 ml of 1% metaphosphoric acid (Union) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichloroindophenol (Sigma) and the absorbance was measured within 15 s at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid.

β-Carotene was extracted and analysed as described by Rundhaug, Pung, Read, and Bertram (1988). Each extract (20 mg) was extracted with a solution of 1% pyrogallol (Wako) in 10 ml of methanol/dichloromethane (1:1, v/v) for 45 min at room temperature, filtered through Whatman No. 4 filter paper and adjusted the volume to 10 ml using the same solution. The filtrate was then passed through a filter unit (13 mm, Lida Corp., Kenosha, WI) and filtered using a 0.45-μm CA filter paper prior to injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Hitachi D-6200 pump, a Hitachi L-5000 LC controller, a Rheodyne 7161 injector, a 20-μl sample loop, a Hitachi D-2500 chromato-integrator, a Hitachi L-4000 UV detector, and a Prodigy 5 ODS-2 column (4.6 × 250 mm, 5 μm, Phenomenex Inc., Torrance, CA). The mobile phase was acetone/methanol/acetonitrile, 1:2:2 (v/v/v), at a flow rate of 0.7 ml min^{-1} and UV detection was at 470 nm. Content of β-carotene was calculated on the basis of the calibration curve of authentic β-carotene (Sigma).

Tocopherols were extracted and analysed according to the method of Carpenter (1979). Each extract (50 mg) was suspended in 6 ml of pyrogallol (6% in 95% ethanol) and 4 ml of 60% potassium hydroxide aqueous solution, and the resulting mixture was saponified at 70 °C for 20 min. Deionised water (15 ml) was added and the mixture was extracted with 15 ml of *n*-hexane. The organic layer was washed with deionised water to neutral, dried over anhydrous sodium sulphate, and rotary evaporated to dryness. The residue was redissolved in 5 ml of *n*-hexane and filtered prior to HPLC injection in the same manner as in the β-carotene assay.

The HPLC system was the same as for the β-carotene assay. The mobile phase was acetonitrile/methanol, 85:15 (v/v), at a flow rate of 1.0 ml min^{-1} and UV detection was at 295 nm. Content of each tocopherol was calculated on the basis of the calibration curve of each authentic tocopherol (Sigma).

Total phenols were determined according to the method of Taga, Miller, and Pratt (1984). Each extract (20 mg) was dissolved in a solution of 5 ml of 1.3% HCl in methanol/deionised water (60:40, v/v) and the resulting mixture (100 μl) was added to 2 ml of 2% aqueous sodium carbonate solution. After 3 min, 100 μl of 50% Folin-Ciocalteau's phenol reagent (Sigma) were added to the mixture. After 30 min standing, the absorbance was measured at 750 nm against a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

2.8. Statistical analysis

For each hot water extract from fruit bodies, mycelia and filtrate, three samples were prepared for assays of every antioxidant attribute and component. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel, Torrie, and Dickey (1997) to determine the least significant difference at the level of 0.05.

3. Results and discussion

3.1. Antioxidant activity

Using hot water as the extractant, the yields were in the descending order of filtrate ≫ fruit bodies – mycelia (Table 1). The higher yield of filtrate was mainly due to the fact that most components contained in the filtrate were small and readily water-soluble. The yields of the

Table 1
Extraction yield of hot water extracts from *Agrocybe cylindracea*

	Amount[a] (g)	Extraction (%)[b] (w/w)
Fruit bodies	4.22 ± 0.44	42.20 B
Mycelia	3.89 ± 0.16	38.91 B
Filtrate	8.48 ± 1.60	84.80 A

[a] Extracted from lyophilized materials (10.00 g). Each value is expressed as mean ± standard deviation ($n = 3$).

[b] Means with different letters within a column are significantly different ($p < 0.05$).

hot-water extracts were higher than those of the methanolic extracts from fruit bodies, mycelia and filtrate (33.5%, 28.0% and 28.0%, respectively) (Huang, Tsai, & Mau, 2002). The use of hot water to extract soluble component from three forms of *A. cylindracea* was to simulate the making of Chinese medicine and the brewing of herbal tea. Therefore, as compared to other solvent extracts, the information obtained by use of hot-water extracts would be more valuable for these products used in human diets.

Using the conjugated diene method, the antioxidant activities of hot-water extracts from *A. cylindracea* fruit bodies, mycelia and filtrate were 32.7%, 22.1% and 20.5% at 1 mg ml^{-1} and 63.6%, 81.6% and 56.8% at 20 mg ml^{-1}, respectively (Fig. 1). However, the antioxidant activities were 99.9% at 0.1 mg ml^{-1} for BHA, 95.1% at 1 mg ml^{-1} for α-tocopherol and 59.3% at 20 mg ml^{-1} for ascorbic acid.

The methanolic extract from fruit bodies of *A. cylindracea* showed moderate (37.0–50.6%) at 0.1–1.0 mg ml^{-1} and high antioxidant activity (90.0–97.3%) at 5–20 mg ml^{-1} (Huang et al., 2002). However, the methanolic extract from its mycelia and filtrate show a rapid and concentration-dependent increase from 21.5% and 24.0% at 0.5 mg ml^{-1} to 67.4% and 90.9% at 20 mg ml^{-1}, respectively (Huang et al., 2002). With regard to antioxidant activity, it seemed that the hot-water extracts from fruit bodies and filtrate were less effective than the corresponding methanolic extracts.

The hot-water extracts from mature and baby Ling chih showed slight (2.97% and 6.57%) at 1 mg ml^{-1} and high antioxidant activities (78.5% and 78.2%, respectively) at 20 mg ml^{-1} (Mau, Tsai, Tseng, & Huang, 2005). The hot-water extract from filtrate showed moderate antioxidant activities (45.8%) at 20 mg ml^{-1} but no activity was found in the hot-water extract from mycelia. (Mau et al., 2005). In addition, the antioxidant activity of the hot-water extract from *Hypsizigus marmoreus* fruit bodies was 9.84% at 1 mg ml^{-1} and 87.2% at 20 mg ml^{-1} (Lee, 2003) whereas that of the hot-water extract from *Pleurotus citrinopileatus* fruit bodies was 43.4% at 1 mg ml^{-1} and 75.6% at 20 mg ml^{-1} (Huang, 2003). However, the hot-water extracts from *P. citrinopileatus* mycelia and filtrate showed low antioxidant activities of 32.4% and 28.7% at 20 mg ml^{-1}, respectively (Huang, 2003). It seemed that with regard to antioxidant activity, the hot-water extracts from *A. cylindracea* were less effective than hot-water extracts from mushrooms mentioned above.

3.2. Reducing power

Reducing powers of hot-water extracts from *A. cylindracea* fruit bodies, mycelia and filtrate were 0.22, 0.20 and 0.33 at 1 mg ml^{-1} and 1.02, 0.86 and 1.14 at 20 mg ml^{-1}, respectively (Fig. 2). However, BHA showed an excellent reducing power of 1.00 at 0.1 mg ml^{-1}, and remained the level of 1.11–1.21 at 20 mg ml^{-1}. At 0.5–20 mg ml^{-1}, ascorbic acid and α-tocopherol showed a slight increase in reducing power from 0.88 to 1.05 and from 0.67 to 0.87, respectively.

Reducing powers of the methanolic extracts from *A. cylindracea* increased in two patterns with increased concentrations, i.e., a fast increase to 0.99 at 5 mg ml^{-1} for fruit bodies and a slow increase to more than 1.0 at 20 mg ml^{-1} for mycelia and filtrate (Huang et al., 2002). It seemed that with regard to reducing power, the hot-water extracts were less effective than the corresponding methanolic extracts.

Reducing powers of hot-water extracts from mature and baby Ling chih, mycelia and filtrate were 0.48, 0.44, 0.23 and 0.42 at 1 mg ml^{-1} and 1.08, 1.04, 0.95 and 1.12 at 20 mg ml^{-1}, respectively (Mau et al., 2005). At 5 mg ml^{-1}, the hot-water extract from *P. citrinopileatus*

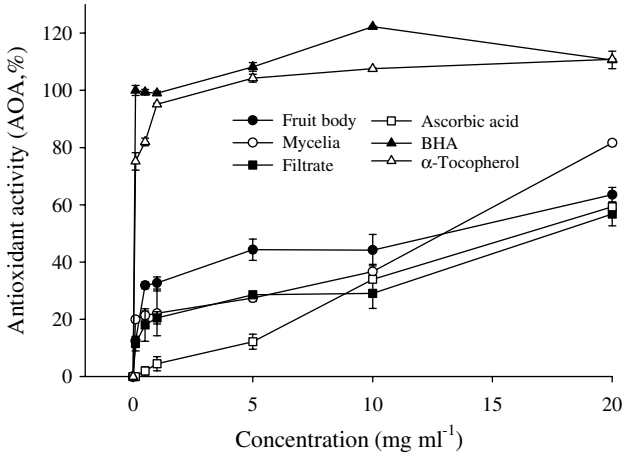

Fig. 1. Antioxidant activity of hot water extracts from *Agrocybe cylindracea* (conjugated diene method). Each value is expressed as mean ± standard deviation ($n = 3$).

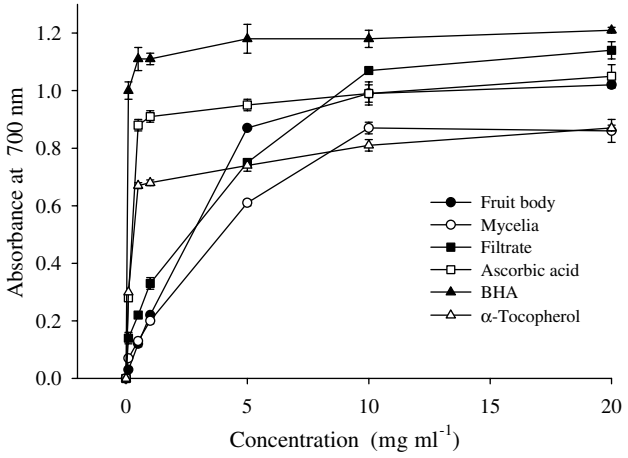

Fig. 2. Reducing power of hot water extracts from *Agrocybe cylindracea*. Each value is expressed as mean ± standard deviation ($n = 3$).

fruit bodies showed a high reducing power of 1.10 whereas those from mycelia and filtrate showed low reducing power of 0.32 and 0.15 (Huang, 2003). However, the hot-water extract from *H. marmoreus* fruit bodies showed a good reducing power of 1.01 at 10 mg ml^{-1} (Lee, 2003). With regard to reducing power, it can be concluded that these hot-water extracts showed higher absorbances at low concentrations.

3.3. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

At 1–20 mg ml^{-1}, the scavenging abilities of hot-water extracts from *A. cylindracea* fruit bodies, mycelia and filtrate on DPPH radicals were in the range of 58.3–66.2%, 47.7–76.1% and 53.5–73.5%, respectively (Fig. 3). Obviously, these hot-water extracts showed moderate activities at the concentrations tested. At 0.1 mg ml^{-1}, BHA and α-tocopherol showed excellent scavenging abilities of 95.0% and 92.6%, respectively. However, at 0.5–20 mg ml^{-1}, ascorbic acid showed a plateau of scavenging ability of 38.3–47.8%.

The scavenging ability of the methanolic extract from *A. cylindracea* fruit bodies was 89.0% at 1 mg ml^{-1} whereas the methanolic extracts from mycelia and filtrate scavenged 91.4% and 94.9% of DPPH radicals at 10 mg ml^{-1}, respectively (Huang et al., 2002). It seemed that the hot-water extracts from *A. cylindracea* fruit bodies, mycelia and filtrate were less effective in scavenging activities than methanolic extracts.

Similarly, at 1–20 mg ml^{-1}, the scavenging abilities of hot-water extracts from mature and baby Ling chih, mycelia and filtrate on DPPH radicals were in the range of 64.6–79.3%, 56.7–80.1%, 52.9–91.2% and 38.8-58.9%, respectively (Mau et al., 2005). Less effectively, at 20 mg ml^{-1}, the scavenging abilities of hot-water extracts from *P. citrinopileatus* fruit bodies, mycelia and filtrate were 52.3%, 48.3% and 23.3%, respectively (Huang, 2003). However, the hot-water extract from *H. marmoreus* fruit bodies showed a moderate scavenging ability of 77.2% at 20 mg ml^{-1} (Lee, 2003).

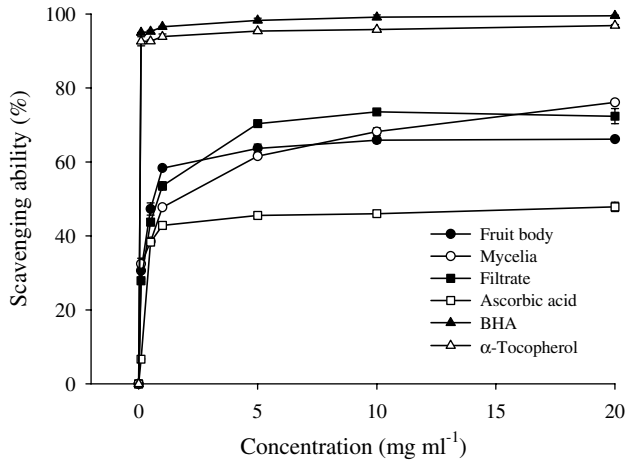

Fig. 3. Scavenging ability of hot water extracts from *Agrocybe cylindracea* on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation ($n = 3$).

3.4. Scavenging ability on hydroxyl radicals

The scavenging abilities of all hot-water extracts from *A. cylindracea* on hydroxyl radicals showed a concentration-dependent increase (Table 2). At 20 mg ml^{-1}, scavenging abilities were in the descending order of filtrate > mycelia > fruit bodies. However, the scavenging ability of BHA was 38.20% at 20 mg ml^{-1}.

The scavenging abilities of methanolic extracts from *A. cylindracea* on hydroxyl radicals were not as good as expected (Huang et al., 2002). At 5 mg ml^{-1}, scavenging abilities were 3.82%, 0% and 3.96% for methanolic extracts from fruit bodies, mycelia and filtrate. With regard to scavenging ability on hydroxyl radicals, the hot-water extracts were much more effective than the corresponding methanolic extracts.

Mau, Chao, and Wu (2001) also indicated that methanolic extracts from ear mushrooms were not good scavengers for hydroxyl radicals. Similarly, methanolic extracts from *Antrodia camphorata* and *Agaricus blazei* did not scavenge hydroxyl radicals (Huang, 2000). At 40 mg ml^{-1}, methanolic extracts from specialty mushrooms scavenged hydroxyl radicals by 39.6–75.0% (Mau, Lin, & Song, 2002), whereas those from commercial mushrooms showed scavenging abilities of 29.2–36.6% (Yang, Lin, & Mau, 2002). It is revealed that most methanolic extracts from mushrooms including fruit bodies and mycelia were not good scavengers for hydroxyl radicals.

At 20 mg ml^{-1}, scavenging abilities on hydroxyl radicals were in the descending order of Ling chih – baby Ling chih > mycelia > filtrate (72.4%, 73.7%, 55.9% and 46.0%, respectively) (Mau et al., 2005). Similarly, the hot-water extracts from *P. citrinopileatus* fruit bodies, mycelia and filtrate scavenged 80.1%, 57.0% and 54.3% of hydroxyl radicals at 20 mg ml^{-1}, respectively (Huang, 2003). However, the hot-water extract from *H. marmoreus* fruit bodies showed a moderate scavenging ability of 51.8% at 20 mg ml^{-1} (Lee, 2003).

Table 2
Scavenging ability of hot water extracts from *Agrocybe cylindracea* on hydroxyl radicals

Amount (mg ml^{-1})	Scavenging ability[a] (%)		
	Fruit bodies	Mycelia	Filtrate
5.0	16.99 ± 0.72 C	8.55 ± 0.65 C	30.87 ± 0.64 C
10.0	31.08 ± 0.66 B	24.69 ± 0.69 B	60.31 ± 0.46 B
20.0	43.39 ± 0.57 A	50.52 ± 0.69 A	78.38 ± 0.07 A

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a column are significantly different ($p < 0.05$).

These results indicated that many hot-water extracts from mushrooms are effective scavengers for hydroxyl free radicals. In addition, Shi et al. (1991) reported hydroxyl-radical scavenging ability of caffeine, and attributed the alleged anticarcinogenic properties of caffeine to this ability. Accordingly, it was anticipated that the moderate to high scavenging ability of hot-water extracts might possess some antimutagenic properties.

3.5. Chelating ability on ferrous ions

The hot-water extracts from *A. cylindracea* fruit bodies and mycelia showed moderate ferrous ion chelating abilities and were 45.8% and 60.2% at 20 mg ml^{-1}, respectively (Fig. 4). In addition, the hot-water extract from filtrate showed a high chelating ability of 92.7% at 20 mg ml^{-1} (Mau et al., 2005). However, EDTA showed an excellent chelating ability of 94.6% at a concentration as low as 0.10 mg ml^{-1}. Citric acid was not a good chelating agent for ferrous ions and its chelating ability was 33.5% at 20 mg ml^{-1}.

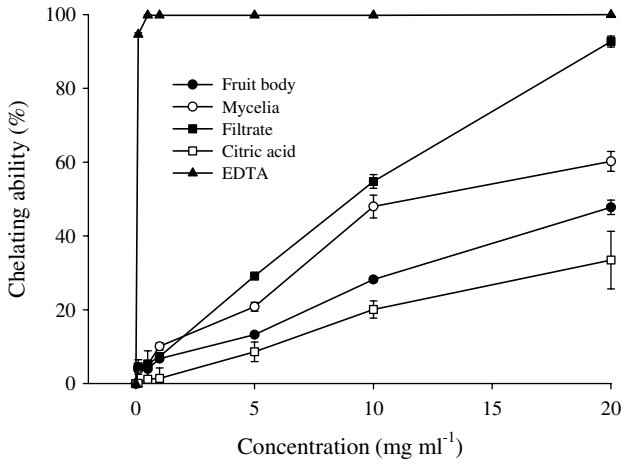

Fig. 4. Chelating ability of hot water extracts from *Agrocybe cylindracea* on ferrous ions. Each value is expressed as mean ± standard deviation ($n = 3$).

All the methanolic extracts from *A. cylindracea* fruit bodies, mycelia and filtrate were good chelators for ferrous ions and their chelating abilities were 90.6%, 84.6% and 96.3% at 5 mg ml^{-1}, respectively (Huang et al., 2002). With regard to ferrous ion chelation, the hot-water extracts were less effective than the corresponding methanolic extracts.

The hot-water extracts from mature and baby Ling chih chelated 42.6% and 39.5% of ferrous ions at 20 mg ml^{-1}, respectively, whereas those from mycelia and filtrate chelated 4.9% and 17.2% of ferrous ions at 20 mg ml^{-1}, respectively (Mau et al., 2005). On the contrary, the hot-water extracts from *P. citrinopileatus* fruit bodies and mycelia chelated 82.1% and 87.9% of ferrous ions at 5 mg ml^{-1}, respectively (Huang, 2003). The hot-water extract from filtrate showed a moderate chelating ability of 46.7–58.3% at 5–20 mg ml^{-1} (Huang, 2003). In addition, the hot-water extract from *H. marmoreus* fruit bodies showed a high chelating ability of 92.6% at 5 mg ml^{-1} (Lee, 2003). Since ferrous ions are the most effective pro-oxidants in the food system (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), the low to moderate ferrous-ion chelating abilities of methanolic extracts from *A. cylindracea* would be somewhat beneficial.

3.6. EC_{50} in antioxidant properties

The antioxidant properties assayed herein were summarized in Table 3, and the results were normalized and expressed as EC_{50} values (mg hot-water extract per ml) for comparison. Effectiveness in antioxidant properties inversely correlated with EC_{50} value. With regard to EC_{50} values in antioxidant activity by the conjugated diene method, the hot-water extracts from fruit bodies and mycelia were better than that from filtrate. Effectiveness in reducing powers was good for three forms of *A. cylindracea* with the hot-water extract from fruit bodies being the most effective.

Scavenging abilities on DPPH radicals were below 2 mg ml^{-1} for hot-water extracts from three forms of *A. cylindracea* and were in a descending order of fruit

Table 3
EC_{50} values of hot water extracts from *Agrocybe cylindracea* in antioxidant properties

	EC_{50} value[a] (mg ml^{-1})		
	Fruit bodies	Mycelia	Filtrate
Antioxidant activity (conjugated diene method)	12.92 ± 1.19 B[b]	13.24 ± 0.24 B	17.54 ± 0.79 A
Reducing power	2.72 ± 0.01 C	3.97 ± 0.05 A	3.09 ± 0.04 B
Scavenging ability on DPPH radicals	0.62 ± 0.06 B	1.66 ± 0.03 A	0.82 ± 0.03 B
Scavenging ability on OH radicals	25.37 ± 0.42 A	19.80 ± 0.27 B	8.26 ± 0.02 C
Chelating ability on ferrous ions	>20	11.60 ± 1.75 A	9.08 ± 0.21 B

[a] EC_{50} value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazyl (DPPH) or hydroxyl (OH) radicals were scavenged by 50%; and ferrous ions were chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.

[b] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row at a specific EC_{50} are significantly different ($p < 0.05$).

bodies – filtrate > mycelia. Scavenging abilities on hydroxyl radicals were in a descending order of filtrate > mycelia > fruit bodies. With regard to EC_{50} values in chelating abilities on ferrous ions, the hot-water extract from filtrate was better than that from mycelia. However, EC_{50} value was not available for the hot-water extract from fruit bodies. From EC_{50} values obtained, it can be concluded that hot-water extracts from three forms of *A. cylindracea* were good in antioxidant properties.

When the extraction yields were taken into consideration, EC_{50} values (mg dried mycelia per ml) in antioxidant activity were 30.6, 34.0 and 20.7 mg ml^{-1} for fruit bodies, mycelia and filtrate, respectively. EC_{50} values in reducing power were 6.45, 10.2 and 3.64 mg ml^{-1} for fruit bodies, mycelia and filtrate, respectively. EC_{50} values in scavenging ability on DPPH radicals were 14.7, 4.27 and 0.97 mg ml^{-1} for fruit bodies, mycelia and filtrate, respectively. EC_{50} values in scavenging ability on hydroxyl radicals were 60.1, 50.9 and 9.74 mg ml^{-1} for fruit bodies, mycelia and filtrate, respectively. EC_{50} values in chelating ability on ferrous ions were 29.8 and 10.7 mg ml^{-1} for mycelia and filtrate, respectively.

Although BHA and α-tocopherol were good in antioxidant activity, reducing power and scavenging ability on DPPH radicals and EDTA was excellent for chelating ferrous ions, they are additives and used or present in mg levels in foods. However, *A. cylindracea* in the form of Ling chih, baby Ling chih, mycelia and filtrate could be used in g levels as food or a food ingredient. Therefore, *A. cylindracea* in human diets might serve as possible protective agents to help human reduce oxidative damage.

3.7. Antioxidant components

Naturally occurring antioxidant components, including ascorbic acid and total phenols, were found in hot-water extracts from fruit bodies, mycelia and filtrate (Table 4). Tocopherols were found in hot-water extracts from fruit bodies and mycelia. Total phenols were the major naturally occurring antioxidant components found in hot-water extracts from *A. cylindracea* and in the range of 23.74–30.16 mg g^{-1}. Total antioxidant components varied among hot-water extracts and were 30.46, 27.72 and 24.57 mg g^{-1} for fruit bodies, mycelia and filtrate, respectively.

Phenols such as BHT and gallate were known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Yen and Duh (1993) found that the antioxidant activity of the methanolic extract from peanut hulls correlated with its content of total phenols. Therefore, the high content of total phenols in all hot-water extracts might explain high antioxidant properties in *A. cylindracea*. To study the antioxidant mechanisms by some specific phenolic components, the fractionation of the hot-water extract and further identification are in progress. Nevertheless, on the basis of the results obtained, upon the consumption of *A. cylindracea*, the alleged antioxidant properties might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage.

Table 4
Contents of ascorbic acid, β-carotene, tocopherols and total phenols of hot water extracts from *Agrocybe cylindracea*

Compound	Content[a] (mg g^{-1})		
	Fruit bodies	Mycelia	Filtrate
Ascorbic acid	0.28 ± 0.01 C	0.42 ± 0.05 B	0.83 ± 0.01 A
β-Carotene	nd[b]	nd	nd
α-Tocopherol	0.01 ± 0.00	nd	nd
γ-Tocopherol	nd	nd	nd
δ-Tocopherol	0.01 ± 0.01 A	0.02 ± 0.02 A	nd
Total phenols	30.16 ± 0.07 A	27.28 ± 0.30 B	23.74 ± 0.25 C

[a] Each value is expressed as mean ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$).
[b] Not detected.

Acknowledgements

We thank the Biotechnology Center of Grape King Inc. for providing the mycelia and fermentation filtrate of *A. cylindracea* and Lung-Kuo Mushroom Farm for fruit bodies.

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