Antioxidant Properties of Methanolic Extracts from Several Ear Mushrooms

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Five kinds of ear mushrooms are commercially available in Taiwan, including black, red, jin, snow, and silver ears. Methanolic extracts were prepared from these ear mushrooms, and their antioxidant properties were studied. For all methanolic extracts from ear mushrooms, the antioxidant activities in the 1,3-diethyl-2-thiobarbituric acid method were moderate (38.6∼74.6%) at 1.0−5.0 mg/mL. Methanolic extracts from red, jin, and snow ears showed excellent antioxidant activities in the conjugated diene method at 5.0 mg/mL. At 5.0 mg/mL, reducing powers of methanolic extracts were in the descending order of snow > black ≈ red ≈ jin > silver ears. The scavenging effect of methanolic extracts from ear mushrooms on 1,1-diphenyl-2-picrylhydrazyl radicals was excellent except for that from silver ears. Ear mushroom extracts were not good scavengers for hydroxyl free radicals but were good chelators for ferrous ions. Naturally occurring antioxidants, including ascorbic acid, tocopherols, and total phenols, were found in the methanolic extracts. However, β-carotene was not detected. Total antioxidant components were 15.69, 30.09, 27.83, 49.17, and 31.70 mg/g for black, red, jin, snow, and silver ears, respectively.

Keywords: *Ear mushrooms; Auricularia; Tremella; antioxidant activity; reducing power; scavenging effect; chelating effect; antioxidant components*

INTRODUCTION

Exogenous chemical and endogenous metabolic processes in the human body or in food systems might produce highly reactive free radicals, especially oxygen-derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage (*1*). Almost all organisms are well-protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol, and glutathione (*2*). When the mechanism of antioxidant protection becomes unbalanced by factors, such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage.

Recently, phytochemicals in food materials and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices, and herbs, have been researched extensively (*3*). Chinese herbs have been used for diet therapy for several millennia. Some of them are alleged to exhibit significant antioxidant activity (*4, 5*). Ear mushrooms are traditional Chinese medicine and are also commonly used as food. Currently, five kinds of ear mushrooms are commercially available in Taiwan, including black ear [*Auricularia mesenterica* (Dickson) Persoon], red ear [*A. polytricha* (Montagne) Saccardo], jin ear [*A. fusco-succinea* (Montagne) Farlow, brown strain], snow ear (*A. fuscosuccinea*, white strain), and silver ear (*Tremella fuciformis* Berkeley). In general, the cultivation methods for these ear mushrooms are parallel to that of shiitake mushrooms (*Lentinula edodes*) on logs or on sterilized sawdust (*6*).

The nutritional values and taste components of these ear mushrooms have been thoroughly studied (*7*). Recently, ear mushrooms including *Auricularia* and *Tremella* spp. were found to be medically active in several therapeutic effects including antiinflammatory, antitumor, blood pressure regulation, hypercholesterolemia, hyperlipidemia, cardiovascular disorders, and chronic bronchitis (*8*). However, little information is available about their antioxidant properties. Our objective was to evaluate the antioxidant properties of these ear mushrooms including antioxidant activity, reducing power, scavenging effects on radicals, and chelating effects on metallic ions. The levels of potential antioxidant components of these ear mushrooms were also determined.

MATERIALS AND METHODS

Mushrooms. Fresh ear mushrooms including black, red, and snow ears were obtained from the Taiwan Agricultural Research Institute, Wufeng, Taichung County, Taiwan. Each fresh ear mushroom was randomly selected into three samples (∼500 g of each) and air-dried in an oven at 40 °C before analysis. Dried jin and silver ears were purchased at a local market in Taichung City, Taiwan. Dried ear mushrooms were also randomly selected into three samples (∼50 g of each).

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After a fine powder (20 mesh) was obtained using a mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany), mushroom sample (10 g) was extracted by stirring with 100 mL of methanol at 25 °C at 150 rpm for 24 h and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 100 mL samples of methanol as described above. The combined methanolic extracts were then rotary evaporated at 40 °C to dryness. The dried extract thus obtained was used directly for analyses of antioxidant components or redissolved in methanol to a concentration of 10 mg/mL and stored at 4 °C for further use.

Antioxidant Activity by 1,3-Diethyl-2-thiobarbituric Acid Method. The antioxidant activity was determined by the 1,3-diethyl-2-thiobarbituric acid (DETBA) method (*9, 10*). To 50 μL of each ear mushroom extract (1∼5 mg/mL) in methanol was added 50 μL of linoleic acid emulsion (Sigma Chemical Co., St. Louis, MO; 2 mg/mL in 95% ethanol). The mixture was incubated in an oven at 80 °C for 60 min and cooled in an ice bath. To the mixture were sequentially added 200 μL of 20 mM butylated hydroxytoluene (BHT; Sigma), 200 μL of 8% sodium dodecyl sulfate (SDS; Merck, Darmstadt, Germany), 400 μL of deionized water, and 3.2 mL of 12.5 mM DETBA (Aldrich Chemical Co., Milwaukee, WI) in sodium phosphate buffer (pH 3.0). The mixture was thoroughly mixed, placed in an oven at 95 °C for 15 min, and then cooled with an ice bath. After 4 mL of ethyl acetate was added, the mixture was mixed and centrifuged at 2000 rpm at 20 °C for 15 min. Ethyl acetate was separated, and its absorbance was measured in a Hitachi 650−40 spectrofluorometer with fluorescence excitation at 515 nm and emission at 555 nm. The antioxidant activity was expressed as the percentage of lipid peroxidation with a control containing no sample being 100%. A higher percentage indicates a lower antioxidant activity. Butylated hydroxyanisole (BHA) was used as control.

Antioxidant Activity by the Conjugated Diene Method. The antioxidant activity was also determined by the conjugated diene method (*11*). Each ear mushroom extract (1∼5 mg/mL) in methanol (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 being incubated for 15 h, 6 mL of 60% methanol (Mallinckrodt Baker, Paris, KY) in deionized water was added, and the absorbance of the mixture was measured at 234 nm in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows: AOA (%) = [(ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control] × 100%. An AOA value of 100% indicates the strongest antioxidant activity.

Reducing Power. The reducing power was determined according to the method of Oyaizu (*12*). Each ear mushroom extract (1∼5 mg/mL) in methanol (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) was added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power.

Scavenging Effect on 1,1-Diphenyl-2-picrylhydrazyl Radicals. Each ear mushroom extract (1∼5 mg/mL) in methanol (4 mL) was mixed with 1 mL of methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma) radicals, so that the final concentration of DPPH was 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in dark, and the absorbance was then measured at 517 nm (*13*).

Scavenging Effect on Hydroxyl Free Radicals. The hydroxyl radical reacted with the nitrone spin trap 5,5-dimethyl pyrroline-*N*-oxide (DMPO; Sigma), and the resultant DMPO−OH adduct was detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing 200 μL of each ear mushroom extract (1−5 mg/mL) in methanol with 200 μL of 10 mM H_2O_2 (Merck), 200 μL of 10 mM Fe^{2+} (Sigma), and 200 μL of 10 mM DMPO using a Bruker EMX-10 EPR spectrometer set at the following conditions: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant, and 200 s scan period (*14*).

Chelating Effects on Ferrous Ions. Chelating effect was determined according to the method of Shimada et al. (*13*). To 2 mL of the mixture consisting of 30 mM hexamine (Wako), 30 mM potassium chloride (Sigma), and 9 mM ferrous sulfate (Union Chemical Works, Hsinchu, Taiwan) was added 2 mL of each ear mushroom extract (1−5 mg/mL) in methanol 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. A lower absorbance indicates a higher chelating power.

Determination of Antioxidant Components. Ascorbic acid was determined according to the method of Klein and Perry (*15*). Dried methanolic extract from ear mushrooms (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 in 15 s at 515 nm. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (Sigma).

β-Carotene was extracted and analyzed as described by Rundhaug et al. (*16*). A dried methanolic extract from ear mushrooms (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 and filtered through Whatman No. 4 filter paper, and the volume was adjusted 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 and UV detection 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 analyzed according to the method of Carpenter (*17*). The dried methanolic extract from ear mushrooms (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. Deionized water (15 mL) was added, and the mixture was extracted with 15 mL of *n*-hexane. The organic layer was washed with deionized water to neutral, dried over anhydrous sodium sulfate, 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 and UV detection 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 et al. (*18*). The dried methanolic extract from ear mushrooms (20 mg) was dissolved in a solution of 5 mL of 1.3% HCl in methanol/deionized 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 reagent (Sigma) was added to the mixture. After the mixture stood for 30 min, absorbance was measured at 750 nm. Content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

Statistical Analysis. For methanolic extracts from ear mushrooms, three samples were prepared for assays of every antioxidant attribute. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel et al. (*19*) to determine the least significant difference at the level of 0.05.

Table 1. Yield of Methanolic Extracts from Several Ear Mushrooms

mushroom	yield (g)[a]	extraction % (w/w)[b]
black ear (*Auricularia mesenterica*)	0.45 ± 0.01	4.51 D
red ear (*Auricularia polytricha*)	0.21 ± 0.04	2.09 E
jin ear (*Auricularia fuscosuccinea* brown strain)	1.08 ± 0.03	10.82 B
snow ear (*Auricularia fuscosuccinea* white strain)	1.16 ± 0.03	11.57 A
silver ear (*Tremella fuciformis*)	0.61 ± 0.01	6.14 C

[a] Extracted from dried ear mushrooms (10.00 g). Each value is expressed as mean ± SD ($n = 3$). [b] Means with different letters within a column are significantly different ($p < 0.05$).

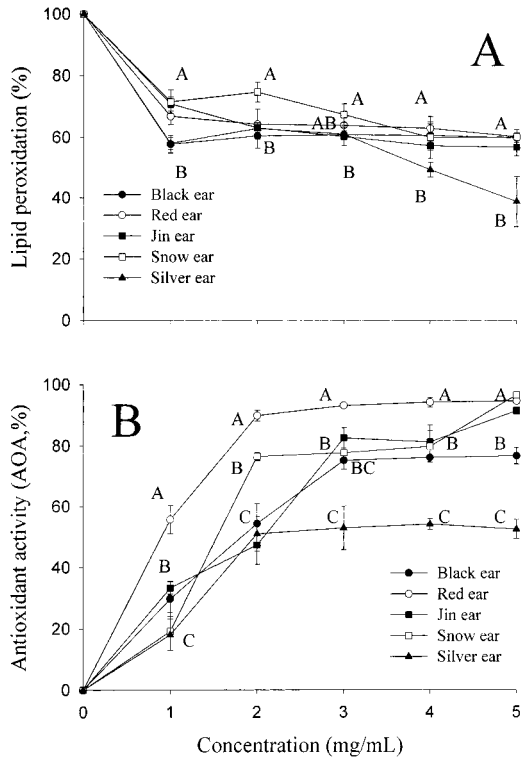

Figure 1. Antioxidant activity of methanolic extracts from several ear mushrooms by 1,3-diethyl-2-thiobarbituric acid (A) and conjugated diene (B) methods. Each value is expressed as mean ± standard deviation ($n = 3$).

RESULTS AND DISCUSSION

Antioxidant Activities. Following the extraction with methanol, two strains of *A. fuscosuccinea* (snow and jin ears) had the highest yields (11.57 and 10.82%, respectively) (Table 1). However, the rest of ear mushrooms had significantly lower yields of 2.09−6.14% and were in the descending order of silver, black, and red ears. Using the DETBA method, all methanolic extracts from ear mushrooms showed moderate antioxidant activities as evidenced by the moderate percentages of lipid peroxidation (38.6−74.6%) at 1.0−5.0 mg/mL (Figure 1A). However, BHA only showed 66.1% of lipid peroxidation at 10 mg/mL.

Huang (*20*) found that methanolic extracts from a medicinal mushroom *Antrodia camphorata* (Chang-chih) showed excellent antioxidant activities as evidenced by 5.88−7.59% of lipid peroxidation at 5 mg/mL, respectively. Huang (*20*) also found that methanolic extract from another medicinal mushroom *Agaricus blazei* (Brazilian mushrooms) showed similar excellent antioxidant activity (6.09% of lipid peroxidation) at 5 mg/mL. Lin (*21*) found that methanolic extracts from other medicinal mushrooms were extremely effective in inhibiting lipid peroxidation [6.41% for *Ganoderma lucidum* (Ling-chih), 2.62% for *G. lucidum* antler, and 2.30% for *G. tsugae* (Sung-shan-ling-chih) at 0.6 mg/mL]. However, the methanolic extract from another medicinal mushroom *Coriolus versicolor* (Yun-chih) showed only 58.56% of lipid peroxidation at 0.6 mg/mL (*21*), which was similar to the findings in Figure 1A.

Among methanolic extracts from four specialty mushrooms at 1.2 mg/mL, only *Dictyophora indusiata* (basket stinkhorn) showed an excellent antioxidant activity (2.26% of lipid peroxidation) (*21*). *Grifola frondosa* (maitake) showed a relatively high antioxidant activity (29.81% of lipid peroxidation), whereas *Hericium erinaceus* (lion's mane) and *Tricholoma giganteum* (white matsutake) showed moderate antioxidant activities (48.45% and 67.02% of lipid peroxidation, respectively) (*21*). Among methanolic extracts from commercial mushrooms at 1.2 mg/mL, *Flammulina velutipes* (winter mushrooms), *Lentinula edodes* (shiitake), *Pleurotus cystidiosus* (abalone mushrooms), and *P. ostreatus* (tree oyster mushrooms) showed moderate to high antioxidant activities (24.71−62.30% of lipid peroxidation) (*21*). It was obvious that the antioxidant activities of methanolic extracts in the DETAB method were excellent for medicinal mushrooms, high for specialty mushrooms, and moderate for commercial and ear mushrooms.

Using the conjugated diene method, all methanolic extracts from ear mushrooms showed different patterns of antioxidant activities at 1.0−5.0 mg/mL (Figure 1B). The antioxidant activities of methanolic extracts from red and silver ears increased as the concentration increased from 1.0 to 2.0 mg/mL and reached a plateau of 89.85−94.62% and 50.94−54.16% at 2.0−5.0 mg/mL, respectively. Similarly, that of methanolic extract from black ears reached a plateau of 75.14−76.60% at 3.0−5.0 mg/mL. In contrast, methanolic extracts from jin and snow ears showed steadily increased antioxidant activities as concentrations increased to 91.44 and 96.56% at 5.0 mg/mL, respectively. In addition, the antioxidant activities of BHA and α-tocopherol at 20 mM (3.6 and 8.6 mg/mL) were 94 and 83%, respectively. Therefore, methanolic extracts from red, jin, and snow ears showed excellent antioxidant activities at 5.0 mg/mL that were similar to that of BHA at 3.6 mg/mL and better than that of α-tocopherol at 8.6 mg/mL. However, antioxidant activities of methanolic extracts from ear mushrooms were in the descending order of red ≈ jin ≈ snow > black > silver ears at 5 mg/mL.

Huang (*20*) mentioned that the methanolic extract from Chang-chih exhibited an outstanding antioxidant activity of 91.24−93.01% at as low as 0.5 mg/mL, whereas that from Brazilian mushrooms also showed an excellent activity of 91.76% at 1.0 mg/mL. As compared to these two well-known medicinal mushrooms, the tested ear mushrooms contained less amounts of components effective for inhibiting the oxidation of linoleic acid.

Reducing Power. The methanolic extract from snow ears showed a high reducing power of 0.73−0.84 at 1.0−5.0 mg/mL (Figure 2). These results might be the reason

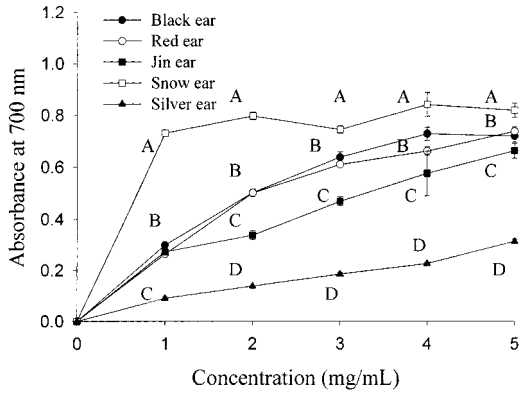

Figure 2. Reducing power of methanolic extracts from several ear mushrooms. Each value is expressed as mean ± standard deviation ($n = 3$).

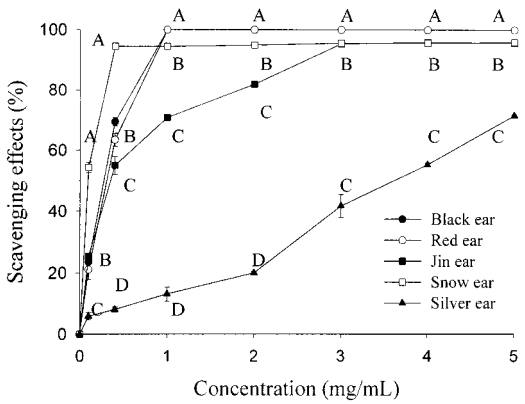

Figure 3. Scavenging effect of methanolic extracts from several ear mushrooms on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation ($n = 3$).

that explains why snow ears, which are a stable translucent white mutant of jin ears (*7*), maintained their white color and faded away the original brown color. The reducing power of methanolic extracts from the other ear mushrooms increased along with the increased concentrations. Methanolic extracts from black, red, and jin ears showed a good reducing power of 0.67−0.74 at 5 mg/mL. However, the methanolic extract from silver ears only exhibited a low reducing power of 0.32 at 5 mg/mL. The reducing power of methanolic extracts from ear mushrooms might be due to their hydrogen-donating ability as described by Shimada et al. (*13*). Accordingly, the methanolic extract from snow ears might contain higher amount of reductone, which could react with free radicals to stabilize and terminate radical chain reactions. In addition, the methanolic extract from silver ears seemed to contain the lowest amount of reductone. However, reducing powers of BHA and α-tocopherol at 20 mM (3.6 and 8.6 mg/mL) were 0.12 and 0.13, respectively.

Huang (*20*) reported that the methanolic extract from Chang-chih showed an excellent reducing power of 0.92−0.94 at 5 mg/mL, whereas that from Brazilian mushrooms showed a reducing power of 0.79 at 5 mg/mL. Methanolic extracts from other medicinal mushrooms including Ling-chih, antler Ling-chih, and Sung-shan-ling-chih exhibited a strong reducing power of 0.81, 1.03, and 1.05 at 1.5 mg/mL, respectively (*21*). However, a good reducing power of 0.79 was observed with the methanolic extract from another medicinal mushroom, Yun-chih, at 4.0 mg/mL (*21*).

Among methanolic extracts from four specialty mushrooms, basket stinkhorn showed an excellent reducing power of 1.09 at 3 mg/mL (*21*). Reducing powers of methanolic extracts from maitake, lion's mane, and white matsutake were 0.88, 0.79, and 0.50 at 6 mg/mL, respectively (*21*). Among methanolic extracts from commercial mushrooms, abalone and tree oyster mushrooms exhibited excellent reducing powers of 0.65 and 0.81 at 5 mg/mL, respectively (*21*). Reducing powers of methanolic extracts from two strains of winter mushrooms were 0.35 and 0.43 at 5 mg/mL, whereas reducing powers of 0.42 and 0.57 were observed with that from two strains of shiitake at 5 mg/mL (*21*). These five ear mushrooms used in this study are commercial mushrooms just like the six commercial mushrooms mentioned above; their reducing powers were somewhat comparable at 5 mg/mL. However, the reducing power of the methanolic extracts from ear mushrooms was only determined up to 5 mg/mL. It is anticipated that the reducing power might increase consistently to a much higher absorbance value as the concentrations increased.

Scavenging Effect on 1,1-Diphenyl-2-picrylhydrazyl Radicals. The scavenging effect of methanolic extracts from ear mushrooms on DPPH radicals was excellent except for that from silver ears (Figure 3). Methanolic extracts from black and red ears showed an outstanding scavenging effect of 100% at 1.0 mg/mL, whereas that from jin ears showed a scavenging effect of 95.4% at 3.0 mg/mL. The scavenging effect of the methanolic extract from snow ears increased with the increased concentrations to a plateau of 94.5−96.0% at 0.4−5.0 mg/mL. In addition, at 5.0 mg/mL, the scavenging effect of the methanolic extract from silver ears was 71.5%. However, the scavenging effect of BHA and α-tocopherol at 20 mM (3.6 and 8.6 mg/mL) was 96 and 95%, respectively.

Excellent scavenging effects (96.3−99.1% and 97.1%) were observed with methanolic extracts from Chang-chih and Brazilian mushrooms at 2.5 mg/mL (*20*), respectively. Scavenging effects of methanolic extracts from other medicinal mushrooms were measured at up to 0.64 mg/mL and were 24.6, 67.6, 74.4, and 73.5% for Yun-chih, Ling-chih, antler Ling-chih, and Sung-shan-ling-chih, respectively (*21*). At 6.4 mg/mL, the methanolic extract from basket stinkhorn scavenged DPPH radicals by 92.1%, whereas scavenging effects of methanolic extracts from other specialty mushrooms were 63.3−67.8% (*21*). In addition, at 6.4 mg/mL, the methanolic extract from tree oyster mushrooms scavenged DPPH radicals by 81.8% (*21*). However, scavenging effects of methanolic extracts from other commercial mushrooms were 42.9−69.9% (*21*). As compared to commercial and specialty mushrooms, ear mushrooms excluding silver ears were good DPPH scavengers.

These results revealed that ear mushrooms excluding silver ears were free radical inhibitors or scavengers, acting possibly as primary antioxidants. Methanolic extracts from ear mushrooms excluding silver ears might react with free radicals, particularly peroxy radicals, which are the major propagators of the autoxidation of fat, thereby terminating the chain reaction (*22*−*24*). Antioxidant activity of natural compounds has been shown to be involved in termination of free radical reactions and reducing power (*13, 25*).

Scavenging Effect on Hydroxyl Free Radicals. The scavenging effects of methanolic extracts from ear

Table 2. Scavenging Effect of Methanolic Extracts from Several Ear Mushrooms on Hydroxyl Free Radicals

amount (mg/mL)	scavenging effect (%)[a]				
	black ear	red ear	jin ear	snow ear	silver ear
0.1	0.00 ± 0.00 C	0.00 ± 0.00 B	0.00 ± 0.00 C	8.11 ± 0.67 C	1.84 ± 0.58 E
0.5	0.00 ± 0.00 C	0.07 ± 0.05 B	7.16 ± 0.23 A	0.00 ± 0.00 E	7.13 ± 0.44 D
1.0	2.90 ± 0.78 B	5.34 ± 0.39 A	0.00 ± 0.00 C	3.49 ± 0.28 D	8.55 ± 0.49 C
2.5	12.04 ± 1.02 A	1.23 ± 1.03 B	3.93 ± 0.34 B	9.21 ± 0.64 B	11.96 ± 0.10 B
5.0	10.85 ± 1.89 A	1.09 ± 0.32 B	0.09 ± 0.08 C	10.52 ± 0.71 A	14.01 ± 0.44 A

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

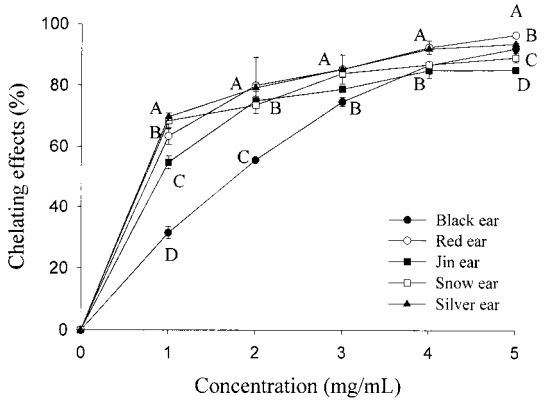

Figure 4. Chelating effect of methanolic extracts from several ear mushrooms on ferrous ions. Each value is expressed as mean ± standard deviation ($n = 3$).

mushrooms on hydroxyl free radicals were not as good as expected (Table 2). At 5 mg/mL, scavenging effects were 10.52−14.01% for methanolic extracts from black, snow, and silver ears, whereas no scavenging effect was observed with methanolic extracts from red and jin ears. In addition, the scavenging effect of BHA at 20 mM (3.6 mg/mL) was 23%, whereas that of α-tocopherol at 20 mM (8.6 mg/mL) was 34%. Similarly, methanolic extracts from Chang-chih and Brazilian mushrooms did not scavenge hydroxyl free radicals (*20*). At 40 mg/mL, methanolic extracts from specialty mushrooms scavenged hydroxyl free radicals by 39.6−75.0%, whereas those from commercial mushrooms scavenged hydroxyl free radicals by 29.2−36.6% (*21*). However, at 16 mg/mL, methanolic extracts from medicinal mushrooms such as Yun-chih, Ling-chih, and Sung-shan-ling-chih scavenged hydroxyl free radicals by 38.0−52.6% (*21*). These results indicated that mushrooms, especially ear mushrooms, are not good scavengers for hydroxyl free radicals.

Chelating Effects on Ferrous Ions. Chelating effects of methanolic extracts from ear mushrooms on ferrous ions increased with the increased concentrations and were 85.1−96.5% at 5 mg/mL (Figure 4). However, at 20 mM (3.6 mg/mL), the chelating effect of BHA was 36%, whereas that of α-tocopherol at 20 mM (8.6 mg/mL) was 92%. Since ferrous ions are the most effective pro-oxidants in the food system (*26*), the higher chelating effects of methanolic extracts from ear mushrooms would be beneficial.

Methanolic extracts from Chang-chih chelated ferrous ions by 64.4−74.5% at 5 mg/mL, whereas that from Brazilian mushrooms showed an excellent chelating effect of 98.6% at 2.5 mg/mL (*20*). The methanolic extract from Yun-chih was not a good ferrous chelator (13.2% at 2.4 mg/mL), whereas other medicinal mushrooms including Ling-chih, antler Ling-chih, and Sung-shan-ling-chih chelated 44.8−67.7% of ferrous ions at 2.4 mg/mL (*21*). Yen and Wu (*27*) reported that the methanolic extract of Sung-shan-ling-chih chelated 95.3% of ferrous ions at 600 ppm (0.6 mg/mL). However, Yen and Wu (*27*) used the method of Decker and Welch (*28*) to determine the chelating effect instead of the method of Shimada et al. (*13*). The methanolic extract from maitake chelated 70.3% of ferrous ions at 6 mg/mL whereas at 24 mg/mL, methanolic extracts from black stinkhorn, lion's mane, and white matsutake chelated ferrous ions by 46.4−52.0% (*21*). For commercial mushrooms including winter, abalone, and tree oyster mushrooms and shiitake, their methanolic extracts chelated 45.6−81.6% of ferrous ions at 1.6 mg/mL. As compared to other mushrooms, methanolic extracts from ear mushrooms were good ferrous ion chelators.

Antioxidant Components. Naturally occurring antioxidant components, including ascorbic acid, tocopherols, and total phenols, were found in methanolic extracts from ear mushrooms (Table 3). However, β-carotene was not detected. Total antioxidant components were 15.69, 30.09, 27.83, 49.17, and 31.70 mg/g for black, red, jin, snow, and silver ears, respectively. Although the contents of total antioxidant components varied widely, the antioxidant properties were comparable among ear mushrooms, excluding silver ears. Furthermore, silver ears contained the second highest amount of total antioxidant components, but their antioxidant properties were less effective than those of other ear mushrooms. Phenols such as BHT and gallate

Table 3. Contents of Ascorbic Acid, β-Carotene, Tocopherols, and Total Phenols of Methanolic Extracts from Several Ear Mushrooms

compound	content (mg/g)[a]				
	black ear	red ear	jin ear	snow ear	silver ear
ascorbic acid	1.63 ± 0.08 E	3.28 ± 0.11 D	11.24 ± 0.63 A	7.99 ± 0.71 B	6.74 ± 0.14 C
β-carotene	nd[b]	nd	nd	nd	nd
α-tocopherol	0.86 ± 0.05 C	16.71 ± 7.39 AB	10.10 ± 3.34 BC	29.54 ± 13.05 A	22.10 ± 3.13 AB
γ-tocopherol	8.59 ± 2.02 A	5.35 ± 0.55 B	1.41 ± 0.19 CD	2.92 ± 1.47 C	0.68 ± 0.54 D
δ-tocophenols	nd	1.55 ± 0.36 A	1.18 ± 0.47 A	nd	1.14 ± 0.34 A
total phenols	4.61 ± 0.21 B	3.20 ± 0.13 D	3.90 ± 0.13 C	8.72 ± 0.58 A	1.04 ± 0.09 E

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

were known to be effective antioxidants (*29*). Therefore, the content of total phenols that was low in the methanolic extract from silver ears might explain the lowest antioxidant properties in ear mushrooms.

At 5 mg/mL, the contents of total antioxidant components in methanolic extracts from ear mushrooms were in the range of 78.5−245.9 μg/mL, which were much less than BHA and α-tocopherol used at 20 mM (3.6 and 8.6 mg/mL). Therefore, it revealed that, in addition to these antioxidant components, some other components were also existed and contributed in part the antioxidant properties to ear mushrooms. To study the antioxidant mechanisms by some other potential antioxidant components, the fractionation of the methanolic extract and further identification are in progress.

Overall, for all methanolic extracts from ear mushrooms, the antioxidant activities in the DETBA method were moderate at 1.0−5.0 mg/mL. At 5.0 mg/mL, the antioxidant activities in the conjugated diene method were in the descending order of red ≈ jin ≈ snow > black > silver ears. At 5.0 mg/mL, reducing powers were in the descending order of snow > black ≈ red ≈ jin > silver ears. In addition, at 5.0 mg/mL, the scavenging effects on DPPH radicals were in the descending order of black ≈ red ≈ snow ≈ jin > snow ears. However, ear mushrooms were not good scavengers for hydroxyl free radicals but were good chelators for ferrous ions.

Generally, silver mushrooms were low in reducing power and scavenging effect on hydroxyl free radicals, moderate in both antioxidant activities and scavenging effect on DPPH radicals, but high in chelating effect on ferrous ions. Excluding silver mushrooms, *Auricularia* mushrooms showed moderate to high antioxidant activities, higher reducing power, excellent scavenging effect on DPPH radicals and chelating effect on ferrous ions, but almost no scavenging effect on hydroxyl free radicals. On the basis of the results obtained, upon the consumption of ear mushrooms, especially *Auricularia* mushrooms, their alleged antioxidant properties might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage.

ABBREVIATIONS USED

AOA, antioxidant activity; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DETBA, 1,3-diethyl-2-thiobarbituric acid; DMPO, 5,5-dimethyl pyrroline-*N*-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EPR, electron paramagnetic resonance; HPLC, high-performance liquid chromatograph; SDS, sodium dodecyl sulfate; TMM, tetramethyl murexide.

LITERATURE CITED

(1) Halliwell, B.; Gutteridge, J. M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **1984**, *219*, 1−4.

(2) Niki, E.; Shimaski, H.; Mino, M. *Antioxidantism-Free Radical and Biological Defense*; Gakkai Syuppan Center: Tokyo, 1994; pp 3−16.

(3) Ho, C. T., Osawa, T., Huang, M. T., Rosen, R. T., Eds. *Food Phytochemicals for Cancer Prevention II*; ACS Symposium Series 547; American Chemical Society: Washington, DC, 1994.

(4) Kim, S. Y.; Kim, J. H.; Kim, S. K.; Oh, M. J.; Jung, M. Y. Antioxidant activities of selected oriental herb extracts. *J. Am. Oil Chem. Soc.* **1994**, *71*, 633−640.

(5) Su, J.-D. Investigation of antioxidative activity and tocopherol contents on Chinese crude drugs of fruits and seeds. *Food Sci.* (*ROC*) **1992**, *19*, 12−24.

(6) Tu, C.-C.; Wu, K.-T. Properties of Jew's ear (*Auricularia* spp.) and silver ear (*Tremella fuciformis*) in Taiwan. In *Proceedings of the Seventh Symposium on Edible Fungi Research and Improvement of Production*; Taiwan Agriculture Research Institute: Taichung, Taiwan, 1989; pp 137−142.

(7) Mau, J.-L.; Wu, K.-T.; Wu, Y.-H.; Lin, Y.-P. Nonvolatile taste components of ear mushrooms. *J. Agric. Food Chem.* **1998**, *46*, 4583−4586.

(8) Wasser, S. P.; Weis, A. L. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspective (review). *Int. J. Med. Mushrooms* **1999**, *1*, 31−62.

(9) Furuta, S.; Nishiba, Y.; Suda, I. Fluorometric assay for screening antioxidative activity of vegetables. *J. Food Sci.* **1997**, *62*, 526−528.

(10) Suda, I.; Furuta, S.; Nishiba, Y. Fluorometric determination of a 1,3-diethyl-2-thiobarbituric acid−malondialdehyde adduct as an index of lipid peroxidation in plant materials. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 14−17.

(11) Lingnert, H.; Vallentin, K.; Eriksson, C. E. Measurement of antioxidative effect in model system. *J. Food Process. Preserv.* **1979**, *3*, 87−103.

(12) Oyaizu, M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307−315.

(13) Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **1992**, *40*, 945−948.

(14) Shi, X.; Dalal, N. S.; Jain, A. C. Antioxidant behaviour of caffeine: Efficient scavenging of hydroxyl radicals. *Food Chem. Toxicol.* **1991**, *29*, 1−6.

(15) Klein, B. P.; Perry, A. K. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *J. Food Sci.* **1982**, *47*, 941−945, 948.

(16) Rundhaug, J. E.; Pung, A.; Read, C. M.; Bertram, J. S. Uptake and metabolism of β-carotene and retinal by C3H/10T1/2 cells. *Carcinogenesis* **1988**, *9*, 1541−1545.

(17) Carpenter, A. P. Determination of tocopherols in vegetable oils. *J. Am. Oil Chem. Soc.* **1979**, *56*, 668−672.

(18) Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928−93.

(19) Steel, R. G.; Torrie, J. H.; Dickey, D. A. *Principles and Procedures of Statistics: A Biometrical Approach*; McGraw-Hill: Singapore, 1997.

(20) Huang, L.-C. Antioxidant properties and polysaccharide composition analysis of *Antrodia camphorata* and *Agaricus blazei*. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan, 2000.

(21) Lin, H.-C. Evaluation of taste quality and antioxidant properties of edible and medicinal mushrooms. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan, 1999.

(22) Frankel, E. N. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **1991**, *54*, 495−511.

(23) Gordon, M. H. The mechanism of antioxidant action in vitro. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier Applied Science: New York, 1990; pp 1−18.

(24) Shahidi, F.; Wanasundara, P. K. J. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−103.

(25) Tanaka, M.; Kuie, C. W.; Nagashima, Y.; Taguchi, T. Application of antioxidative Maillard reaction products from histidine and glucose to sadine products. *Nippon Suisan Gakkaishi* **1988**, *54*, 1409−1414.

(26) Yamaguchi, R.; Tatsumi, M. A.; Kato, K.; Yoshimitsu, U. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* **1988**, *52*, 849−850.
(27) Yen, G.-C.; Wu, J.-Y. Antioxidant and radical scavenging properties of extracts from *Ganoderma tsugae*. *Food Chem.* **1999**, *65*, 375−379.
(28) Decker, E. A.; Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* **1990**, *38*, 674−677.
(29) Madhavi, D. L.; Singhal, R. S.; Kulkarni, P. R. Technological aspects of food antioxidants. In *Food Antioxidants: Technological, Toxicological, and Health Perspectives*; Madhavi, D. L., Deshpande, S. S., Salunkhe, D. K., Eds.; Marcel Dekker: New York, 1996; pp 159−265.

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