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Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia

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Abstract

Three species of mushroom mycelia are commercially available in Taiwan, namely *Grifola frondosa* (maitake), *Morchella esculenta* (morel), and *Termitomyces albuminosus* (termite mushroom). Methanolic extracts were prepared from these three mycelia and their antioxidant properties were studied. Methanolic extracts from the three mycelia showed high antioxidant activities (85.4–94.7%) at 25 mg ml^{-1}. Reducing powers of the three methanolic extracts were 0.97–1.02 at 25 mg ml^{-1}. Scavenging effects on 1,1-diphenol-2-picrylhydrazyl radicals were 78.8–94.1% at 10 mg ml^{-1}. These three mycelia showed no scavenging effect on hydroxyl radicals. Chelating effects on ferrous ions were high (90.3–94.4%) at 10 mg ml^{-1}. Total phenols were the major naturally occurring antioxidant components found in methanolic extracts. Contents of ascorbic acid and tocopherols were similar for these three mycelia. All EC_{50} values were below 10 mg ml^{-1}, indicating that the three mycelia had good antioxidant properties except for the scavenging effect on hydroxyl radicals.

Keywords: *Grifola frondosa*; *Morchella esculenta*; *Termitomyces albuminosus*; Antioxidant activity; Reducing power; Scavenging effect; Chelating effect; Antioxidant components

1. Introduction

Mushrooms have been used as food and food-flavouring material in soups and sauces for centuries, due to their unique and subtle flavour. Mushrooms have recently become attractive as functional foods and as a source of physiologically beneficial medicine. Currently, three species, including maitake, morel and termite mushrooms, are highly valued in Taiwan, partially due to their rareness and difficulty in cultivation.

Grifola frondosa (Dickson: Fries) Gray (maitake) is also called the king of mushrooms and the hen of the woods (Stamets, 1993). The cultivation of this mushroom has been developed by the Taiwan Agricultural Research Institute, Wufeng, Taiwan. Currently, this mushroom is not yet available in the fresh market in Taiwan. *Morchella esculenta* (L.: Fries) Persoon (morel) is a mushroom of high gastronomic quality (Phillips, 1991) and has been defying attempts at domestication. However, morel is not available in Taiwan.

Termitomyces albuminosus (Berkeley and Broome) Heim (termite mushroom), also called chicken julienne mushroom, is a symbiotic fungus found in tropical Africa and Asia. Termites cultivate this fungus in their nests as food (Heim, 1977). The fruit bodies form inside the tunnels and bore through the very hard layer of inert matter, forcing their way through it with a special umbo (Kendrick, 2001). This rare termite mushroom is also found in Taiwan and is extremely tasty. Like morel, termite mushrooms are not always available.

In addition to dried mushrooms imported from China or Japan, alternative or substitute mushroom products are mycelia of these three mushrooms, mainly prepared from submerged culture. These mycelia are used as food and food-flavouring materials, and also in the formulation of nutraceuticals and functional foods.

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The nutritional values and taste components of these mycelia have been studied (Weng, 2003). Fruit bodies, as well as mycelia of several mushrooms were found to have good antioxidant properties (Huang, Huang, & Chen, 1999a, 1999b; Mau, Chao, & Wu, 2001, 2002a, 2002b; Tsai, 2002; Yang, Lin, & Mau, 2002). Accordingly, our objective was to evaluate the antioxidant properties of methanolic extracts from these three mushroom mycelia, including antioxidant activity, reducing power, scavenging effects on radicals, and chelating effects on ferrous ions. The contents of potential antioxidant components in methanolic extracts from these mycelia were also determined.

2. Materials and methods

2.1. Mushroom mycelia

Freeze-dried mycelia of *G. frondosa*, *M. esculenta* and *T. albuminosus* were obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan. Each mycelium was randomly selected into three samples (~50 g each). After a fine powder (20 mesh) was obtained using a mill (Restsch ultra centrifugal mill and sieving machine, Haan, Germany), a subsample (10 g) was extracted by stirring with 100 ml of methanol at 25 °C at 20*g* for 24 h and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 100 ml portions of methanol, as described above. The combined methanolic extracts were then rotary-evaporated at 40 °C to dryness. The dried extract was used directly for analyses of antioxidant components or redissolved in methanol 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 (0.5–25 mg ml^{-1}) 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 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 (%) = [(ΔA_{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 ml^{-1}) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis. Ascorbic acid, butylated hydroxyanisole (BHA) and α-tocopherol were used as controls.

2.3. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (0.5–25 mg ml^{-1}) 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 Chemical Co., St. Louis, MO), 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 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 effect on 1,1-diphenyl-2-picrylhydrazyl radicals

Each extract (0.5–25 mg ml^{-1}) in methanol (4 ml) was mixed with 1 ml of methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (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 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 effect on hydroxyl radicals

The hydroxyl radical 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 200 μl of each extract (0.5–25 mg ml^{-1}) in methanol 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 effects on ferrous ions

Chelating effect 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), was added 2 ml of each extract (0.5–25 mg ml^{-1}) 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 against a blank. A lower absorbance indicates a higher chelating power. EC_{50} value (mg 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 and ethylenediaminetetraacetic acid (EDTA) were used as controls.

2.7. Determination of antioxidant components

Ascorbic acid was determined according to the method of Klein and Perry (1982). Each dried methanolic 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 at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (Sigma).

β-carotene was extracted and analysed as described by Rundhaug, Pung, Read, and Bertram (1988). Each dried methanolic 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 the volume 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^{-1} 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 analysed according to the method of Carpenter (1979). Each dried methanolic 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 dried methanolic 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 reagent (Sigma) were added to the mixture. After 30 min standing, 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 methanolic extract from mycelia, 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

Following the extraction with methanol, the yields from mycelia were in the descending order: *G. frondosa* ≈ *T. albuminosus* > *M. esculenta* (Table 1). The yields from mycelia of *Ganoderma tsugae* and *Agrocybe cylindracea* were 40.6% and 28.0%, respectively (Tsai, 2002). The yields from mycelia of *Antrodia camphorata* and *Agaricus blazei* were 31.1 and 35.1–41.2%, respectively (Huang, 2000). Generally, the yields from mycelia were higher than those from some fruit bodies that contained high contents of crude fibre, such as *G. tsugae*, *Lentinula edodes* and *Pleurotus* spp. (Tsai, 2002; Yang et al., 2002). However, the yield from *G. frondosa* mycelia (24.5%) was not markedly higher than that from fruit bodies (20.8%) (Mau et al., 2002b), due to the fact that the fibre contents in the mycelia and fruit bodies

Table 1
Extraction yield of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia

Mycelia	Yield[a] (g)	Extraction % (w/w)
G. frondosa	2.45 ± 0.12	24.5 A[b]
M. esculenta	1.35 ± 0.11	13.5 B
T. albuminosus	2.42 ± 0.06	24.2 A

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

were 11.7% and 10.1%, respectively (Chang, 2002; Mau et al., 2001).

Using the conjugated diene method, the methanolic extracts from the three mycelia showed low (19.1–29.8% at 0.5 mg ml^{-1}) and high antioxidant activities (85.4–94.7% at 25 mg ml^{-1}) (Fig. 1). Obviously, the antioxidant activities were concentration-dependent for the three extracts. Starting from 1.0 mg ml^{-1} and onwards, the extract from *T. albuminosus* showed the highest antioxidant activity among the three extracts. However, the antioxidant activities were 36.9%, 80.5% and 98.1% at 0.5 mg ml^{-1} for ascorbic acid, α-tocopherol and BHA, respectively.

Huang (2000) found that the methanolic extracts from mycelia of *A. camphorata* and *A. blazei* exhibited high antioxidant activities of 87.7% and 93.6%, respectively, at concentrations as low as 0.5 mg ml^{-1}. The methanolic extract from *A. cylindracea* mycelia showed a slight increase in antioxidant activity, from 21.5% at 0.5 mg ml^{-1} to 67.4% at 20 mg ml^{-1} (Tsai, 2002). Surprisingly, the methanolic extract from *G. tsugae* mycelia exhibited a poor antioxidant activity of 7.6–19.3% at 0.5–20 mg ml^{-1} (Tsai, 2002). With regard to the antioxidant activity of methanolic extracts, these three mycelia were better than mycelia of *A. cylindracea* and *G. tsugae* but less effective than mycelia of *A. camphorata* and *A. blazei*.

3.2. Reducing power

The reducing power of the methanolic extract from *M. esculenta* mycelia increased with increased concentration and was 0.11 at 0.5 mg ml^{-1} and 0.97 at 25 mg ml^{-1} (Fig. 2). The methanolic extracts from mycelia of *G. frondosa* and *T. albuminosus* showed no reducing power at 0.5–1.0 mg ml^{-1}, whereas reducing powers of these two extracts were 0.30–0.37 at 5 mg ml^{-1} and 1.01–1.02 at 25 mg ml^{-1}. However, reducing powers were 0.80, 0.89 and 0.92 at 1.0 mg ml^{-1} for ascorbic acid, α-tocopherol and BHA, respectively.

Mau et al. (2002b) found that, at 9 mg ml^{-1}, the methanolic extract from fruit bodies of *G. frondosa* showed a high reducing power of 1.19, much more effective than that from its mycelia (0.62 at 10 mg ml^{-1}) (Fig. 2). Huang et al. (1999a) reported that the methanolic extract from *A. camphorata* mycelia showed a high reducing power of 0.90 at 10 mg ml^{-1}, whereas that from *A. blazei* mycelia GK4 showed a high reducing power of 0.95 at 5 mg ml^{-1} (Huang et al., 1999b). Tsai (2002) found that the reducing power of the methanolic extract from *G. tsugae* mycelia increased rapidly from 0.29 at 0.5 mg ml^{-1} to 1.05 at 5 mg ml^{-1}. However, the reducing power of the methanolic extract from *A. cylindracea* increased steadily from 0.04 at 0.5 mg ml^{-1} to 1.26 at 20 mg ml^{-1} (Tsai, 2002). With regard to reducing power of methanolic extracts, these three mycelia were comparable to mycelia of *A. cylindracea*, but much lower than mycelia of *A. camphorata*, *A. blazei* and *G. tsugae*.

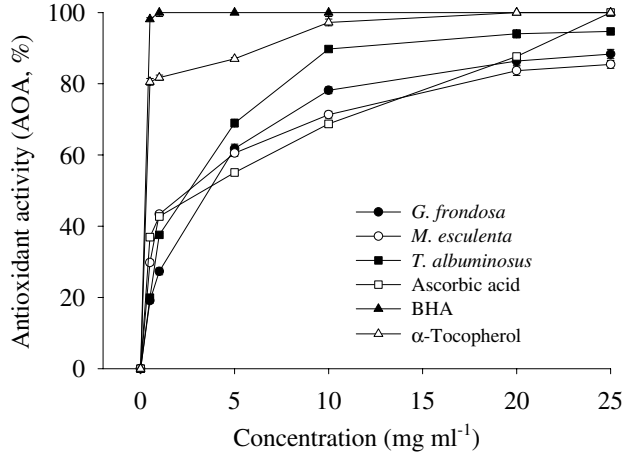

Fig. 1. Antioxidant activity of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia (conjugated diene method). Each value is expressed as mean ± standard deviation ($n = 3$).

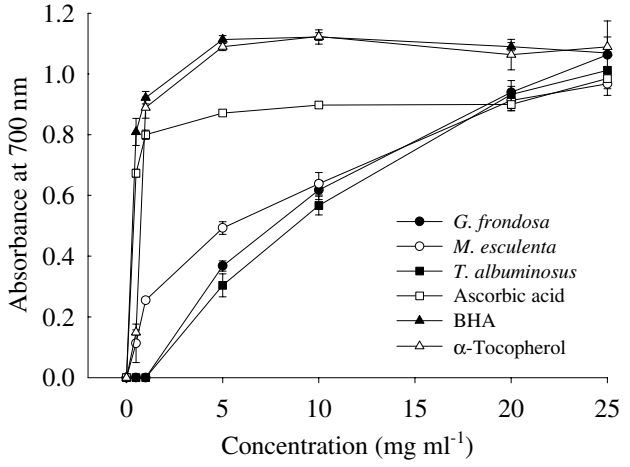

Fig. 2. Reducing power of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Each value is expressed as mean ± standard deviation ($n = 3$).

3.3. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals

Scavenging effects of these three methanolic extracts on DPPH radicals sharply increased from 0.5 to 10 mg ml^{-1} and were 78.8%, 79.4% and 94.1% for *T. albuminosus*, *G. frondosa* and *M. esculenta* at 10 mg ml^{-1}, respectively (Fig. 3). At concentrations below 10 mg ml^{-1}, the extract from *M. esculenta* showed the highest scavenging effect on DPPH radicals among the three extracts, while scavenging effects of the extracts from *G. frondosa* and *T. albuminosus* were low but comparable. However, scavenging effects were 41.8%, 91.6% and 93.9% at 0.5 mg ml^{-1} for ascorbic acid, α-tocopherol and BHA, respectively.

Huang (2000) found that the methanolic extracts from mycelia of *A. camphorata* and *A. blazei* scavenged DPPH radicals by 97.1% and 98.8% at 5 mg ml^{-1}, respectively. At 10 mg ml^{-1}, the methanolic extracts from mycelia of *A. cylindracea* and *G. tsugae* scavenged DPPH radicals by 91.4% and 95.6%, respectively (Tsai, 2002). Apparently, with regard to scavenging effects of methanolic extracts on DPPH radicals, *M. esculenta* mycelia were comparable to mycelia of *A. cylindracea* and *G. tsugae*. However, all the three mycelia were less effective in scavenging effects than mycelia of *A. camphorata* and *A. blazei*.

At 0.5–10 mg ml^{-1}, the scavenging effects of methanolic extracts from the three mycelia on hydroxyl radicals were 0–0.5%, 0–2.1% and 0–5.5% for *G. frondosa*, *M. esculenta* and *T. albuminosus*, respectively. Evidently, these three mycelia showed no scavenging effect on hydroxyl radicals. However, at 1 mg ml^{-1}, the scavenging effect of BHA was only 12.2%. Tsai (2002) found that the methanolic extracts from mycelia of *A. cylindracea* and *G. tsugae* showed slight scavenging effects. Mau et al. (2001) also indicated that ear mushrooms were not good scavengers for hydroxyl radicals. Lin (1999) found that the methanolic extracts from fruit bodies of commercial, speciality and medicinal mushrooms scavenged hydroxyl radicals by 29.2–36.6% at 40 mg ml^{-1}, 39.6–75.0% at 40 mg ml^{-1} and 38.0–52.6% at 16 mg ml^{-1}, respectively. Among speciality mushrooms, the scavenging effect of fruit bodies of *G. frondosa* increased from 19.5 at 5 mg ml^{-1} to 39.6% at 40 mg ml^{-1} (Lin, 1999), much better than that of mycelia in this study.

3.4. Chelating effects on ferrous ions

The chelating effects of the methanolic extracts on ferrous ions were low and insignificant at 0.5–1.0 mg ml^{-1}, but notably high at 5–25 mg ml^{-1} (Fig. 4). However, EDTA showed excellent chelating ability, of 100% at a concentration as low as 0.5 mg ml^{-1}. Citric acid was not a good chelating agent for ferrous ions and its chelating effect was 32.9% at 25 mg ml^{-1}. The most effective pro-oxidants present in food systems are ferrous ions (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988) and the high chelating effect of methanolic extracts would be beneficial if mycelia were formulated into foods.

The methanolic extract from fruit bodies of *G. frondosa* chelated ferrous ions by 55.1% at 3 mg ml^{-1} and by 91.5% at 21 mg ml^{-1} (Mau et al., 2002b), less effective than that of mycelia in this study. The methanolic extract from *A. camphorata* mycelia chelated 89.0% of ferrous ions at 10 mg ml^{-1} (Huang et al., 1999a), whereas that of *A. blazei* mycelia chelated 93.3–97.2% of ferrous ions at 2.5 mg ml^{-1} (Huang et al., 1999b). Tsai (2002) reported that the chelating effects of

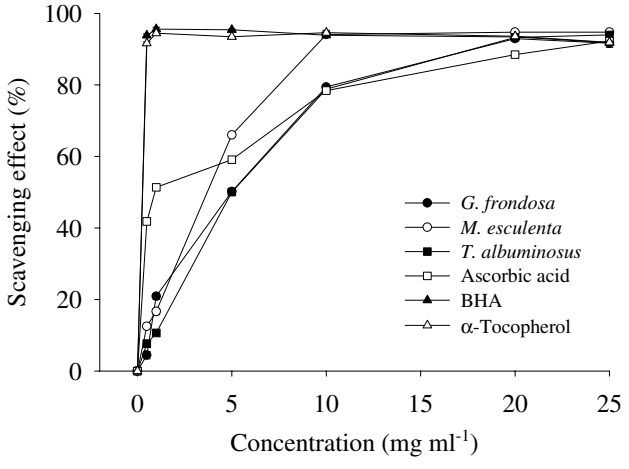

Fig. 3. Scavenging effect of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± standard deviation ($n = 3$).

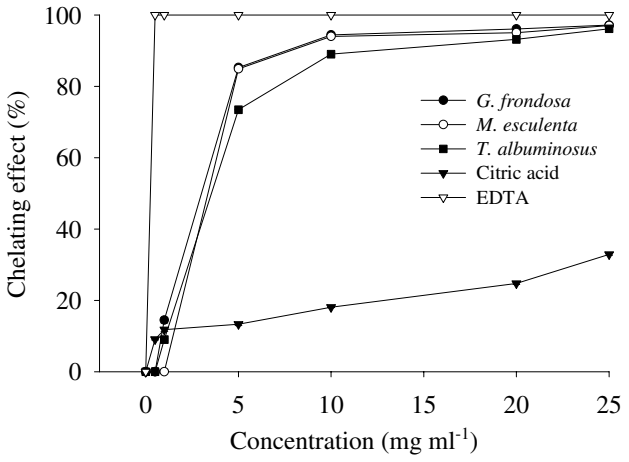

Fig. 4. Chelating effect of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia on ferrous ions. Each value is expressed as mean ± standard deviation ($n = 3$).

methanolic extracts from mycelia of *G. tsugae* and *A. cylindracea* were, respectively, 80.2% and 84.6% at 5 mg ml^{-1}. With regard to chelating effects of methanolic extracts on ferrous ions, these three mycelia were less effective than mycelia of *A. blazei*, comparable to mycelia of *G. tsugae* and *A. cylindracea*, and more effective than mycelia of *A. camphorata*.

3.5. EC_{50} values in antioxidant properties

The antioxidant properties assayed herein are summarized in Table 2 except for the scavenging effect on hydroxyl radicals, and the results are normalized and expressed as EC_{50} values for comparison. Generally, all EC_{50} values were below 10 mg ml^{-1}, indicating that the three mycelia were good in these antioxidant properties. With regard to EC_{50} values in antioxidant activities of methanolic extracts, the three mycelia were different but their values were much below those of mycelia of *G. tsugae* and *A. cylindracea* (>20 and 19.5 mg ml^{-1}, respectively) (Tsai, 2002). However, EC_{50} values of *A. camphorata* and *A. blazei* in antioxidant activities were calculated to be 0.29 and 0.27 mg ml^{-1}, respectively (Huang, 2000).

The reducing power of the methanolic extract from *G. frondosa* mycelia was less effective than that from fruit bodies, as shown by EC_{50} values (3.67 versus 2.02 mg ml^{-1}) (Mau et al., 2002b). With regard to EC_{50} values in reducing power, the efficacies of methanolic extracts from the mycelia were in the descending order: *G. tsugae* (0.93) > *M. esculenta* (1.25) > *A. blazei* (1.96) > *G. frondosa* (3.67) > *A. camphorata* (4.06) > *T. albuminosus* (4.72) > *A. cylindracea* (6.89 mg ml^{-1}) (Huang et al., 1999a, 1999b; Tsai, 2002).

EC_{50} values, in scavenging effect on DPPH radicals, were comparable for methanolic extracts from mycelia and fruit bodies of (4.95 and 4.65 mg ml^{-1}, respectively) (Lin, 1999). EC_{50} values of methanolic extracts from these three mycelia were higher than those from mycelia of *A. camphorata* and *A. blazei* (2.26 and 1.55 mg ml^{-1}, respectively) (Huang, 2000), but similar to those from mycelia of *G. tsugae* and *A. cylindracea* (4.28 and 3.95 mg ml^{-1}, respectively) (Tsai, 2002). EC_{50} values of methanolic extracts, in chelating effect on ferrous ions, were better from fruit bodies than from mycelia of *G. frondosa* (2.00 versus 3.49 mg ml^{-1}) (Mau, 2002b). EC_{50} values of methanolic extracts from these three mycelia were higher than those from mycelia of *A. blazei* (0.37–0.81), *G. tsugae* (1.10), *A. cylindracea* (1.79), and *A. camphorata* (2.06 mg ml^{-1}) (Huang et al., 1999a, 1999b; Tsai, 2002).

Although BHA and α-tocopherol were good in antioxidant activity, reducing power and scavenging effect on DPPH radicals, as well as EDTA was for chelating ferrous ions, they are additives and used or present in mg levels in foods. However, *G. frondosa*, *M. esculenta* and *T. albuminosus*, in the form of mycelia, could be used in g levels as food or a food ingredient. Therefore, these three mycelia might serve as possible protective agents in human diets to help reduce oxidative damage.

3.6. Antioxidant components

Total phenols were the major naturally occurring antioxidant components found in methanolic extracts from these three mycelia (Table 3). However, β-carotene was not detected. Contents of ascorbic acid and tocopherols were similar for these three mycelia. Total contents of antioxidant components were 1.87, 3.93 and 2.09 mg g^{-1} for *G. frondosa*, *M. esculenta* and *T. albuminosus*, respectively.

Generally, total phenols were the major antioxidant components found in the methanolic extracts from other mushroom fruit bodies and mycelia (Huang, 2000; Lin, 1999; Mau et al., 2001; Tsai, 2002). Contents of total phenols were 12.3 mg g^{-1} in the methanolic extract from fruit bodies of *G. frondosa* (Lin, 1999), much higher than in that from its mycelia (1.59 mg g^{-1}). Similarly, Huang (2000) mentioned that contents of total phenols were higher in the methanolic extract from fruit bodies than in those from mycelia of *A. blazei* and *A. camphorata*. However, contents of total phenols in the methanolic extracts from mycelia were 19.8, 18.6, 23.5 and 24.0 mg g^{-1} for *A. blazei*, *A. camphorata*, *A. cylindracea* and *G. tsugae*, respectively (Huang, 2000; Tsai, 2002). Therefore, contents of total phenols in

Table 2
EC_{50} values of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia in antioxidant properties

	EC_{50}[a] (mg ml^{-1})		
	G. frondosa	*M. esculenta*	*T. albuminosus*
Antioxidant activity	3.63 ± 0.10 A[b]	2.78 ± 0.14 B	2.59 ± 0.04 C
Reducing power	3.67 ± 0.24 B	1.25 ± 0.06 C	4.72 ± 0.11 A
Scavenging effect on DPPH radicals	4.95 ± 0.08 A	3.71 ± 0.03 C	5.04 ± 0.08 A
Chelating effect on ferrous ions	3.49 ± 0.01 B	3.55 ± 0.01 A	3.01 ± 0.02 C

[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) 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 are significantly different ($p < 0.05$).

Table 3
Contents of ascorbic acid, β-carotene, tocopherols and total phenols of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia

Compound	Content (mg g^{-1})		
	G. frondosa	*M. esculenta*	*T. albuminosus*
Ascorbic acid	0.14 ± 0.01 A[a]	0.13 ± <0.01 A	0.13 ± <0.01 A
β-carotene	nd[b]	nd	nd
α-tocopherol	0.05 ± 0.01 C	0.07 ± <0.01 B	0.10 ± 0.01 A
γ-tocopherol	0.05 ± 0.01 A	0.06 ± 0.02 A	0.03 ± 0.01 B
δ-tocopherol	0.04 ± 0.01 A	0.04 ± <0.01 A	0.03 ± <0.01 A
Total phenols	1.59 ± 0.01 B	3.63 ± 0.31 A	1.80 ± 0.01 B

[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.

methanolic extracts from the three mycelia were relatively low (1.59–3.63 mg g^{-1}) compared to the contents mentioned above.

Phenols, such as BHT and gallates, are known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Therefore, contents of total phenols in the three methanolic extracts might explain their high antioxidant properties. Yen and Duh (1993) found that the antioxidant activity of the methanolic extract from peanut hulls correlated with its content of total phenols. However, it seems that the contents of antioxidant components did not correlate well with antioxidant properties in the methanolic extracts from these mycelia as mentioned above. On the basis of the results obtained, these three mycelia might be somewhat beneficial to the antioxidant protection system of the human body. For application in the food industry, the fractionation of methanolic extracts and further identification are areas of further investigation.

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