

Antioxidant properties of methanolic extracts from two kinds of *Antrodia camphorata* mycelia

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Abstract

Red and white mycelia of *Antrodia camphorata* (Zang and Su) were obtained due to different fermentation operations. The antioxidant properties and antioxidant components of methanolic extracts from these two mycelia were studied. Using the conjugated diene method, the antioxidant activity of methanolic extracts from white mycelia was better than that from red mycelia (EC_{50} 3.11 vs. 19.8 $mg\ ml^{-1}$). Both mycelia were efficient in the reducing power and scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals but white mycelia showed significantly lower EC_{50} values (1.56 and 1.70 $mg\ ml^{-1}$, respectively). At 7.5–10 $mg\ ml^{-1}$, the chelating effects on ferrous ions reached a plateau of 95.7–98.7% for both mycelia. However, the EC_{50} value was lower for red mycelia. Contents of antioxidant components were found to be in the order of tocopherols > total phenols > ascorbic acid > β -carotene. Methanolic extracts from *A. camphorata* red and white mycelia were good in the antioxidant properties tested, except for the scavenging effect on hydroxyl ions. Although contents of total antioxidant components were relatively high in red mycelia, the antioxidant properties were better with the methanolic extract from white mycelia.

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Keywords: *Antrodia camphorata*; Antioxidant activity; Reducing power; Scavenging effect; Chelating effect; Antioxidant components

1. Introduction

Antrodia camphorata (Zang and Su) (Chang-chih or niu-chang-ku) in the Polyporaceae (Aphylophorales) causes brown heart rot of endemic evergreen *Cinnamomum kanehirai* Hay (Lauraceae) in Taiwan (Wu, Ryvarden, & Chang, 1997). “Niu-chang” is the Chinese common name for *C. kanehirai*, which is one of the endangered species in Taiwan; “ku” in Chinese means mushroom and “chih” means *Ganoderma*-like fungus. Chang-chih, specifically referred to as the fruit body of *A. camphorata*, is well known in Taiwan as an expensive medicinal material, and is commonly used as an antidote, anticancer, antiitching and hepatoprotective drug. The red to light cinnamon, resupinate, effused-reflexed to pileate fruit bodies of *A. camphorata* are very bitter in

taste and have a mild camphor oil odour like the host woods (Chang & Chou, 1995). The mycelia isolated from the fruit body forms orange red, orange brown to light cinnamon colonies (Chang & Chou, 1995).

Chien, Chiang, and Chen (1997) first examined solid and liquid mycelia cultures of *A. camphorata* and found new triterpenoids therefrom. In 1998, a submerged mycelial culture was established on a 50-ton scale in Grape King Inc. under standard fermentation procedure for mushrooms. Contrary to that observed on plates, the harvested mycelia were white in colour. However, antioxidant properties of *A. camphorata* were evaluated using fruit bodies and white mycelia (Huang, 2000; Huang, Huang, Chen, & Mau, 1999). Also, the non-volatile taste components of *A. camphorata* white mycelia were determined (Chang, Chao, Chen, & Mau, 2001). Due to the unacceptable white colour, a specific fermentation procedure was then established for *A. camphorata*, in which citric acid was added to maintain the pH of culture at pH 4.5; the culture turned orange red in the fermentor at

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days 8–10 and mycelia thus harvested at days 13–16 were called red mycelia (Chen et al., 2001).

Fruit bodies of *A. camphorata* are expensive and less available, partially due to their rareness and difficulty in cultivation. Thus, *A. camphorata* is mainly prepared from submerged culture in the form of mycelium for use in the formulation of nutraceuticals and functional foods. Currently, two kinds of mycelia are commercially available in Taiwan. It is of great interest to examine the discrepancy in chemical or biological properties of two mycelia. Accordingly, our objective was to study and compare the antioxidant properties of methanolic extracts from red and white mycelia of *A. camphorata*. Antioxidant properties were assayed in terms of antioxidant activity (AOA) by the conjugated diene method, reducing power, scavenging effects on radicals and chelating effect on ferrous ions. The contents of potential antioxidant components in these two mycelia were also determined.

2. Materials and methods

2.1. Mushroom mycelia

Freeze-dried red and white mycelia of *A. camphorata* were obtained from the Biotechnology Center, Grape King Inc., Chungli, Taiwan. For each kind of mycelia, three samples (~50 g each) were randomly selected and prepared for analyses. 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 20g 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 AOA was determined by the conjugated diene method (Lingnert, Vallentin, & Eriksson, 1979). Each mycelial extract (1–50 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 were added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The AOA was calculated as follows: AOA (%) = $[(\Delta A_{234} \text{ of control} - \Delta A_{234} \text{ of sample}) / \Delta A_{234} \text{ of control}] \times 100\%$. An AOA value of 100% indicates the

strongest AOA. EC₅₀ (mg ml⁻¹) is the effective concentration at which the AOA was 50% and was obtained by interpolation from linear regression analysis. Butylated hydroxyanisole (BHA) and α -tocopherol (vitamin E) were used as controls.

2.3. Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each mycelial extract (0.5–10 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 200g 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. EC₅₀ (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. BHA and α -tocopherol were used as controls.

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

Each mycelial extract (0.5–10 mg ml⁻¹) in methanol (4 ml) was mixed with 1 ml of a methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (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). EC₅₀ (mg ml⁻¹) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. BHA and α -tocopherol were used as controls.

2.5. Scavenging effect on hydroxyl radicals

The hydroxyl radical reacted with the nitron 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 mycelial extract (0.5–5 mg ml⁻¹) in methanol with 200 µl of 10 mM H₂O₂ (Merck, Darmstadt, Germany), 200 µl of 10 mM Fe²⁺ (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), were added 2 ml of each mycelial extract (0.5–10 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. EC₅₀ (mg ml⁻¹) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. Ethylenediaminetetraacetic acid (EDTA) was used as a control.

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 s at 515 nm against 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; 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⁻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 dried methan-

olic extract (50 mg) was suspended in 6 ml of pyrogallol (6% in 95% ethanol) and 4 ml of 60% aqueous potassium hydroxide 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–Ciocalteu reagent (Sigma) were added to the mixture. After 30 min of 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 methanolic extracts from each mycelium, 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 methanol as the extractant, the yield from red mycelia was higher (Table 1). Also, the yield from white

Table 1
Extraction yield of methanolic extracts from *Antrodia camphorata* mycelia

Mycelia	Yield ^a (g)	Extraction % ^b (w/w)
Red	3.78 ± 0.79	37.8A
White	2.75 ± 0.56	27.5B

^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).

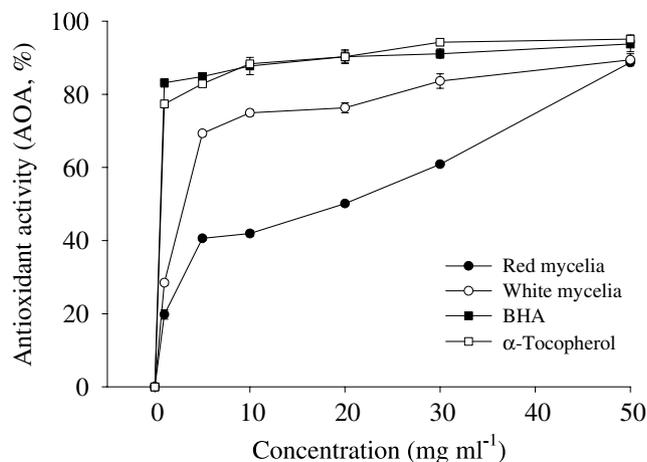


Fig. 1. Antioxidant activity of methanolic extracts from *Antrodia camphorata* mycelia. Each value is expressed as mean \pm standard deviation ($n = 3$).

mycelia was lower than that from white mycelia (31.10%) in Huang's (2000) report. Using the conjugated diene method, methanolic extracts from two mycelia showed two different increasing patterns of antioxidant activities (Fig. 1). Generally, the AOA of methanolic extracts from white mycelia was better than that from red mycelia but lower than those of BHA and α -tocopherol. At 5 mg ml⁻¹, the antioxidant activities were 40.6% and 69.3% for red and white mycelia, respectively. However, at 50 mg ml⁻¹, the antioxidant activities were similar in the two mycelia. Huang (2000) found that methanolic extracts from white mycelia, fresh and air-dried fruit bodies, showed good antioxidant activities of 87.7%, 93.0% and 91.2% at concentrations as low as 1 mg ml⁻¹, respectively. However, the effective concentrations in this study seemed to be much higher than those found by Huang (2000).

3.2. Reducing power

Reducing powers of methanolic extracts from the two mycelia increased rapidly at low concentrations from 0.5 to 2.5 mg ml⁻¹, after which reducing powers became parallel plateau with different levels to 7.5 mg ml⁻¹ (Fig. 2). At 2.5–7.5 mg ml⁻¹, the reducing powers from white mycelia were higher than those from red mycelia. Nevertheless, at 10 mg ml⁻¹, the reducing power from red mycelia increased to 1.24 and was higher than that from white mycelia. Overall, reducing powers of all methanolic extracts were in the range of moderate to high (>0.57) at concentrations higher than 2.5 mg ml⁻¹. However, the reducing powers of BHA and α -tocopherol maintained the levels of 0.95–1.02 and 0.70–0.78 at 0.50–10 mg ml⁻¹, respectively.

Huang et al. (1999) found that methanolic extracts from fresh and air-dried fruit bodies showed excellent reducing powers of 0.94 and 0.92 at 5 mg ml⁻¹, respec-

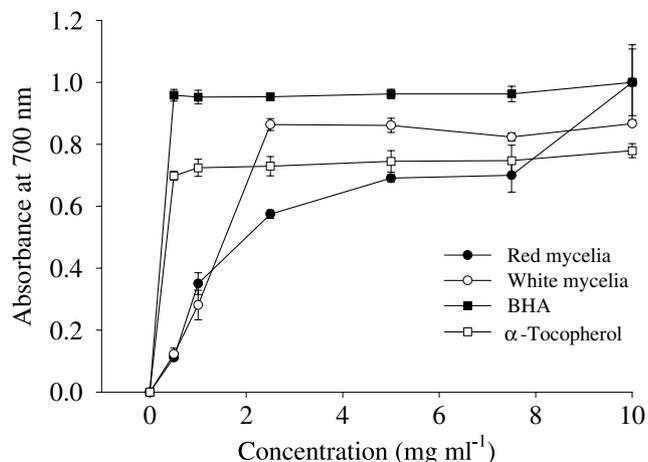


Fig. 2. Reducing power of methanolic extracts from *Antrodia camphorata* mycelia. Each value is expressed as mean \pm standard deviation ($n = 3$).

tively, whereas the reducing power of methanolic extracts from white mycelia was 0.62 at 5 mg ml⁻¹. At 2.5 mg ml⁻¹, those from white mycelia, fresh and air-dried fruit bodies showed low to moderate reducing powers of 0.30, 0.64 and 0.54, respectively (Huang et al., 1999).

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

The scavenging effect of methanolic extracts from both mycelia on DPPH radicals rapidly increased from 0.5 to 5.0 mg ml⁻¹ (Fig. 3). At 5.0 mg ml⁻¹, the scavenging effects were 97.0% and 98.4% for red and white mycelia, respectively. However, the scavenging effects were 95.7% and 93.4% for BHA and α -tocopherol at 0.5 mg ml⁻¹. Huang et al. (1999) found that methanolic extracts from fresh and air-dried fruit bodies scavenged

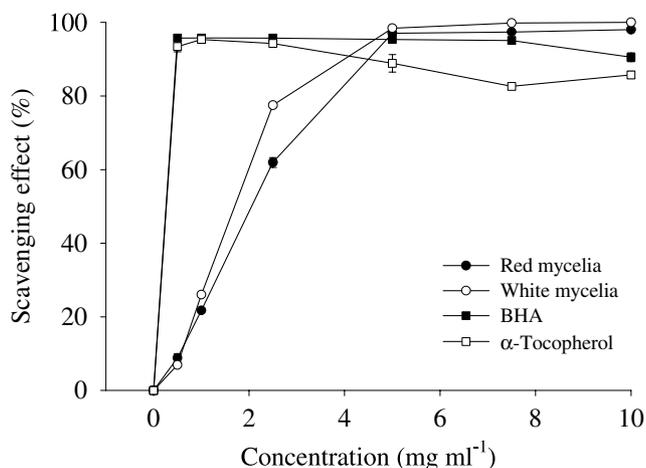


Fig. 3. Scavenging effect of methanolic extracts from *Antrodia camphorata* mycelia on DPPH radicals. Each value is expressed as mean \pm standard deviation ($n = 3$).

DPPH radicals by 99.1% and 96.3% at 2.5 mg ml⁻¹, respectively. In addition, the scavenging effect was 97.1% at 5.0 mg ml⁻¹ for white mycelia (Huang et al., 1999). Overall, two mycelia showed similar effect on DPPH radicals.

3.4. Scavenging effect on hydroxyl radicals

None to little scavenging effect on hydroxyl radicals was observed with methanolic extracts from white mycelia whereas those from red mycelia showed slight scavenging effect (Table 2). However, the scavenging effect of BHA at 1 mg ml⁻¹ was 12.2%. Obviously, the components possessing the scavenging ability on hydroxyl radicals might be present in red mycelia. The difference between incubation conditions for the two mycelia was the pH whereas the observed difference between mycelia was the colour. Therefore, red components might be responsible for the slight scavenging effect on hydroxyl radicals. However, Huang (2000) found that none to little scavenging effect on hydroxyl radicals was observed with methanolic extracts from fresh and air-dried fruit bodies, whereas the scavenging effect of that from white mycelia was low (1.46–23.07%) and not concentration-dependent.

3.5. Chelating effects on ferrous ions

The chelating effects of methanolic extracts from the two mycelia on ferrous ions increased with increased concentration (Fig. 4). At 7.5–10 mg ml⁻¹, the chelating effects reached a plateau of 95.7–98.7% for both mycelia. However, the chelating effect of EDTA at 1 mg ml⁻¹ was 100%. Since ferrous ions are the most effective pro-oxidants in the food system (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), the high chelating effects of methanolic extracts from mycelia would be beneficial. The chelating effects of methanolic extracts from white mycelia, fresh and air-dried fruit bodies were also concentration-dependent (Huang et al., 1999). At 10 mg ml⁻¹, the methanolic extracts from white mycelia, fresh and air-dried fruit bodies chelated ferrous ions by 89.0%, 81.5% and 88.9%, respectively (Huang et al., 1999).

Table 2
Scavenging effect of methanolic extracts from *Antrodia camphorata* mycelia on hydroxyl radicals

Amount (mg ml ⁻¹)	Scavenging effect (%) ^a	
	Red mycelia	White mycelia
0.5	5.88 ± 0.38A	0.00 ± 0.00B
1.0	18.4 ± 0.24A	5.11 ± 0.29B
5.0	7.19 ± 0.15A	0.00 ± 0.00B

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

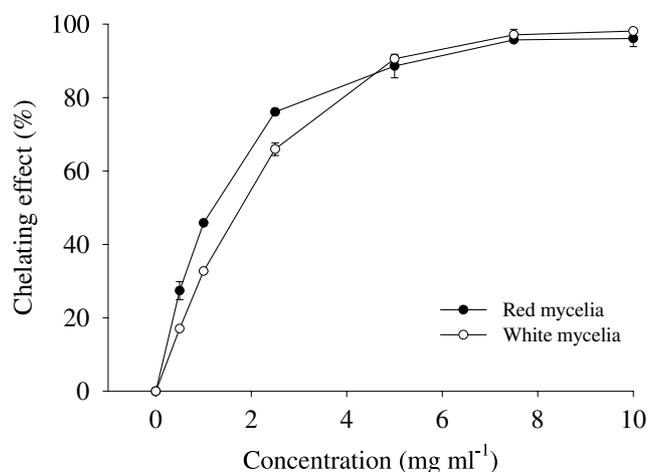


Fig. 4. Chelating effect of methanolic extracts from *Antrodia camphorata* mycelia on ferrous ions. Each value is expressed as mean ± standard deviation ($n = 3$).

3.6. EC₅₀ in antioxidant properties

The antioxidant properties assayed herein are summarized in Table 3 except for the scavenging effect on hydroxyl radicals, and the results are normalized and expressed as EC₅₀ values (mg methanolic extract per ml) for comparison. With regard to EC₅₀ values in the AOA of methanolic extracts by the conjugated diene method, white mycelia were much better than red mycelia (3.11 vs. 19.8 mg ml⁻¹). However, Huang (2000) indicated that EC₅₀ values in the AOA were 0.29 and 0.27 mg ml⁻¹ for white mycelia and fruit bodies, respectively.

Both mycelia were good in the reducing power and scavenging effect on DPPH radicals but white mycelia showed significantly lower EC₅₀ values (1.56 and 1.70 mg ml⁻¹, respectively). Huang et al. (1999) reported that EC₅₀ values for the reducing power were 4.06 and 1.49–

Table 3
EC₅₀ of methanolic extracts from *Antrodia camphorata* mycelia for antioxidant properties

	EC ₅₀ ^a (mg ml ⁻¹)	
	Red mycelia	White mycelia
Antioxidant activity	19.8 ± 0.60A ^b	3.11 ± 0.05B
Reducing power	2.00 ± 0.04A	1.56 ± 0.08B
Scavenging effect on DPPH radicals	2.06 ± 0.03A	1.70 ± 0.01B
Chelating effect on ferrous ions	1.21 ± <0.01B	1.78 ± 0.01A

^a EC₅₀, the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; DPPH radicals were scavenged by 50%, and ferrous ions were chelated by 50%, respectively. EC₅₀ 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 ($p < 0.05$).

2.33 mg ml⁻¹ for white mycelia and fruit bodies, respectively. Furthermore, Huang et al. (1999) found that EC₅₀ values in the scavenging effect were 2.26 and 0.49–0.82 mg ml⁻¹ for white mycelia and fruit bodies, respectively. In addition, both mycelia were good in the chelating effect on ferrous ions. However, the EC₅₀ value was lower for red mycelia. Huang et al. (1999) found that EC₅₀ values in the chelating effect were 2.19 and 1.77–3.54 mg ml⁻¹ for white mycelia and fruit bodies, respectively.

Based on EC₅₀ values obtained, both red and white mycelia were comparable to the fruit bodies reported by Huang et al. (1999) in the reducing power and scavenging effect on DPPH radicals, and less effective in AOA and chelating effect on ferrous ions. Based on the antioxidant properties assayed, both mycelia were good and might be a substitute for the natural fruit bodies as an ingredient for nutraceuticals and functional foods. However, to make sure whether the mycelia are a good substitute for the fruit bodies or not, other chemical and biological properties of mycelia and fruit bodies are needed to study and compare further.

When the extraction yields were taken into consideration, EC₅₀ values (mg dried mycelia per ml) in the AOA, reducing power, scavenging and chelating effects were 52.4, 5.29, 5.45 and 3.20 mg ml⁻¹ for red mycelia and 11.3, 5.67, 6.18 and 6.47 mg ml⁻¹ for white mycelia. Surprisingly, EC₅₀ values for reducing power and scavenging effect were lower for red mycelia. Therefore, red mycelia were better in the reducing power, and scavenging and chelating effects, based on the weight of dried mycelia.

3.7. Antioxidant components

Tocopherols were the major naturally occurring antioxidant components found in the methanolic extracts from *A. camphorata* red and white mycelia (Table 4). Generally, contents of antioxidant components were found to be in the order: tocopherols > total phe-

nols > ascorbic acid > β-carotene. White mycelia were high in ascorbic acid and β-carotene contents. The total phenol contents were similar for both mycelia. Phenols such as BHT and gallate are known to be effective antioxidants (Madhavi, Singhal, & Kulkarni, 1996). Total tocopherol contents were 32.6 and 18.8 mg ml⁻¹ for red and white mycelia, respectively. Total antioxidant component contents were 37.8 and 25.2 for red and white mycelia, respectively.

Huang (2000) found the ascorbic acid content in the methanolic extract from white mycelia to be 2.39 mg ml⁻¹, similar to those found in Table 4, whereas the contents in methanolic extracts from fruit bodies (0.02–0.16 mg ml⁻¹) were much lower. Contrary to Table 4, contents of β-carotene, tocopherols and total phenols in the methanolic extract from white mycelia were 2.15, 7.61 and 18.6 mg ml⁻¹, respectively (Huang, 2000). However, the contents of β-carotene (6.87 and 2.89 mg ml⁻¹), tocopherols (25.3 and 24.3 mg ml⁻¹) and total phenols (21.2 and 23.3 mg ml⁻¹) were high in methanolic extracts from fresh and air-dried fruit bodies, respectively (Huang, 2000). The considerable discrepancies in the profile of antioxidant compounds might be the main reason for the differences in antioxidant properties assayed between white mycelia in this study and that in Huang (2000).

As shown in Table 3, methanolic extracts from *A. camphorata* red and white mycelia were good in the antioxidant properties tested, except for the scavenging effect on hydroxyl ions. With regard to AOA, reducing power and scavenging effect on DPPH radicals, the white mycelia were good, based on the weight of methanolic extracts. In addition, red mycelia were better in the scavenging effect on hydroxyl radicals and chelating effects on ferrous ions. On the other hand, contents of total antioxidant components were relatively high in red mycelia. However, based on the weight of dried mycelia, red mycelia were better in the antioxidant properties assayed, except for AOA. Before conclusion on the unacceptability of the white mycelia, more information is needed. The difference between antioxidant properties of red and white mycelia is a result of the difference in pH conditions during growth. To understand the discrepancy between the two mycelia, other chemical and biological properties of mycelia need study. Although the mechanism by which the antioxidant properties are affected is not yet known, the association of incubation conditions with the antioxidant properties is another area of investigation.

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Table 4

Contents of ascorbic acid, β-carotene, tocopherols and total phenols of methanolic extracts from *Antrodia camphorata* mycelia

Compound	Content (mg g ⁻¹) ^a	
	Red mycelia	White mycelia
Ascorbic acid	2.06 ± 0.06B	2.35 ± 0.05A
β-Carotene	0.32 ± 0.05B	1.03 ± 0.15A
α-Tocopherol	1.02 ± <0.01B	1.25 ± 1.18A
γ-Tocopherol	30.4 ± 0.86A	16.6 ± 1.37B
δ-Tocopherol	1.17 ± <0.01A	0.99 ± <0.01B
Total phenols	2.87 ± 0.02A	2.90 ± 0.05A

^a Each value is expressed as mean ± standard deviation (*n* = 3). Means with different letters within a row are significantly different (*p* < 0.05).

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