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Antioxidant properties of methanol extract of a new commercial gelatinous mushrooms (white variety of *Auricularia fuscosuccinea*) of Taiwan

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White variety of *Auricularia fuscosuccinea* is a newly cultivated gelatinous mushrooms which is found only in Taiwan. In this study, total phenolic and total flavonoid content of methanol extract of white variety of *A. fuscosuccinea* was estimated, and *in vitro* antioxidant properties and antioxidant enzyme activities were also evaluated. When compared with two other common gelatinous mushrooms, *A. polytricha* and *Tremella fuciformis*, white variety of *A. fuscosuccinea* had the highest total phenolic [7.88 mg gallic acid equivalents (GAE)/g] and total flavonoid [1.60 mg quercetin equivalents (QE)/g]. Among all methanol extracts analyzed, white variety of *A. fuscosuccinea* had the lowest EC_{50} value on reducing g power (0.305 mg/ml) and scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (0.150 mg/ml) had the highest total sugars [44.73 mg dextrose equivalents (DEX)/g] and the lowest EC_{50} value on chelating effect on ferrous ions (0.427 mg/ml). The methanol extracts from white variety of *A. fuscosuccinea* possessed the highest superoxide dismutase activity (2.10 U/mg) and total antioxidant capacity (2.26 mM/g). The glutathione reductase activity (7.97 U/g) of *A. polytricha* was the highest. The analyses of the antioxidant contents phenolic compounds are mainly responsible for the antioxidant effect of gelatinous mushrooms.

Key words: *Auricularia fuscosuccinea*, antioxidant activity, reducing power, scavenging effect, chelating effect.

INTRODUCTION

Butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) are the two most commonly used synthetic antioxidants. The processing costs of synthetic antioxidants used in the food industry are high, while selected synthetic antioxidants may be harmful to human life. Their toxicity and ability to induce DNA damage led them to be restricted when applied in food industry (Sasaki et al., 2002). Nevertheless, mushroom species could provide the antioxidant capacity in *in vitro* systems (Ribeiro et al., 2006, 2007). Therefore, natural antioxidants from mushroom extracts have attracted increasing interest due to their safety (Mau et al., 2004). Mushrooms have been used as a sort of food ingredient for centuries. The unique and subtle flavor of mushrooms is responsible for their use in seasoning and flavoring.

Research conducted during the last decades has indicated that mushrooms exert a number of nutritional and nutraceutical properties and they are source of beneficial bioactive compounds (Ferreira et al., 2009; Yaltirak et al., 2009; Jayakumar et al., 2009). Mushrooms contain significant amounts of bioactive substances such as vitamins and vitamin precursors, minerals and trace

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Figure 1. Appearance of white variety of *A. fuscosuccinea*.

elements (Kalač, 2009), specific β-glucans, and exert antioxidant properties which are mainly attributed to their phenolic content (Ferreira et al., 2009; Yaltirak et al., 2009). A large body of evidence supports the implication of oxidative stress in the pathogenesis of several chronic and degenerative diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, cancer and aging (Halliwell, 1996; Valko et al., 2007). Therefore, the enhancement of the antioxidant systems for the prevention of cellular oxidative damage via the consumption of antioxidant rich foods is of great interest.

Gelatinous mushrooms are believed to be of high nutritional value since it has the high content of carbohydrates, amino acids, trace elements and vitamins, thus to be processed into a variety of foods (Fan et al., 2006). In previous studies, polysaccharides of gelatinous mushroom were found to have the potent antioxidant activity in both *in vitro* (Fan et al., 2006; Kho et al., 2009) and *in vivo* assay (Chen et al., 2008a, 2008b, 2008c; Wu et al., 2010).

A current research also reported that polysaccharides had the potential application as a new antioxidant agent in food industry (Fan et al., 2006). Other antioxidant compounds in mushrooms such as phenolics (Bendini et al., 2006; Quezada et al., 2004), flavonoids and organic acids (Ribeiro et al., 2007) are used in dietary supplement and by the pharmaceutical industry. White variety of *A. fuscosuccinea* is a white mutant of *A. fuscosuccinea*, originally selected and generated in Taiwan Agricultural Research Institute.

After populari-zation, it could be successfully cultivated in organic farm. The fresh fruit body of white variety of *A. fuscosuccinea* has a shape of dancing skirt and a glossy velvety coat outer surface (Figure 1). After boiled into a sweet soup, the smooth texture tasted like edible nest of cliff swallows and was called “bird’s nest soup for vegetarian”. However, in Taiwan, the rareness and the expensiveness keeps consumers from purchase. On the other hand, the similar appearance of dry fruit body between white variety of *A. fuscosuccinea* and *T. fuciformis* makes it hard for identification by consumers. We investigated the white variety of *A. fuscosuccinea* because its hot water extract has lot of benefits for the proliferation of probiotics. Therefore, it is of great interest to explore other beneficial effects of this mushroom, and the analysis results might be the basis for its application as in functional food.

Mau et al (2001) had examined the antioxidant properties of methanol extracts from several oven-dried ear mushrooms (Mau et al., 2001). However, the investigation about the influence of freeze-drying to the antioxidant contents and corresponding antioxidant activity of gelatinous mushrooms is rather limited. To better interpret the data obtained from white variety of *A. fuscosuccinea*, the antioxidant values were compared with that of two commonly used gelatinous mushrooms, *T. fuciformis* and *A. polytricha*. Because of their beneficial properties like antiinflammatory and antitumor properties, both of these two mushrooms have been widely used in many countries for years in therapeutics for blood pressure regulation, hypercholesterolemia, hyperlipide-mia, cardiovascular disorders and chronic bronchitis (Yang et al., 2002; Zhang et al., 2007).

Since food composition in bioactive or potentially bioactive com-pounds is recognized as critical for throwing light upon the association between diet and health, this study will shed lights on the correlations between the antioxidant contents and antioxidant activity. We further compared these results with commercial antioxidants (BHA, BHT and EDTA) to assess the dietary supplements potential of white variety of *A. fuscosuccinea*. Therefore, these results will help us know the utility of white variety of *A. fuscosuccinea* in functional food industry.

In the present work, we also demonstrate if white variety of *A. fuscosuccinea* contains enzymatic antioxi-dant defense systems (SOD, GPx and GRd) to remove the reactive oxygen stress (ROS).

MATERIALS AND METHODS

Mushroom fruit bodies

Fresh fruit bodies of white variety of *A. fuscosuccinea* and *A. polytricha* were harvested from organic mushroom farm (Wufeng, Taiwan and Caotun, Taiwan). Fresh *T. fuciformis* fruit bodies (approximately 1 kg each) were purchased from supermarkets. Fresh mushrooms were freeze-dried at -50± 2°C.

Methanol extract of mushrooms

A coarse powder was obtained using a mill (20 mesh, Retsch ultracentrifugal mill and sieving machine, Haan, Germany). These powders of dried fruit bodies were placed in Tupperware® boxes and stored in refrigerator at 4°C for further use. For the methanol extraction of mushrooms, a subsample (10 g) was extracted using 100 ml of methanol in a glass conical flask using a shaker at 25°C

for 24 h and then the mixture was filtered through filter paper. The residue was then extracted with an additional 100 ml of methanol as described for the first extraction. the two methanol extracts were combined; 10 ml of the mixture were evaporated at 50°C by oven drying. Total dry matter of methanol extracts was determined gravimetrically as residue remaining after drying. Each methanol extract was adjusted by methanol to 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg/ml. All diluted solutions were maintained in the dark until tested.

Determinations of total phenolic, flavonoid and sugars contents

Total phenolic content was determined using the Folin-Ciocalteu reagent method described by Lai et al. (2001). Each methanol extract (100 mg) was dissolved in 5 ml of 0.3% HCl in methanol/water (60:40, v/v), and the resulting mixture (100 µl) was added to 2 ml of aqueous sodium carbonate solution. After 2 min, 100 µl of 50% Folin-Ciocalteau reagent (Sigma) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm against a blank. The content of total phenolic was calculated on the basis of the calibration curve of gallic acid (Sigma).

Total flavonoid content was determined using a method described by Jia et al. (1999). 0.5 ml of each methanol extract was mixed with 1.5 ml of deionized water, 0.1 ml of 1 mg/ml $Al(NO_3)_3 \cdot 9H_2O$ (Wako), and 0.1 ml of 1 M CH_3COOK (Wako). After 40 min at room temperature in the dark, the absorbance of the mixture was determined at 415 nm against a blank. A higher absorbance indicates higher flavonoid content. Content of total flavonoid was calculated on the basis of the calibration curve of quercetin (Sigma). Total sugar content was determined by the modified phenol-sulfuric acid method described by Dubois et al. (1956).

0.2 ml of each methanol extract was mixed with 0.2 ml 5% w/v phenol solution. Added 1 ml concentrated sulfuric acid rapidly and directly on the sample and left for 10 min. The contents were stirred and incubated at 25°C for 30 min and the absorbance of the mixture was determined at 490 nm against a blank. Content of total sugars was calculated on the basis of the calibration curve of D-glucose (Merck).

Determination of reducing power

The reducing power was determined by a method described by Oyaizu (1986), which measured the ability of sample to reduce ferricyanide to ferrocyanide. Each methanol extract (0.4 ml) was mixed with 0.4 ml of a 0.2 M phosphate buffer (pH 6.6; Merck) and 1% $K_3Fe(CN)_6$ (Sigma). The mixture was incubated at 50°C for 20 min, followed by addition of 0.4 ml of 10% trichloroacetic acid (Merck) and then centrifuged for 5 min at 10,000 rpm. The upper layer of solution (1.5 ml) was mixed with 1.5 ml of deionized water and 0.3 ml of 1 mg/ml $FeCl_3$ (Sigma). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. Methanol was used as a control. BHA was used for comparison, and the concentration used was the same as the sample.

Determination of DPPH radicals scavenging activity

The DPPH scavenging activity was determined using the method described by Shimada et al. (1992). Each methanol extract (4 ml) was mixed with 1 ml of a 10 mM DPPH (Sigma) methanol solution. After 30 min incubation at room temperature in the dark, the absorbance of the mixture was determined at 517 nm against a blank. A lower absorbance indicates a higher scavenging activity. Methanol was used as a control. BHA and BHT were used for comparison, and the concentrations used were the same as with the sample. The following equation was used to determine the scavenging effect:

$$\text{Scavenging effect (\%)} = \frac{\triangle A_{517} \text{ of control} - \triangle A_{517} \text{ of sample}}{\triangle A_{517} \text{ of control}} \times 100$$

Ability of chelating ferrous ions

The chelating effects were determined using a method by Dinis et al. (1994). Each methanol extract (0.1 ml) was mixed with 3.7 ml of methanol and 0.1 ml of 2 mM $FeCl_2 \cdot 4H_2O$ (Merck). The reaction was initiated by adding in 0.2 ml of 5 mM ferrozine (Sigma). After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance indicated a higher chelating power. Methanol was used as a control. EDTA was used for comparison, and the concentration used was the same with sample. The following equation was used to determine the chelating effect:

$$\text{Chelating effect (\%)} = \frac{\triangle A_{562} \text{ of control} - \triangle A_{562} \text{ of sample}}{\triangle A_{562} \text{ of control}} \times 100$$

Determination of total antioxidant capacity

The 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was determined at 600 nm by using a Randox total antioxidant status kit (Randox Laboratories Ltd., Crumilin Co. Antrim, UK) according to the method of Miller et al. (1993). In the TAS assay, ABTS is incubated with metmyoglobin and hydrogen peroxide to produce the ABTS· radical cation with a relative stable blue-green color. Antioxidants present in the sample cause a reduction in absorption proportional to their concentration. The total antioxidant capacity (TAC) value was expressed as an equivalent of the concentration (mM) of Trolox solution.

Determination of antioxidant enzyme activity

SOD activity was determined at 505 nm using a Ransod kit (Randox Laboratories Ltd., UK) according to the method of Delmas-Beauvieux et al. (1995). GPx activity was determined at 340 nm using a Ransel kit (Randox Laboratories Ltd., UK) according to the method of Paglia and Valentine (1967). SOD and GPx activities were expressed as U/mg extract. GRd activity was determined at 340 nm using a Rangrd kit ((Randox Laboratories Ltd., UK) according to the method of Goldberg and Spooner (1983) and was expressed as U/g extract.

Statistical analysis

All data were showed as mean ± standard deviation (SD) of three replicated determinations. Data were analyzed using the statistical analysis system (Ver. 9.1 for Windows, 2010) software package. Analysis of variance was performed using one-way ANOVA procedures, analysis of variance. Significant differences ($\rho<0.05$) between means were determined using the Duncan's new multiple

Table 1. Antioxidant contents from methanol extracts of three gelatinous mushrooms.

Content	Content concentrations (mg/g of dry weight)		
	White variety of *A. fuscosuccina*	*A. polytricha*	*T. fuciformis*
Total flavonoid	2.18 ± 0.12^{x}	0.49 ± 0.14^{z}	0.78 ± 0.15^{y}
Total phenolic	10.85 ± 1.00^{x}	2.98 ± 0.44^{y}	1.12 ± 0.28^{z}
Total sugars	22.40 ± 3.13^{x}	18.64 ± 1.36^{y}	24.42 ± 1.33^{x}

Each value is expressed as means ± standard deviation of three replicates. $^{x-z}$Different superscripts with the same row indicate significantly different ($\rho < 0.05$).

range test.

RESULTS

Extraction yield

Methanol was used to extract the dry mushroom material, white variety of *A. fuscosuccinea* showed the highest yield (13.77%), whereas *T. fuciformis* (7.28%) and *A. polytricha* (5.46%) showed less yield.

Determination of total phenolic, flavonoid and sugars contents

Naturally occurring antioxidant components such as phenolics, flavonoids and sugars were found in the methanol extracts from the gelatinous mushrooms (Table 1). Total phenolic contents (per gram of dry weight) in the methanol extracts from the three gelatinous mushrooms ranged from 1.12 to 10.85 mg GAE/g. The descending order of total phenolic was: white variety of *A. fuscosuccinea* (10.82 mg GAE/g)> *A. polytricha* (2.98 mg GAE/g) > *T. fuciformis* (1.12 mg GAE/g). The flavonoid values were in the following descending order: white variety of *A. fuscosuccinea* (2.18 mg QE/g)> *T. fuciformis* (0.78 mg QE/g) > *A. polytricha* (0.49 mg QE/g). The contents of total sugars from the methanol extracts were in the following order: *T. fuciformis* (24.42 mg DEX/g)> white variety of *A. fuscosuccinea* (22.40 mg DEX/g)> *A. polytricha* (18.64 mg DEX/g).

Determination of reducing power

For measurement of the reductive ability, the Fe^{3+} to Fe^{2+} transformation in the three gelatinous mushrooms was investigated. The reducing power of the methanol extracts was concentration dependent. As the concentration increased from 0.1 to 3.5 mg/ml, there was an increase in absorbance.

Amongst the methanol extracts from the three gelatinous mushrooms, the reducing power of white variety of *A. fuscosuccinea* dramatically increased until it reached a plateau status as follows: 1.87 at 1.0 mg/ml, 2.16 at 1.5 mg/ml, 2.19 at 2.0 mg/ml, and 2.21 at 3.5 mg/ml (Figure 2). On the contrary, reducing power of methanol extracts from *A. polytricha* and *T. fuciformis* increased slowly along with increasing concentrations as follows: 0.77 and 0.16 at 1.0 mg/ml, 1.21 and 0.39 at 2.0 mg/ml; 1.99 and 0.42 at 3.5 mg/ml, respectively. These results imply that the reducing power is increasing with increasing concentration of the methanol extracts from gelatinous mushrooms.

Determination of DPPH radical scavenging activity

The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-colored methanol solution of DPPH. The methanol extract from white variety of *A. fuscosuccinea* had a robust scavenging effect on DPPH radicals resulting in a final plateau as follows: 90.65% at 0.4 mg/ml, 91.14% at 0.5 mg/ml and 92.52% at 3.5 mg/ml (Figure 3).

The scavenging effects on DPPH radicals of *A. polytricha* slowly increased along with concentrations until it reached a final plateau and were as follows: 34.84% at 0.4 mg/ml, 72.66% at 1.0 mg/ml, 90.30% at 1.5 mg/ml and 92.74% at 3.5 mg/ml.

The scavenging activities of white variety of *A. fuscosuccinea* and *A. polytricha* increased along with concentrations. However, an exception was observed with *T. fuciformis*, which did not level off with concentration and had lower scavenging activity than white variety of *A. fuscosuccinea* and *A. polytricha*. The *T. fuciformis* scavenging effect was 6.92 at 1.0 mg/ml and 17.63 at 3.5 mg/ml; whereas, the scavenging activities of positive controls, BHA and BHT, at 0.1 mg/ml were 95.18 and 91.07%, respectively.

These results indicate that the antioxidants in the methanol extract of *T. fuciformis* are weak DPPH radical scavengers and therefore required high concentration to have a significant effect.

Ability of chelating ferrous ions

The absorbance of the Fe^{2+}-ferrozine complex linearly decreased in a dose dependent manner, and the

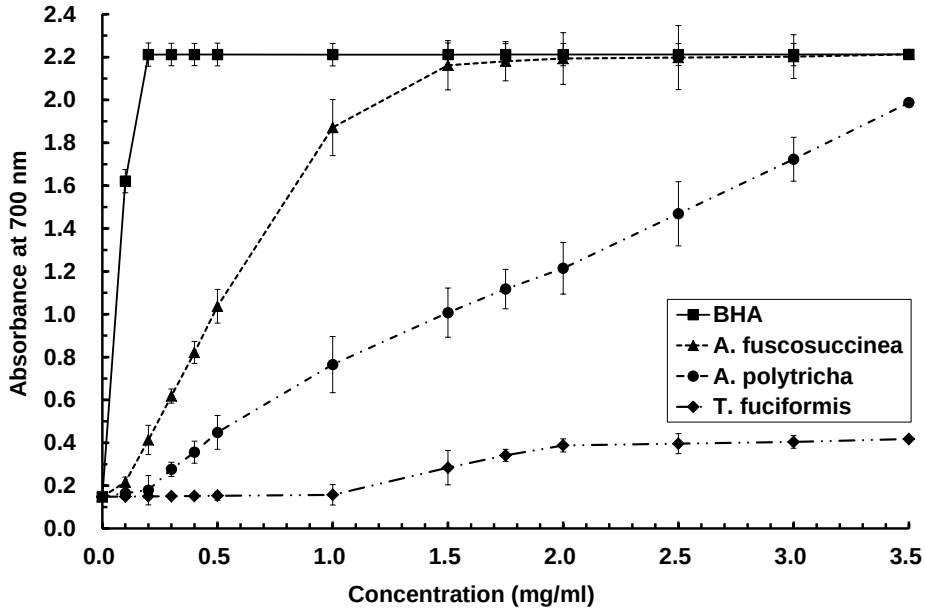

Figure 2. The reducing power of methanol extracts from three gelatinous mushrooms. Each symbol with vertical bars represents the mean ± standard deviation of three replicates. ■, BHA; ▲, methanol extract from white variety of *A. fuscosuccinea;* ●, methanol extract from *A. polytricha*; ◆, methanol extract from *T. fuciformis*.

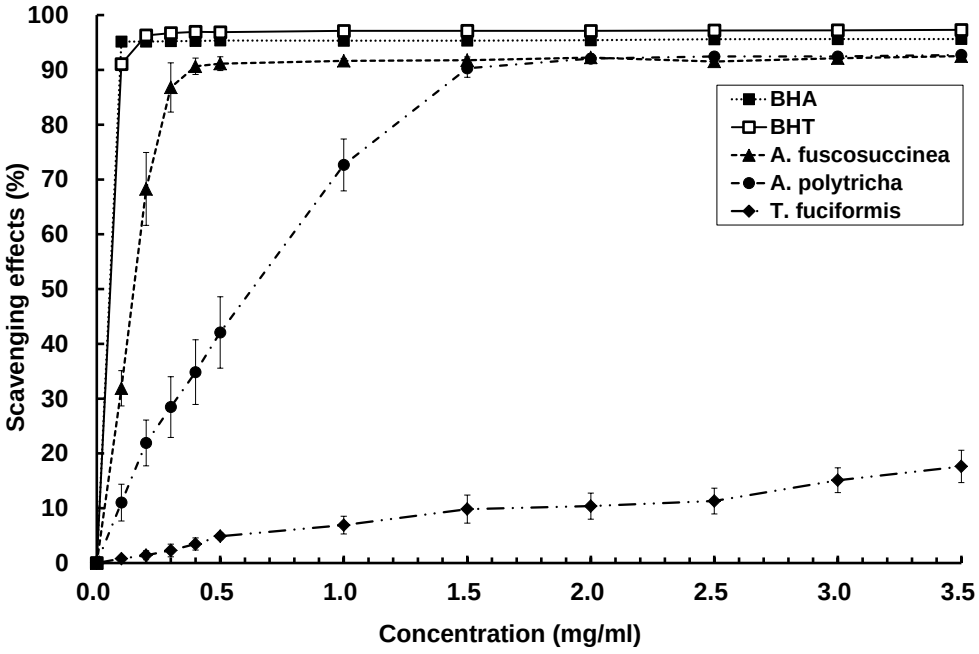

Figure 3. Scavenging effects of methanol extracts from three gelatinous mushrooms on α,α-diphenyl-2-picryl hydrazyl (DPPH) radicals. Each symbol with vertical bars represents the mean ± standard deviation of three replicates. ■, BHA; □, BHT; ▲, methanol extract from white variety of *A. fuscosuccinea*; ●, methanol extract from *A. polytricha*; ◆, methanol extract from *T. fuciformis*.

chelating abilities on ferrous ions of the methanol extracts from the three gelatinous mushrooms increased with concentrations (Figure 4). The chelating effects for white variety of *A. fuscosuccinea*, *A. polytricha* and *T. fuciformis*

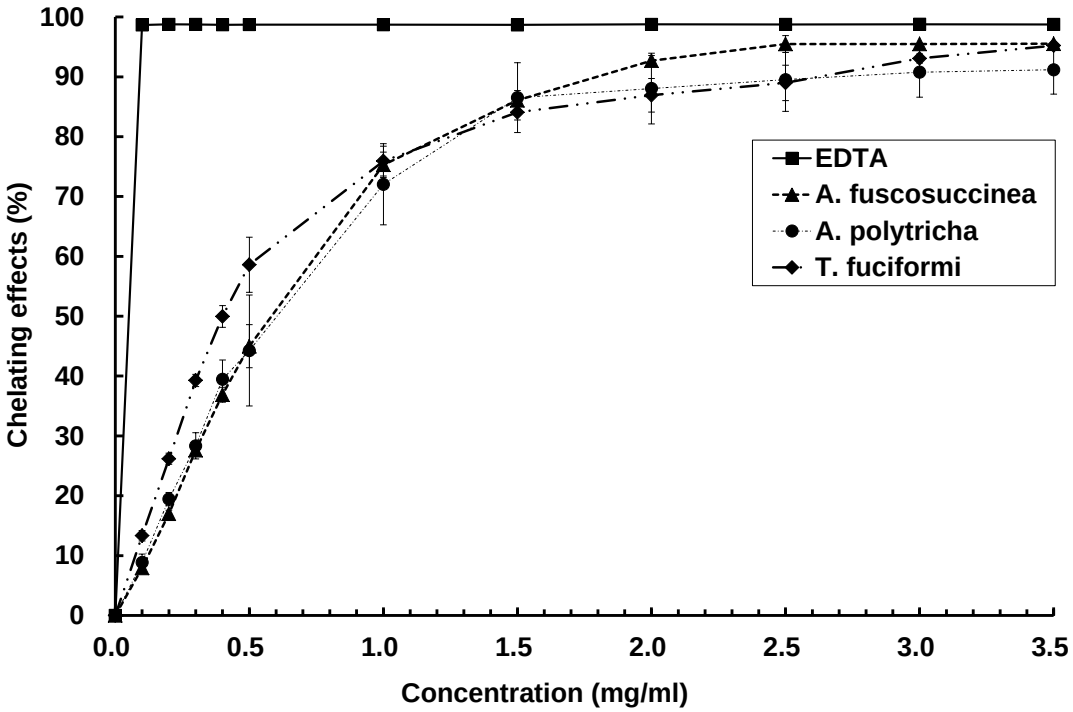

Figure 4. Chelating effects of methanol extracts from three gelatinous mushrooms against ferrous ions. Each symbol with vertical bars represents the mean ± standard deviation of three replicates. ■, BHA; ▲, methanol extract from white variety of *A. fuscosuccinea*; ●, methanol extract from *A. polytricha*; ◆, methanol extract from *T. fuciformis*.

Table 2. EC_{50} values of methanol extracts from three gelatinous mushrooms in antioxidant properties.

Analysis method	EC_{50} values[a,b] (mg extract/ml)		
	White variety of *A. fuscosuccinea*	*A. polytricha*	*T. fuciformis*
Reducing power	0.305 ± 0.085^{x}	0.576 ± 0.018^{y}	N. E.[c,z]
Scavenging effects on DPPH radicals	0.150 ± 0.012^{x}	0.630 ± 0.021^{y}	N. E.[c,z]
Chelating effects on ferrous ions	0.582 ± 0.008^{y}	0.602 ± 0.015^{z}	0.427 ± 0.014^{x}

[a]EC_{50} values, the effective concentration at which the antioxidant activity was 50%; the absorbance was 1.0 for reducing power; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50%, and the ferrous ions were chelated by 50%. EC_{50} values were obtained by interpolation from linear regression analysis. [b]Each value is expressed as means ± standard deviation of three replicates. [c]N. E. = No effect, means not achieve EC_{50} value in determined concentration. [x-z]Different superscripts with the same row indicate significantly different ($p < 0.05$).

were as follows: 44.99, 44.27 and 58.61% at 0.5 mg/ml; 91.28, 88.04 and 86.92% at 2.0 mg/ml; 95.53, 91.19 and 95.22% at 3.5 mg/ml, respectively. However, EDTA was an excellent chelating agent for ferrous ions and it showed a chelating effect was 98.66% at 0.1 mg/ml.

EC_{50} values in antioxidant properties

The antioxidant properties that were assayed from the three gelatinous mushrooms are summarized in Table 2, and the results were normalized and expressed as EC_{50} value (mg dry weight of extracts per ml) for comparison. The EC_{50} value is the effective concentration at which the antioxidant activity is 50%. The lower EC_{50} values of reducing power, scavenging of DPPH radicals and chelating effects on ferrous ions indicated that the methanol extracts of gelatinous mushrooms were more effective. With regard to the reducing power, the EC_{50} value of methanol extracts from white variety of *A. fuscosuccinea* and *A. polytricha* were 0.305 and 0.576 mg/ml, respectively, except for the value of *T. fuciformis* in which we fail to achieve an EC_{50} value with a determined concentration. With regard to the scavenging effect on DPPH radicals, the EC_{50} value of methanol extracts from white variety of *A. fuscosuccinea* and *A.*

Table 3. Enzyme activity from methanol extracts from three gelatinous mushrooms in antioxidant properties.

Enzyme	Enzyme activity[a]		
	White variety of *A. fuscosuccinea*	*A. polytricha*	*T. fuciformis*
SOD[b]	2.10 ± 0.25[x]	1.27 ± 0.15[y]	0.98 ± 0.04[y]
GPx[b]	19.33 ± 0.16	19.50 ± 0.39	19.57 ± 0.28
GRd[c]	5.13 ± 1.10[y]	7.97 ± 0.80[x]	5.37 ± 0.51[y]
TAC[d]	2.26 ± 0.02[x]	1.82 ± 0.07[y]	1.62 ± 0.01[z]

[a]Each value is expressed as means ± standard deviation of three replicates. [b]The activities of SOD and GPx were expressed as U/mg extract. [c]The activity of GRD was expressed as U/g extract. [d]The activity of TAC was expressed as mM/g extract. [x-z]Different superscripts with the same row indicate significantly different (ρ< 0.05).

polytricha were 0.150 and 0.630 mg/ml, respectively, except for the value of *T. fuciformis* in which we failed to achieve an EC_{50} value with a determined concentration. The chelating effect on ferrous ions from *T. fuciformis* was 0.427 mg/ml, which was better than the effects from white variety of *A. fuscosuccinea* (0.582 mg/ml) and *A. polytricha* (0.602 mg/ml).

Determination of antioxidant enzyme activity

SOD activity

The methanol extract of white variety of *A. fuscosuccinea* (2.10 U/mg) contained the highest SOD activity when compared to the SOD activities of *A. polytricha* (1.27 U/mg) and *T. fuciformis* (0.98 U/mg) (Table 3).

GPx activity

With regard to GPx activities (Table 3), there were no significant differences among the methanol extracts from the three gelatinous mushrooms (ranging from 19.33 to19.57 U/mg, ρ>0.05).

GRd activity

The methanol extract of *A. polytricha* (7.97 U/g) had the highest GRd activity whereas *T. fuciformis* (5.37 U/g) and white variety of *A. fuscosuccinea* (5.13 U/g) were significantly lower (ρ<0.05).

Determination of total antioxidant capacity (TAC)

The antioxidant efficiency of the three gelatinous mushrooms was expressed with a TAC value [as an equivalent of Trolox concentration (mM)] according to Miller et al. (1993). The TAC value for the methanol extract from white variety of *A. fuscosuccinea* (2.26 mM/g) was higher than *A. polytricha* (1.82 mM/g) and *T. fuciformis* (1.62 mM/g).

DISCUSSION

Correlation between antioxidant contents and antioxidant activity

Phenolic and flavonoid compounds attract food and medical scientists' attention because of their strong *in vitro* and *in vivo* antioxidant activities and the ability to scavenge free radicals, break radical chain reaction and chelate metals. It is usually speculated that in plant extracts that there is a direct correlation between total phenolic contents, total flavonoid contents and antioxidant activity of the extracts.

According to Orhan and Üstün (2011), polar or phenolic compounds cause higher antioxidant activity (Orhan and Üstün, 2011). A number of studies showed that antioxidant activity of plant extracts is correlated with total phenolic rather than with any individual phenolic compound (Frankel et al., 1995; Meyer et al., 1997; Prior et al., 1998). In addition to, it is rather difficult to characterize every phenolic compound and assess or compare their antioxidant activities (Su and Silva, 2006; Wong et al., 2006).

Therefore, in this study, total phenolic and total flavonoid were measured instead of the individual compounds. Total phenolics of *Lentinula edodes* (0.55 mg GAE/g) (Choi et al., 2006) was significantly lower when compared to ear mushrooms like *A. fuscosuccinea* white strain (8.72 mg GAE/g), *A. auricular-judae* (5.37 to 14.90 mg GAE/g), *A. mesenterica* (4.61 mg GAE/g), *A. fuscosuccinea* brown strain (3.90 mg GAE/g), *A. polytricha* (3.20 mg GAE/g) and *T. fuciformis* (1.04 mg GAE/g) (Mau et al., 2001; Kho et al., 2009).

In contrast, *Dictophora indusiata* (Mau et al., 2002b), *Agrocybe aegerita* var. *alba* (Lo and Cheung, 2005) and *Lactarius deliciosus* (L.) gray (Ferreira et al., 2007) contained 16.28, 15.3 and 17.25 mg GAE/g, respectively. Natural plant sources that contain lower total phenolic content than in gelatinous musrooms include fresh corn (Asami et al., 2003), different colors of fresh peppers (Zhang and Hamauzu, 2003b), and dried Greek aromatic plants (Proestos et al., 2006). These natural sources possessed only 0.25, 0.55 to 0.65, and 0.03 to 0.28 mg GAE/g, respectively. In contrast, total phenolic content in

seeds (Soong and Barlow, 2004) and medicinal plants (Djeridane et al., 2006; Matkowski and Piotrowska, 2006) range between 3.1 to 117 mg GAE/g of dry sample. Salah et al. (1995) demonstrated that the plant phenolic extracts act as agents of other mechanisms contributing to anticarcinogenic actions. High phenolic compounds consumption has been connected with a reduced risk of cardiovascular diseases and some cancers (Marja et al., 1999; Tapiero et al., 2000).

The amount of flavonoids content of *Macrolepiota mastoidea* was observed as 2.84 mg QE/g of extract (Shirmila and Radhamany, 2013). The flavonoid contents were found in the methanol extract of *Boletus aestivalis* [1.53 mg rutin equivalents (RE)/g], *Leccinum carpini* (1.48 mg RE/g) and *B. edulis* (1.46 mg RE/g) (Marijana et al., 2012). Total flavonoid compound was higher in *Pleurotus platypus* [4.46 to 4.73 mg tannic acid equivalents (TAE)/g] when compared to *P. eous* (3.75 to 3.97 mg TAE/g) (Sathyaprabha et al., 2011). The potential benefits of flavonoids on human health include antiviral, antiallergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities (Jia et al., 1999).

Total flavonoids showed significant correlation with antioxidant action through scavenging or chelating process (Ribeiro et al., 2006). Numerous techniques are available to evaluate the antioxidant activity of a sample because there are different types of antioxidants with different antioxidant mechanisms. Besides, this is also due to the following reasons: 1) an antioxidant will turn into a pro- oxidant at critical condition, and 2) an antioxidant will be saturated and become a free radical source when it is not able to step into the chain of electron transport. Therefore, it can be considered that “A single antioxidant is not an antioxidant!” (Truscott, 1996). In this study, reducing power, DPPH free radical scavenging activity and ferrous ions chelating activity were chosen in order to evaluate the antioxidant capacities of white variety of *A. fuscosuccinea*.

Since the reducing power assay can only determine total nonenzymatic antioxidant activity of an extract, it is not able to detect free radical scavenging activity. Hence, the scavenging activity of mushroom extracts in the DPPH free radical assay were assessed to complement the determination of the scavenging effect on free radicals by the extract (Kanatt et al., 2007). Since synergistic action may occur among the different antioxidants in an extract, the relationships among the three different types of assay plus the determination of total phenolic, total flavonoid, and total sugar contents may be useful for the elucidation of the antioxidant properties of white variety of *A. fuscosuccinea*. According to Mau’s (2001) results, the reducing power of methanol extracts from five ear mushrooms was dose-dependent, and the descending order was: white variety of *A. fuscosuccinea* > *A. mesenterica* > *A. polytricha* > *A. fuscosuccinea* (0.67 to 0.74 at 5 mg/ml) > *T. fuciformis* (0.32 at 5 mg/ml). Huang (2000) reported that the methanol extract from *Taiwanofungus camphoratus* showed an excellent reducing power of 0.92 to 0.94 at 5 mg/ml, whereas, that from *Agaricus blazei* showed a reducing power of 0.79 at 5 mg/ml. Methanol extracts from other medicinal mushrooms including *Ganoderma lucidum*, *G. lucidum* antler and *G. tsugae* exhibited a strong reducing power of 1.62, 2.28 and 2.38 at 4.0 mg/ml, respectively (Mau et al., 2002a). However, a good reducing power 0.79 was observed with the methanol extract from another medicinal mushroom, *Coriolus versicolor*, at 4.0 mg/ml.

Reducing powers of methanolic extracts from *Grifola frondosa*, *Hericium erinaceus* and *Tricholoma giganteum* were 1.18, 1.01 and 0.63 at 9 mg/ml, respectively. Among methanol extracts from commercial mushrooms, *P. cystidiosus* and *P. ostreatus* exhibited excellent reducing powers of 1.00 and 1.19 at 10 mg/ml (Mau et al., 2002b). The reducing power of three gelatinous mushrooms in our study agreed with Mau’s results. Mau et al. (2001) also demonstrated that the high reducing power of white variety of *A. fuscosuccina* makes it maintained its white color and faded the original brown color.

Reports suggested that the reducing power is generally asso-ciated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Lillian et al., 2009). Shimada et al. (1992) reported that the antioxidant activity and reducing power are related, and the reducing power may result from their hydrogen donating ability. Duh (1998) reported that the reducing properties are associated with reductones, which may inhibit lipid peroxidation products (LPO) by donating a hydrogen atom and then terminating the free radical chain reaction (Gordon, 1990). Hence, the high reducing power of white variety of *A. fuscosuccinea* may be associated with high amount of reductones like phenolics and flavonoids.

At 1 mg/ml, the methanol extracts from *A. mesenterica* and *A. polytricha* scavenged DPPH radical completely (100%), whereas, those from *A. fuscosuccinea* white strain and *A. fuscosuccinea* brown strain scavenged DPPH radical by 94.5% at 0.4 mg/ml and 95.4% at 3 mg/ml, respectively. However, *T. fuciformis* was not effective in scavenging DPPH radical (71.5% at 5 mg/ml) (Mau et al., 2001). Excellent scavenging effects (96.3 to 99.1 and 97.1%) were observed with methanol extracts from *T. camphoratus* and *A. blazei* at 2.5 mg/ml, respectively (Huang, 2000).

At 6.4 mg/ml, the methanol extract from *Dictyophora indusiata* savenged DPPH radical by 92.1%, whereas scavenging effects of methanol extracts from other specialty mushrooms were 63.3 to 67.8% (Mau et al., 2002b). The methanol extract from *P. ostreatus* at 6.4 mg/ml scavenged DPPH radical by 81.8%, whereas scavenging effects of extracts from other commercial mushrooms were 42.9 to 69.9% (Mau et al., 2002a). Scavenging effects of methanolic extracts from other me-

dicinal mushrooms were measured at up to 0.64 mg/ml and were 24.6, 67.6, 74.4 and 73.5% for *Trametes versicolor*, *G. lucidum*, *G. lucidum antler* and *G. tsugae* (Mau et al., 2002a). These results revealed that gelatinous mushrooms were free radical inhibitor or scavengers, acting possibly as primary antioxidants.

Methanol extracts from gelatinous mushrooms react with free radicals, particularly peroxy radicals, which are the major propagators of the antioxidation of fat, thereby terminating the chain reaction (Shahidi and Wanasundara, 1992). Some researchers referred a high correlation between DPPH radical-scavenging activities and total phenolics (r = 0.971) (Liu and Ng, 2000; Siriwardhana et al., 2003). Kumar et al. (2008) reported that the linear regression analysis of DPPH scavenging with the total phenolic content (GAE) gave an r value of 0.937 which indicated a statistically significant correlation. The data presented in this study indicated that the methanol extracts of white variety of *A. fuscosuccinea* had the highest amount of phenolics, which may explain the high scavenging ability on DPPH.

Mau et al. (2001) reported that the methanol extracts of ear mushrooms were decent chelators for ferrous ions. The chelating effects of methanol extracts from white variety of *A. fuscosuccinea*, *A. mesenterica*, *A. polytricha*, *A. fuscosuccinea* and *T. fuciformis* were 89.16, 92.05, 96.53, 85.13 and 93.64%, respectively at 5 mg/ml. Methanol extracts from *T. camphoratus* chelated ferrous ions by 64.4 to 74.5% at 5 mg/m, whereas that from *A. blazei* showed an excellent chelating effect of 98.6% at 2.5 mg/ml (Huang, 2000). The methanolic extract from *T. versicolor* was not a good ferrous chelator (13.2% at 2.4 mg/ml), whereas, other medicinal mushrooms including *G. lucidum*, *G. lucidum antler*, *G. tsugae* and *C. versicolor* chelated 55.5, 67.7, 44.8 and 13.2% of ferrous ions at 2.4 mg/ml (Mau et al., 2002a).

The methanol extract from *G. frondosa* chelated 70.3% of ferrous ion at 6 mg/ml, whereas at 24 mg/ml, methanol extracts from *D. indusiata*, *H. erinaceus* and *T. giganteum* chelated ferrous ion by 46.4 to 52.0% (Mau et al., 2002b). For commercial mushrooms including *Flammulina velutipes*, *P. cystidiosus*, *P. ostreatus* and *L. edodes* chelated ferrous ion at 1.6 mg/ml (Mau et al., 2002a). Since ferrous ions are the most effective pro-oxidants in the food system (Yamaguchi et al., 1988), the higher chelating effect of methanol extracts from gelatinous mushrooms would be beneficial. The metal chelating abilities of three gelatinous mushrooms and standard antioxidants were determined by evaluating their capacity to compete against ferrozine for the ferrous ions. Therefore, measurement of color reduction rate allows estimation of the chelating efficiency of the coexisting chelator (Yamauchi et al., 1988). It has been reported that chelating agents act as effective secondary antioxidants through reducing redox potential to stabilize oxidized form of metal ions (Gardner et al., 2000). Antioxidant properties of mushrooms were usually related to low-molecular-weight compounds, in particular to the phenolic fractions (Nickavar et al., 2007; Pan et al., 2008).

Past studies indicated that correlations have been found among total phenolic contents, TAC values and nitric oxide (NO) scavenging effects (Tsushida et al., 1994). In this study, compared to the other gelatinous mushrooms, white variety of *A. fuscosuccinea* had significant higher total phenolic (approximately twice than *A. polytricha* and almost four times than *T. fuciformis*) and relatively high total flavonoid content, which is comparable to that detected in *T. fuciformis*. These results might provide a possible explanation why white variety of *A. fuscosuccinea* had highest reducing power, scavenging effect on DPPH radicals. The DPPH and ABTS radicals had been used widely to investigate the scavenging activities of several natural compounds such as phenolic compounds or crude extracts of plants.

The model of scavenging the stable DPPH radical is widely used to evaluate antioxidant activities over a relatively short time compared to other methods. The antioxidant properties and antioxidant enzyme activities of *T. fuciformis* were less effective when compared to the activities of white variety of *A. fuscosuccinea* and *A. polytricha*. This may be explained by *T. fuciformis* having the lowest content of total phenolics. Similar results were reported by Mau et al. (2001). As for the better chelating effects on ferrous ions of the methanol extract of *T. fuciformis*, this study referred that *T. fuciformis* has significant higher total sugar (approximately 1.6 times that of *A. polytricha* and almost 2.5 times that of white variety of *A. fuscosuccinea*); and there is a high correlation between ferrous ions chelating effects and total sugars (r = 0.987).

In this assay, the methanol extracts of the three gelatinous mushrooms and standard antioxidant compounds interfer the formation of the ferrous and ferrozine complex. These results suggest that they have chelating activities and capable to capture ferrous ions before ferrozine. Ferrous ions are the most effective pro-oxidants and are commonly found in foods (Yamaguchi et al., 1988). Therefore, the high ferrous ion chelating abilities of methanol extracts from these mushrooms are beneficial. Among the methanol extracts, white variety of *A. fuscosuccinea* was more effective than *A. polytricha* and *T. fuciformis* in reducing power and scavenging effect on DPPH radicals.

In contrast, *T. fuciformis* was more effective than white variety of *A. fuscosuccinea* and *A. polytricha* in chelating effects on ferrous ions. The methanol extracts had high antioxidant activities, indicating that the methanol extracts may contain some potential natural antioxidant components that are relatively effective. BHA and BHT as a pure/concentrated synthetic phenolic antioxidant can scavenge reactive oxygen species such as DPPH free radicals by donating labile hydrogen and leaving an oxidized phenolic ion stabilized by the inherent resonance of the benzene ring. This may be the reason why BHA

demonstrated a relatively higher free radical scavenging activity than the extracts of *A. auricula judae*. In addition, the extracts tested in this study were crude.

Generally, EC_{50} values lower than 1 mg/ml indicate that the methanol extracts have effective antioxidant properties. Although, BHA and BHT had inhibitory effects on reducing powers and scavenging effects of DPPH radicals and EDTA was able to chelate ferrous ions, they are additives and are present in low levels (mg) in foods. The three gelatinous mushrooms in this study may be used of higher levels (g) in food or food ingredients. Therefore, these mushrooms may serve as possible protective agents in human diet to help humans in reducing oxidative damage.

The mechanism of SOD is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet}$), that is produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye.

The SOD activity is measured by the degree of inhibition of this reaction. One unit of SOD is the amount that causes a 50% inhibition of the rate of INT reduction. The high SOD activities that were found in the methanol extracts of white variety of *A. fuscosuccinea* indicated that the extract contained compounds to inhibit the reduction of INT.

GPx catalyses the oxidation of reduced glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form (GSH) with a concomitant oxidation of NADPH to $NADP^+$. Hydrogen peroxide is accumulated during dopamine metabolism. GPx activity plays an important role to scavenge the elevated levels of hydrogen peroxide.

This result suggests that the three gelatinous mushrooms all have good scavenging activities of hydrogen peroxide. GRd catalyses the reduction of GSSG in the presence of NADPH, which is oxidized to $NADP^+$. Increasing GRd activity may occur to maintain GSH in a reduced form helps.

The methanol extracts of *A. polytricha* had the highest GRd-like activity to remove the toxic GSSG. There are less research shed lights on the correlations between enzyme activities and the antioxi-dant activities of mushrooms.

The present study shows that the methanol extract form white variety of *A. fuscosuccinea* had highest SOD activity and TAC activity compared to *A. polytricha* and *T. fuciformis*. *T. fuciformis* did not show good activities on reducing power and scavenging effect on DPPH radicals. All antioxidant enzymes are essential catalysts which stimulate chemical reactions without becoming consumed or integrated in the reaction. Antioxidant enzymes may also stop the free radical from forming in the first place. In addition, they may interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. Significant differences were detected in antioxidant activities and antioxidant enzyme activities among three gelatinous mushrooms.

Conclusion

According to this study, the consumption of white variety of *A. fuscosuccinea* may be beneficial to the antioxidant protection system of the human body against oxidative damage. Therefore, in addition to these antioxidant components, other components may contributeto the antioxidant properties of white variety of *A. fuscosuccinea*. To study the mechanisms of other potential antioxidant components, the fractionation of methanol extracts and further identification should be in progress.

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