Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 89 (2005) 533–539
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba

K.M. Lo, Peter C.K. Cheung *

Department of Biology, Food and Nutritional Sciences Programme, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, PR China

Received 26 January 2004; received in revised form 4 March 2004; accepted 4 March 2004

Abstract

Antioxidant activity of the methanol crude extract and its fractions, isolated by liquid–liquid partition, from the fruiting bodies of *Agrocybe aegerita*, an edible mushroom, was evaluated by scavenging activity of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation ($ABTS^{\cdot+}$) and inhibition of lipid peroxidation of rat brain homogenate. The ethyl acetate (EA) fraction, which showed the most potent antioxidant activity in the above two assays, was further fractionated by a Sephadex LH-20 column into four subfractions (EA1–EA4). EA3 exhibited the strongest radical-scavenging activity in the $ABTS^{\cdot+}$ and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and showed a similar extent of in vitro inhibition of human LDL oxidation to caffeic acid. Significant correlation was found between the total phenolic content and the antioxidant activity ($p < 0.01$) in the EA fraction and its subfractions.

Keywords: Mushroom; Antioxidant; Phenolic compounds; Radical scavenging

1. Introduction

Free radicals are defined as any molecules or atoms with one or more unpaired electrons (Yashikawa, Naito, & Kondo, 1997). With the possession of the unpaired electrons, free radicals are usually unstable and highly reactive. Peroxyl radical is a key step in lipid peroxidation and is an important cause of cell membrane destruction and thus tissue damage (Halliwell, 1995). It has also been suggested that oxidative modification of low-density lipoprotein (LDL) is the main cause of atherosclerosis which is highly related to peroxyl radical formation (Brown & Goldstein, 1983). Antioxidants can scavenge free radicals and inhibit lipid peroxidation. Vegetables and fruits are rich sources of antioxidants such as vitamin C, vitamin E and beta-carotene, which are suggested to be antiatherogenic in epidemiological studies (Enstrom, Kanim, & Klein, 1992; Rimm et al., 1993; Stampfer et al., 1993). Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular diseases, especially atherosclerosis. Phenolic compounds are other type of antioxidant that possess a strong inhibition effect against lipid oxidation through radical scavenging (Frankel, Kanner, German, Parks, & Kinsella, 1993). Some common edible mushrooms, which are widely consumed in Asian culture, have currently been found to possess antioxidant activity which is well correlated with their total phenolic content (Cheung, Cheung, & Ooi, 2003). Two novel prenylated phenolics, asiaticusin A and asiaticusin B, have been isolated in the fruiting bodies of an edible mushroom, *Boletinus asiaticus* (Wada, Hayashi, & Shibata, 1996). Another mushroom, *Paxillus panuoides*, was found to contain two *p*-terphenyls which showed potent inhibition effects lipid peroxidation

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid); DCM, dichloromethane; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EA, ethyl acetate; LDL, low-density lipoprotein; MDA, malondialdehyde; TBHQ, *tert*-butylhydroquinone; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; TCA, trichloroacetic acid.

* Corresponding author. Tel.: +86-852-2609-6144; fax: +86-852-2603-5646.
E-mail address: petercheung@cuhk.edu.hk (P.C.K. Cheung).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.006

(Yun, Lee, Kim, & Yoo, 2000). These findings suggest that the edible mushrooms might be a potential source of phenolic antioxidants. However, investigations of antioxidants derived from lesser-known edible mushrooms, which are produced by artificial cultivation methods, are still relatively rare. As these mushrooms are only recently available in the market, their health benefits, especially antioxidative properties, are of intense research interest. Recently, we have screened the antioxidant activity of the methanol crude extract of 14 lesser-known edible mushrooms cultivated in Fujian Province of China (unpublished data). Among these mushrooms, the *Agrocybe aegerita* var. alba showed the most potent antioxidant activity. In this study, the antioxidant activity of this mushroom was investigated in relation to its total phenolic content.

2. Materials and methods

2.1. Chemicals

The Trolox (6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and tetramethoxypropane were from Aldrich Chemical (Milwaukee, WI). The iron (II) sulphate was an AnalaR chemical (Poole, England). EDTA was Fluka Chemical (Buchs, Switerland). Sodium azide was from Merck (Darmstadt, Germany). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Extraction and fractionation of antioxidants

The dried fruiting bodies of *A. aegerita* were provided by the Sanming Mycological Institute in Fujian Province of China. The mushroom (200 g) was milled into powder through a 0.5 mm sieve using a hammer mill (IKA-WERKE MF10, Selangor, Malaysia), and extracted with methanol (2 l) in a Soxhlet extractor for 24 h. The solvent of the methanol crude extract was removed under reduced pressure and the residue was re-dissolved in distilled water and then sequentially extracted with dichloromethane, ethyl acetate (EA) and water-saturated butanol, using liquid–liquid partition. After removal of the solvents, four fractions were obtained, including the dichloromethane (DCM), EA, butanol and water fractions, with the extraction yields being 0.133%, 0.237%, 1.57% and 6.03% of the dry weight of mushroom, respectively. The scavenging activities of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation ($ABTS^{\cdot+}$) and the lipid peroxidation (of rat brain homogenate) inhibition of the methanol crude extract and these four fractions were evaluated.

Because of its high antioxidant activity, the EA fraction (at 20 mg/ml) was further fractionated by a column (2.5×45 cm) packed with Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and eluted with methanol at a flow rate of 0.8 ml/min (Shahidi, Amarowicz, He, & Wettasinghe, 1997). The eluates were collected in test tubes using a fraction collector (GradiFrac, Pharmacia, Uppsala, Sweden). The eluates were combined according to the results of UV absorbance and thin-layer chromatography (TLC), developed by a solvent system of EA/propanol/ammonia (9:7:4, v/v/v) (Babitskaya, Shcherba, Oleshko, & Osadchaya, 1996) to produce four subfractions: EA1, EA2, EA3 and EA4; the EA1 subfraction was the first eluted subfraction and EA4 was the last eluted one. Solvent in each subfraction was removed by a stream of nitrogen. The scavenging activity of $ABTS^{\cdot+}$ and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as well as the inhibition of human LDL oxidation of the EA fraction and its subfractions (EA1–EA4) were evaluated.

2.3. Scavenging activity of $ABTS^{+}$ radical cation

The scavenging activity of $ABTS^{\cdot+}$ was measured according to the method described by Re et al. (1999) with some modifications. The ABTS reagent was prepared by mixing 5 ml of 7 mM ABTS with 88 μl of 140 mM $K_2S_2O_8$. After the mixture was kept in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with 95% ethanol so that its absorbance was adjusted to 0.70 ± 0.05 at 734 nm using a spectrophotometer (Genesys5, Spectronic Instruments, Rochester, NY). To determine the scavenging activity, 1 ml ABTS reagent was mixed with 10 μl of sample or negative control (methanol) and the absorbance was measured at 734 nm 6 min after the initial mixing, using ethanol as blank. The inhibition percentage of the samples was calculated by the following equation:

$$\text{Inhibition percentage } (\%) = \{1 - (\text{Ab}_{734} \text{ sample}/\text{Ab}_{734} \text{ control})\} \times 100\%, \quad (1)$$

where Ab_{734} was the absorbance at 734 nm.

Trolox, with a concentration range of 0.5–2 mM, was prepared as a standard. A dose–response curve of inhibition percentage against different concentrations of Trolox standard was prepared. The antioxidant activity of samples was expressed as Trolox equivalent antioxidant capacity (TEAC) which represented the concentration (mM) of Trolox, having the same activity as 1 mg of sample.

2.4. Scavenging activity of DPPH radical cation

The scavenging activity of DPPH radical was assayed according to the method described by Chu, Chang, and Hus (2000) with some modifications. An aliquot of 1.0 ml of 0.1 mM DPPH radical solution dissolved in

methanol was mixed with 0.5 ml sample extract of various concentrations (0.05–2 mg/ml) or negative control (methanol). The reaction mixture was mixed and its absorbance at 520 nm was measured. *tert*-Butylhydroquinone (TBHQ) (1.5 g/ml) was used as standard. The DPPH radical-scavenging activity (%) was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \{1 - (\text{Ab}_{520}\ \text{sample}/\text{Ab}_{520}\ \text{control})\} \times 100\%. \quad (2)$$

The scavenging activity of sample was expressed as 50% effective concentration (EC_{50}) which represented the concentration of sample having 50% of DPPH radical scavenging effect.

2.5. Lipid peroxidation assay

The inhibition of lipid peroxidation of rat brain homogenate was assayed according to the method described by Ng, Liu, and Wang (2000) with some modifications. Brain tissues obtained from male Sprague–Dawley (SD) rats weighing 150 g were homogenized with a Polytron homogenzier in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) homogenate. The homogenate was centrifuged at 3000*g* for 10 min. An aliquot (0.1 ml) of supernatant was mixed with 0.2 ml of mushroom fractions and methanol crude extract of various concentrations, followed by addition of 0.1 ml of 10 μM $FeSO_4$ and 0.1 ml of 0.1 mM L-ascorbic acid; the mixture was incubated at 37 °C for 1 h. The reaction was terminated by adding 0.5 ml trichloroacetic acid (TCA, 28%, w/v), followed by 0.38 ml thiobarbituric acid (TBA, 2%, w/v), with heating at 100 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the absorbance of the supernatant containing the thiobarbituric acid-reactive substances (TBARS) was measured at 532 nm by a spectrophotometer. Caffeic acid (0.25 mg/ml) was used as a standard and Tris–HCl buffer was used instead of sample as the negative control. The inhibition percentage of lipid peroxidation of the sample was calculated by the following equation:

$$\text{Inhibition percentage (\%)} = \{1 - (\text{Ab}_{532}\ \text{sample}/\text{Ab}_{532}\ \text{control})\} \times 100\%, \quad (3)$$

where Ab_{532} was the absorbance at 532 nm.

The inhibition of lipid peroxidation of sample was expressed as 50% inhibition concentration (IC_{50}) which represented the concentration of sample having 50% inhibition effect on lipid peroxidation of the rat brain tissue.

2.6. Inhibition of human LDL oxidation

LDL was prepared according to the method described by Zhang, Chan, Luk, Ho, and Chen (1997). The LDL was isolated by sequential flotation ultracentrifugation and the protein content was determined by Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951). The stock LDL solution (0.5 mg/ml) was dialyzed against 100 volumes of degassed dialysis buffer, pH 7.4, containing 0.05% NaN_3, 0.9% NaCl, 0.1 M sodium phosphate and 10 μM EDTA for 24 h. The dialysis buffer was changed four times over the period of 24 h. The dialyzed LDL was then diluted to 250 μg/ml using a dilution buffer which had the same chemical constituents as the dialysis buffer but without EDTA and NaN_3. An aliquot of 0.4 ml diluted LDL was incubated with 50 μl of 50 μM $CuSO_4$ and 50 μl of EA fraction and subfractions (0.5 mg/ml) or ultra pure water (negative control) for a time period of 4, 12 on 36 h at 37 °C. The reaction was stopped by the addition of 25 μl of EDTA (1%, w/v) and the mixture was cooled at 4 °C. The TBARS was formed by adding 2 ml TCA–TBA–HCl solution followed by heating at 95 °C for 1 h. After the mixture was cooled, the formation of TBARS was determined by measuring absorbance at 532 nm. A standard calibration curve with a concentration range of 1.25–30 μM was constructed by using malondialdehyde (MDA), prepared from tetramethoxypropane (Aldrich). The extent of TBARS was expressed as nmol MDA/mg LDL. Caffeic acid (0.5 mg/ml) was used as a positive control.

2.7. Total phenolic content

The total phenolic content of the methanol crude extract and its fractions, as well as the EA subfractions was determined by the Folin–Ciocalteu method with some modifications (Singleton & Rossi, 1965). One millilitre of sample solution was mixed with 1 ml of Folin–Ciocalteu reagent. After 3 min of incubation at room temperature, 1 ml of saturated Na_2CO_3 (35% aqueous solution) was added to the mixture, followed by the addition of 7 ml of distilled water. The mixture was kept in the dark for 90 min and its absorbance at 725 nm was then measured. A calibration curve, using gallic acid with a concentration range of 0.01–0.4 mM, was prepared. The total phenolic content of the samples was expressed as gallic acid equivalents (GAE), which reflected the phenolic content as the amount of gallic acid (mg) in 1 g of sample.

2.8. Statistical analysis

All results were obtained in triplicate and data were expressed as mean ± SD. The mean values of data were also analyzed by one-way analysis of variance (one-way ANOVA) and Tukey's pairwise means comparison test (Tukey) was used to detect significant difference ($p < 0.05$) between the mean values that had more than two groups. For comparison of data between two

groups, the Student's *t*-test was carried out to detect any significant differences ($p < 0.05$). Correlations were found by Pearson's correlation coefficient in bivariate correlations. The statistical analysis was done by the Statistical Package for Social Sciences (SPSS 11.0, 2001).

3. Results and discussion

3.1. Total phenolic content and antioxidant activity of methanol crude extract and fraction

Phenolic compounds in plants are powerful free radical-scavengers which can inhibit lipid peroxidation by neutralizing peroxyl radicals generated during the oxidation of lipids (Shahidi, Janitha, & Wanasundra, 1992). Since mushrooms (edible fungi) also possess phenolic compounds (Cheung et al., 2003; Wada et al., 1996), it is interesting to investigate the antioxidant activity of mushroom in relation to their total phenolic contents. The total phenolic content of different fractions of *A. aegerita* is shown (Table 1) with the GAE value of the EA fraction being the highest ($p < 0.05$). Except for the water fraction which had a significantly lower GAE value, all the other fractions showed a significantly higher GAE value than that of the methanol crude extract ($p < 0.05$) (Table 1), indicating that the phenolic compounds in *A. aegerita* were mainly soluble in organic solvents, especially EA.

Table 1
Total phenolic content and scavenging activity of $ABTS^{\cdot+}$ radical cation of the methanol crude extract and its fractions from *A. aegerita*

Extract/fractions	GAE[a]	TEAC[b]
Methanol crude extract	15.3 ± 0.24D	0.096 ± 0.004BC
DCM fraction	41.3 ± 0.30B	0.162 ± 0.024B
EA fraction	51.2 ± 0.38A	0.254 ± 0.021A
Butanol fraction	30.5 ± 0.71C	0.163 ± 0.049B
Water fraction	12.9 ± 0.41E	0.063 ± 0.022C

[a] Total phenolic content is expressed as gallic acid equivalents (GAE; mg/g of GAE). Each value is the mean ± SD of triplicate measurements. Values within a column with different letters (A–E) differ significantly ($p < 0.05$).

[b] TEAC, expressed as mM Trolox per mg of sample. Each value is the mean ± SD of triplicate measurements. Values within a column with different letters (A–C) differ significantly ($p < 0.05$).

The antioxidant activities of the methanol crude extract and its fractions isolated from *A. aegerita* were evaluated by two assays. In the scavenging activity of $ABTS^{\cdot+}$, the relatively long-lived $ABTS^{\cdot+}$, generated by the direct oxidation of ABTS with potassium persulfate (Frankel & Meyer, 2000), was decolorized during the reaction with hydrogen-donating antioxidant (Leong & Shui, 2002). The $ABTS^{\cdot+}$ scavenging activity was expressed in term of a TEAC value with a higher value indicating a more potent radical-scavenging effect. Among the methanol crude extract and its fractions, the TEAC value of the EA fraction was the highest ($p < 0.05$) (Table 1), with its radical scavenging effect being 3.3 times higher than that of the methanol crude extract (Table 1). Similarly, the lipid peroxidation of rat brain homogenate induced by Fe^{2+}/ascorbate, was strongly inhibited by the EA fraction as indicated by its lowest IC_{50} value (Table 2). The inhibition effect of the EA fraction was 7.5 times higher than its parent methanol crude extract (Table 2) and its inhibition effect (95.3%) of lipid peroxidation at 0.25 mg/ml was similar ($p > 0.05$) to that of caffeic acid (96.4%) at 0.25 mg/ml (data not shown in table). In contrast, the $ABTS^{\cdot+}$ radical scavenging activity and inhibition of lipid peroxidation (at 0.5 mg/ml) of the water fraction was the lowest ($p < 0.05$) among the four fractions (Tables 1 and 2). These findings suggested that antioxidants with hydrogen-donating and lipid peroxidation-inhibiting properties in the methanol crude extract of *A. aegerita* were selectively extracted into the EA fraction. Since a strong positive correlation was found between the total phenolic content and TEAC value (Table 1) ($R_2 = 0.903$, $p < 0.01$), as well as the inhibition of lipid peroxidation at 0.5 mg/ml (Table 2) ($R_2 = 0.863$, $p < 0.01$) for the methanol crude extract and its fractions, the strong antioxidant potency of the EA fraction might be highly contributed by the abundant phenolic compounds that it contained.

3.2. Yield of ethyl acetate subfractions

Due to the potent antioxidant activity of the EA fraction, it was further fractionated by a Sephadex LH-20 column into four subfractions, based on size-exclu-

Table 2
The IC_{50} and the inhibition percentage of the methanol crude extract and its fractions from *A. aegerita* in lipid peroxidation of rat brain homogenate

Extract/fractions	Inhibition percentage[a] (%)			IC_{50} (mg/ml)
	0.5 (mg/ml)	1 (mg/ml)	2 (mg/ml)	
Methanol crude extract	48.7 ± 0.68D	73.9 ± 3.15B	92.2 ± 1.28A	0.378
DCM fraction	68.4 ± 2.13B	94.3 ± 0.69A	93.4 ± 0.60A	0.478
EA fraction	93.8 ± 0.57A	96.1 ± 0.86A	94.9 ± 0.56A	0.0502
Butanol fraction	62.1 ± 0.45C	95.3 ± 0.24A	95.5 ± 0.42A	0.457
Water fraction	7.47 ± 1.23E	6.28 ± 8.94C	11.3 ± 5.64B	>2

[a] Values within a column with different letters (A–E) differ significantly ($p < 0.05$).

Table 3
The yield, total phenolic content, scavenging activity of $ABTS^{\cdot+}$ radical cation and the 50% effective concentration (EC_{50}) in DPPH radical-scavenging of the different subfractions of the EA fraction from *A. aegerita*

Fraction/subfractions	Yield[a] (%)	GAE[b]	TEAC[c]	EC_{50} of DPPH (mg/ml)
EA	–	51.2 ± 0.38D	0.254 ± 0.021D	1.16
EA1	58.4	23.9 ± 1.13E	0.132 ± 0.004E	1.92
EA2	8.94	80.9 ± 2.01C	0.594 ± 0.068C	0.438
EA3	2.44	192 ± 2.50A	0.934 ± 0.0301A	0.139
EA4	2.39	122 ± 1.80B	0.813 ± 0.023B	0.269

[a] Extraction yield is expressed as percentage dry weight of EA fraction.
[b] Total phenolic content is expressed as gallic acid equivalents (GAE; mg/g GAE). Each value is the mean ± SD of triplicate measurements. Values within a column with different letters (A–E) differ significantly ($p < 0.05$).
[c] TEAC, expressed as mM Trolox per mg of sample. Each value is the mean ± SD of triplicate measurements. Values within a column with different letters (A–E) differ significantly ($p < 0.05$).

sion and normal-phase chromatographic separation mechanisms, with methanol being used as the mobile phase (Henke, 1995). The yield of the 4 subfractions decreased from EA1 to EA4 (Table 3), showing that the earlier eluted subfractions had relatively higher yield. According to the separation principles of normal-phase and size-exclusion chromatography, the compounds in EA1 might be relatively more non-polar and have higher molecular weights than those in EA4. This might indicate that the amount of non-polar and high molecular weight compounds in the EA fraction was larger than the polar and low molecular weight ones.

3.3. Antioxidant activity and total phenolic content of the ethyl acetate fraction and its subfractions

Concerning the total phenolic content of the subfractions, it was found that the GAE values of EA2, EA3 and EA4 were significantly higher than that of the EA fraction ($p < 0.05$), with EA3 having the highest GAE value (Table 3). On the other hand, EA1 showed a significantly lower GAE value than the EA fraction ($p < 0.05$). These findings indicated that the phenolic compounds in the EA fraction, which were mainly found in EA3, seemed to be relatively more polar and had lower molecular weight.

Comparing the scavenging activity of $ABTS^{\cdot+}$, the TEAC values of EA2, EA3 and EA4 were 2.3–3.7 times higher than that of the EA fraction, with EA3 and EA1 having the highest and lowest radical scavenging activity ($p < 0.05$), respectively (Table 3). Concerning the scavenging activity of DPPH radical, this assay also evaluated the ability of sample to scavenge the DPPH radical by a hydrogen-donating mechanism. The use of the stable DPPH radical has the advantage of being unaffected by side reactions, such as enzyme inhibition and metal chelation (Wettasinghe & Shahidi, 1999). The EC_{50} values of EA3, EA4 and EA2 were about 8-fold, 4-fold and 3-fold, respectively, smaller than that of the EA fraction, with the EC_{50} value (0.139 mg/ml) of EA3 being the lowest, indicating that this subfraction exhibited the highest radical scavenging effect (Table 3).

From the above 2 assays, a strong positive correlation between the total phenolic content and the TEAC value ($R^2 = 0.943$, $p < 0.01$) (Table 3) as well as the DPPH radical-scavenging activity at 0.5 mg/ml (data not shown) ($R^2 = 0.974$, $p < 0.01$) was found for the EA fraction and its subfractions, suggesting that the phenolic compounds might be the major antioxidant components present in the EA2, EA4 and especially EA3 which could effectively scavenge both DPPH and $ABTS^{\cdot+}$ radicals by the neutralization of radicals through hydrogen donation.

In LDL oxidation, the amount of TBARS, the breakdown product of LDL during lipid peroxidation, can be used as an index of lipid peroxidation in LDL (Ernster, 1993). Our results showed that, after 4 h of incubation, the EA fraction, EA2, EA3 and EA4 showed significantly lower TBARS levels than that of the control ($p < 0.05$) but not EA1 ($p > 0.05$) (Table 4), indicating that the samples, except EA1, could effectively inhibit the LDL oxidation, at least for a short period. At the 12th hour of incubation, probably due to the exhaustion of antioxidants for protecting the LDL against oxidation, the EA fraction showed ineffective inhibition as its TBARS level was the same as that of the control ($p > 0.05$) (Table 4). In contrast, the EA2, EA3 and EA4 subfractions continued to show effective inhibition, as

Table 4
The formation of TBARS in LDL oxidation for the EA fraction and its subfractions from *A. aegerita* at 0.5 mg/ml

Samples/standard	TBARS formation[a] (nmole MDA/mg LDL protein)		
	4th hour	12th hour	36th hour
EA	3.09 ± 0.38A	28.0 ± 0.64B	23.8 ± 0.22B
EA1	22.4 ± 1.72B	26.7 ± 0.52B	23.0 ± 0.22B
EA2	2.05 ± 0.29A	4.78 ± 0.79A	8.28 ± 1.4A
EA3	2.74 ± 0.14A	5.02 ± 0.52A	6.77 ± 0.25A
EA4	3.04 ± 0.22A	5.54 ± 0.28A	8.11 ± 0.54A
Control	28.8 ± 0.14C	27.4 ± 0.25B	22.6 ± 0.50B
Caffeic acid	2.69 ± 0.16A	6.53 ± 0.58A	7.47+0.52A

[a] Each value is the mean ± SD of triplicate measurements. Values within a column with different letters (A–C) differ significantly ($p < 0.05$).

their TBARS levels were significantly lower than that of the control, even at the 36th hour of incubation ($p < 0.05$) (Table 4). These results suggested that the persistency of these EA subfractions in inhibition of LDL oxidation was much stronger than that of its parent EA fraction (Table 4) Also, the TBARS levels of EA2, EA3 and EA4 were similar to that of caffeic acid throughout the whole incubation period ($p > 0.05$), indicating that the antioxidant activity of these subfractions, in terms of inhibition of LDL oxidation, was as potent and persistent as caffeic acid at the same concentration (0.5 mg/ml) (Table 4). Besides, it was found that a negative and statistically significant correlation ($R^2 = 0.811$, $p < 0.01$) was demonstrated between the TBARS formation (Table 4) and total phenolic content (Table 3) for the EA fraction and its subfractions at the 36th hour of incubation, suggesting that the phenolic compounds present in EA2, EA4 and especially EA3, might be at least one of the active components inhibiting the LDL oxidation. As phenolic antioxidants were suggested to act as inhibitors of LDL oxidation by means of free radical scavenging (Frankel & Meyer, 2000), the phenolic compounds presented in these subfractions might inhibit LDL oxidation by mean of hydrogen donation to scavenge the peroxyl radical in a similar way to that observed in the two radical scavenging assays mentioned above. As a result, the EA2, EA4 and especially EA3 subfractions were most effective in inhibition of LDL oxidation in vitro. As this event is the key step for the formation of oxidative modified LDLs which later causes the foam cell formation and finally atherosclerosis (Frei, 1995), these subfractions, especially EA3, may have a preventive effect against atherosclerosis by inhibiting LDL oxidation through radical scavenging. Thus, they might be a potential source of antioxidants in the prevention of atherosclerosis.

3.4. Conclusion

The EA fraction of the methanol crude extract of *A. aegerita* was most potent in the scavenging of $ABTS^{\cdot+}$ radical and inhibition of lipid peroxidation among the four fractions. Three of the four subfractions isolated from EA, the EA2, EA4 and especially EA3 subfractions, showed potent scavenging activity of DPPH and $ABTS^{\cdot+}$ radicals as well as strong a inhibition effect against human LDL oxidation in vitro ($p < 0.05$). The antioxidant activity was found to be positively correlated with the total phenolic content among the EA fraction and its subfractions in the three antioxidant assays, suggesting that phenolic compounds probably having low molecular weight and relatively high polarity, might play an important role in radical scavenging and inhibition of LDL oxidation. The possible mechanism by which for the EA subfractions inhibit LDL oxidation might be radical-scavenging, through hydrogen donation.

However, there is no study so far concerning the characterization of the active antioxidative components in *A. aegerita*. Since phenolic compounds might be a possible class of antioxidant in this mushroom, future work on the isolation and structural characterization of the active components is needed. Also, the antioxidant activity of these components, in regard to the mechanisms for radical scavenging and protection against lipid peroxidation, will be the primary objective of further investigation.

Acknowledgements

We thank Mr. C.H. Lee for the isolation of human low-density lipoproteins. This research was funded by the Direct Grant administered by the Research Committee of the Chinese University of Hong Kong.

References

Babitskaya, V. G., Shcherba, V. V., Oleshko, V. S., & Osadchaya, O. V. (1996). *Pleurotus ostreatus* produces a complex of biologically active compounds. *Applied Biochemistry and Microbiology, 32*, 203–210.

Brown, M. S., & Goldstein, J. (1983). Lipoprotein metabolism in the macrophage. *Annual Review of Biochemistry, 52*, 223–261.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry, 81*, 249–255.

Chu, Y. H., Chang, C. L., & Hus, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Enstrom, E., Kanim, L. E., & Klein, M. A. (1992). Vitamin C and mortality among a sample of the US population. *Epidemiology, 3*, 194–198.

Ernster, L. (1993). Lipid peroxidation in biological membranes: Mechanisms and implications. In K. Yagi (Ed.), *Active oxygens, lipid peroxides and antioxidants* (pp. 1–38). Tokyo, Japan: Japan Scientific Societies Press.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*, 1925–1941.

Frankel, E. N., Kanner, J., German, J. B., Parks, E., & Kinsella, J. E. (1993). Inhibition of human low-density lipoprotein by phenolic substances in red wine. *Lancet, 341*, 454–457.

Frei, B. (1995). Cardiovascular disease and nutrient antioxidants: Role of low-density lipoprotein oxidation. *Critical Review of Food Science and Nutrition, 35*, 83–98.

Halliwell, B. (1995). Antioxidant characterization. *Biochemical Pharmacology, 49*, 1341–1348.

Henke, H. (1995). Mechanism of separation on Sephadex LH-20. In *Preparative gel chromatography on Sephadex LH-20* (pp. 220–283). Heidelberg, Germany: Hüthig.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry, 76*, 69–75.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. (1951). Protein measurement with the folin-phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Ng, T. B., Liu, F., & Wang, Z. T. (2000). Antioxidative activity of natural products from plants. *Life Science, 66*, 709–723.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rimm, E. B., Stampfer, M. J., Ascherio, A., Giovannucci, E., Colditz, G. A., & Willett, W. C. (1993). Vitamin E consumption and the risk of coronary heart disease in men. *New England Journal of Medicine, 328*, 1450–1456.

Shahidi, F., Amarowicz, R., He, Y., & Wettasinghe, M. (1997). Antioxidant activity of phenolic extracts of evening primrose (*Oenothera biennis*): A preliminary study. *Journal of Food Lipids, 4*, 75–86.

Shahidi, F., Janitha, P. K., & Wanasundra, P. D. (1992). Phenolic antioxidants. *Critical Review of Food Science and Nutrition, 32*, 67–103.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Stampfer, M. J., Rimm, E. B., Hennekens, C. H., Manson, J. E., Colditz, G. A., Rosner, B., & Willett, W. C. (1993). Vitamin E consumption and the risk of coronary disease in women. *New England Journal of Medicine, 328*, 1444–1449.

Wada, T., Hayashi, Y., & Shibata, H. (1996). Asiaticusin A and B, novel prenylated phenolics from *Boletinus asiaticus* and *B. paluster* (Boletaceae) fungi. *Bioscience Biotechnology and Biochemistry, 60*, 120–121.

Wettasinghe, M., & Shahidi, F. (1999). Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) Seeds. *Food Chemistry, 63*, 399–414.

Yashikawa, T., Naito, Y., & Kondo, M. (1997). Free radicals and disease. In M. Hiramatsu, T. Yoshikawa, & M. Inoue (Eds.), *Food and free radicals* (pp. 11–19). New York: Plenum Press.

Yun, B. S., Lee, I. K., Kim, J. P., & Yoo, I. D. (2000). Two *p*-terphenyls from mushroom *Paxillus panuoides* with free radical scavenging activity. *Journal of Microbiology and Biotechnology, 10*, 233–237.

Zhang, A., Chan, P. T., Luk, Y. S., Ho, W. K. K., & Chen, Z. Y. (1997). Inhibitory effect of jasmine green tea epicatechin isomers on LDL-oxidation. *Nutritional Biochemistry, 8*, 334–340.