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Original article

Antioxidant activities of extracts from five edible mushrooms using different extractants

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ABSTRACT

Extractions were performed of the total phenolic and flavonoid contents and antioxidant properties of five edible mushroom samples—*Lentinus edodes*, *Volvariella volvacea*, *Pleurotus eous*, *Pleurotus sajor-caju* and *Auricularia auricular*—using three different extractants. Among the three different extractants, 50% (volume per volume; v/v) ethanol was the most suitable for antioxidant extraction from the mushroom samples. The 50% (v/v) ethanolic extract of dried *L. edodes* contained higher total phenolic and flavonoid contents than in the other mushroom extract samples. The antioxidant activities of 50% (v/v) ethanolic extract of dried *L. edodes* showed the strongest 2,2-diphenyl-1-picrylhydrazyl radical-scavenging assay (64.34%) compared to butylated hydroxyanisole (BHA) and α-tocopherol at 500 μg/mL. The ethanolic extract showed a lower reducing power of 0.10 compared to BHA and α-tocopherol at 500 μg/mL. Moreover, the *L. edodes* ethanolic extract also had the highest chelating ability (66.28%) which was lower than for ethylenediaminetetraacetic acid at 500 μg/mL and showed the strongest superoxide radical-scavenging activity (64.17%) compared to BHA and α-tocopherol. Therefore, the 50% (v/v) ethanolic extract of *L. edodes* could be used as a potential natural antioxidative source or as an ingredient in the fish and fishery product industries.

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Introduction

Edible mushrooms (cultivated and wild mushrooms) are widely consumed in many countries and the amount consumed has greatly increased because of their good taste, ease of purchase and attraction as functional foods since they are low in calories, sodium, fat and cholesterol while high in protein, carbohydrate, fiber, vitamins and the important content of essential amino acids (Mattila et al., 2000). In addition to their nutritional value, some edible mushrooms have been found to be medically active in several therapies because they are rich in bioactive compounds that contain a variety of secondary metabolites including phenolic compounds, polyketides, terpenes and steroids (Kues and Liu, 2000). Different bioactive compounds of edible mushrooms are responsible for their antioxidants properties. From many reasons, mushrooms are considered to be a good source of natural antioxidants and seem useful as a natural source of potential antioxidant additives.

This study evaluated the antioxidant activity of five edible mushroom species—*Lentinus edodes* (known as hed-hom in Thai), *Volvariella volvacea* (hed-fang), *Pleurotus eous* (hed-nangfha-bhutan), *Pleurotus sajor-caju* (hed-nangfha) and *Auricularia auricular* (hed-hunu)—that are popular for consumption in Thailand because of their availability all year round and their good taste. Even though there have been many studies on edible mushrooms in many countries, few have reported on the antioxidant activities of these five, edible mushroom species. Yang et al. (2002) who determined the antioxidant properties of *L. edodes* and *P. eous* found high antioxidant activities. In addition, Cheung et al. (2003) reported that the antioxidant activities of the water extract of *L. edodes* showed the most potent radical-scavenging activity in assays consisting of the β-carotene bleaching method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method and inhibition of erythrocyte hemolysis. Caglarirmak (2007) reported that the nutritional value of *L. edodes*, *P. ostraetus* and *P. sajor-caju* included minerals, vitamin C, folic acid, niacin and vitamins B1 and B2. Moreover, *L. edodes* was found to have a high antioxidant

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component which possessed strong antioxidant activity and applications. High levels of crude protein, amino acids (leucine and lysine), cadmium and eritadenine provide cholesterol-lowering properties, inhibitors of HMG-CoA reductase and anti-hypertensive effects (Guillamon et al., 2010). Mishra et al. (2013) reported on the antioxidant activity of *Pleurotus citrinopileatus*, *P. djamor*, *P. flabellatus*, *P. eryngii*, *P. florida*, *Pleurotus ostreatus P. sajor-caju* and *Hypsizygus ulmarius* with their DPPH radical-scavenging activities in the range 13.63–69.67 percent and with chelating activities in the range 60.25–82.7 percent. As previously mentioned, some studies on the antioxidant activities of the same genera of mushrooms reported different results because of the content of bioactive compounds (which are responsible for the antioxidant properties) may vary considerably in edible mushrooms, since the concentrations of these substances are affected by differences in strain, substances, cultivation, developmental stage, age of the fresh mushrooms, storage conditions and the extraction method, especially, the type of solvent extractant (Mishra et al., 2013). Therefore, the objectives of this study were to determine the total phenolic and flavonoid contents of extracts from five edible mushroom species found in Thailand using three different extractants (water, 50% (volume per volume; v/v) ethanol and diethyl ether) and to evaluate the antioxidant activities.

Materials and methods

Materials

Five edible mushroom samples (*L. edodes*, *V. volvacea*, *P. eous*, *P. sajor-caju* and *A. auricula*) at the mature stage (cap opened) were collected from local markets in Thailand. All of the fresh mushroom samples were immediately freeze-dried (ScanVac CoolSafe Pro, Labogene, Lynge, Denmark) and kept at 4 °C in hermetically vacuum-sealed plastic bags until analysis.

Folin-Ciocalteu's phenol reagent was purchased from Sigma-Aldrich, Switzerland.

Butylated hydroxyanisole (BHA), ferrozine, methionine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron(II) chloride and nitroblue tetrazolium (NBT) were purchased from Sigma-Aldrich, USA. Trichloroacetic acid, gallic acid and α-tocopherol were purchased from Sigma-Aldrich, Germany. Ethanol, methanol and diethyl ether were purchased from BDH, USA. All other chemicals were analytical grade and obtained from one of the Sigma-Aldrich suppliers.

Methods

Preparation and extraction of mushrooms

Dried mushroom samples were finely milled to produce mushroom powder. The powder was extracted using three different extractants—water, 50 percent (v/v) ethanol and diethyl ether. For water extraction (WE), powdered samples (10 g) were boiled in water (500 mL) for 30 min and centrifuged at 12,000 revolutions per minute (rpm) for 15 min; then, supernatants were filtered through a Buchner funnel with Whatman No. 4 filter paper and the filtrate was collected. The obtained extract was concentrated under vacuum at 40 °C using a rotary evaporator (Rotavapor R-124; Bucchi Labortechnic; Flawil, Switzerland) and then adding 100 mL of distilled water, mixed well and transferred into a dark plastic bottle and stored at −20 °C until analysis.

For 50 percent (v/v) ethanol extraction (50% EE), each powdered sample (10 g) mixed with 100 mL of 50 percent (v/v) ethanol was shaken at 150 rpm at room temperature for 24 h then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a Buchner funnel with Whatman No.4 filter paper and the filtrate was collected. The residue was re-extracted under the same conditions. The obtained extract was concentrated under vacuum at 40 °C using the rotary evaporator and 50 percent EE (100 mL) was added, mixed well and transferred into a dark plastic bottle and stored at −20 °C until analysis.

For diethyl ether extraction (DE), each powdered sample (10 g) mixed with 100 mL of diethyl ether was shaken at 150 rpm at room temperature for 24 h and then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a Buchner funnel with Whatman No.4 filter paper and the filtrate was collected. The residue was re-extracted under the same conditions. The combined diethyl ether extract was transferred into a dark plastic bottle and concentrated by flushing with 99.995 percent nitrogen gas and stored at −20 °C until analysis. When using a dried diethyl ether extract for analysis, 100 mL of diethyl ether was added and mixed well before analysis.

Determination of total phenolic compounds in mushroom extracts

The total phenolic compounds of mushroom extract were determined according to Turkoglu et al. (2007) with slight modifications. Briefly, the extract (1 mL) in a volumetric flask was diluted with distilled water (46 mL). Folin-Ciocalteu reagent (1 mL) was added and the contents of the flask were mixed thoroughly for 3 min; then, Na_2CO_3 (2% (v/v), 3 mL) was added. The mixture was allowed to stand for 90 min with intermittent shaking at room temperature. The absorbance of each mixture was measured at 760 nm. The concentration of total phenolic compounds was measured by plotting the calibration curve of a gallic acid standard, determined as milligrams of gallic acid equivalents per gram of dried mushroom.

Determination of total flavonoid contents in mushroom extracts

The flavonoid contents of the mushroom extract were measured according to the method of Turkoglu et al. (2007). The extract (1 mL) was diluted with 4.3 mL of 80 percent (v/v) aqueous ethanol containing 0.1 mL of 10 percent (v/v) aluminum nitrate and 0.1 mL of 1 M aqueous potassium acetate and allowed to stand for 40 min at room temperature. The absorbance was determined spectrophotometrically at 415 nm. The total flavonoid contents were measured by plotting the calibration curve of a quercetin standard, determined as milligrams of quercetin equivalents per gram of dried mushroom.

Determination of antioxidant activities in mushroom extracts

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity

The free radical-scavenging activities of mushroom extract were conducted using the method of Devi et al. (2008). Briefly, 3 mL of each mushroom extract with different concentrations (50 mg/mL, 100 mg/mL, 150 mg/mL, 250 mg/mL, 500 mg/mL) were mixed with 1 mL of DPPH (0.1 mM) solution in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature and the absorbance was then measured with a quartz glass cuvette (Hellma; Mullheim, Germany) at 517 nm against a blank using a UV-visible spectrophotometer (Pharma Spec UV-1700; Shimadzu; Kyoto, Japan). A low absorbance of the reaction mixture indicated a high free-radical-scavenging activity. BHA and α-tocopherol were used as positive controls. The capability to scavenge the DPPH radical was calculated using Equation (1):

$$\text{DPPH scavenging effect (\%)} = (A_{blank} - A_{sample}) / A_{blank} \times 100 \quad (1)$$

where A_{blank} and A_{sample} are the absorbance of the control reaction (containing all reagents except the test extract) and the absorbance of the test extract, respectively.

Reducing power

The reducing power of mushroom extract was determined according to the modified method of Barros et al. (2008). Various concentrations (50 mg/mL, 100 mg/mL, 150 mg/mL, 250 mg/mL, 500 mg/mL) of mushroom extract (2.5 mL) were mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1 percent (v/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and 2.5 mL of 10 percent (v/v) trichloroacetic acid was added to the mixture and centrifuged at 1000 rpm for 8 min. The upper layer of solution (5 mL) was mixed with distilled water (5 mL) and 1 mL of 0.1 percent (v/v) ferric chloride ($FeCl_3$). The absorbance of the test extract was measured at 700 nm; a higher absorbance indicates a higher reductive capability. BHA and α-tocopherol were used as the positive controls.

Superoxide anion radical-scavenging activity

Superoxide radicals of mushroom extract were determined according to Elmastasa et al. (2007). Each extract (1 mL) with different concentrations (50 mg/mL, 100 mg/mL, 150 mg/mL, 250 mg/mL, 500 mg/mL) was mixed with 1 mL of phosphate buffer (0.05 M; pH 7.8), riboflavin (1 mL; 3×10^{-6} M), methionine (1 mL; 1×10^{-2} M) and nitroblue tetrazolium (NBT; 1 mL; 1×10^{-4} M). The photo-induced reactions were performed in an aluminum, foil-lined box with two fluorescent lamps (20 W) and the distance between the reactant and the lamps was adjusted until the intensity of illumination reached 4000 lx and the reactant was illuminated at 25 °C for 25 min. The photochemically reduced riboflavins generated O_2^-, which reduced NBT to form blue formazan. The absorbance of the reaction mixture was measured at 560 nm. BHA and α-tocopherol were used as positive controls. The degree of scavenging was calculated using Equation (2):

$$\% \text{ Scavenging} = (A_{control} - A_{sample}) / A_{control} \times 100 \quad (2)$$

where $A_{control}$ and A_{sample} are the absorbance of un-illuminated reaction mixture and the absorbance of mushroom extract added with reaction mixture, respectively.

Chelating effect on ferrous ions

The chelating effect on the ferrous ions of the mushroom extract was estimated using the method of Yaltirak et al. (2009). Each extract (1 mL) with different concentrations (50 mg/mL, 100 mg/mL, 150 mg/mL, 250 mg/mL, 500 mg/mL) was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine. The mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the mixture was measured spectrophotometrically at 562 nm against a blank; ethylenediaminetetraacetic acid (EDTA) was used as the positive control. The results were expressed as the percentage of inhibition of the ferrozine-Fe^{2+} complex formation which was calculated using Equation (3):

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/A_{control} \times 100 \quad (3)$$

where $A_{control}$ and A_{sample} are the absorbance of the ferrozine-Fe^{2+} complex and the absorbance of test extract, respectively.

Statistical analysis

ANOVA was performed to determine differences in data between samples using Duncan's test at the 0.05 level of significance.

Results and discussion

Total phenolic compounds

Phenolic compounds are a large group of secondary plant metabolites which play a major role in the protection of oxidation processes (Croft, 1999). Phenolic compounds have antioxidant properties and can act as free radical scavengers, hydrogen donators and singlet oxygen quenchers (Croft, 1999). Numerous studies have conclusively demonstrated that mushrooms also contain many phenolics which are important plant constituents because of their scavenging ability (Elmastasa et al., 2007; Turkoglu et al., 2007). In addition, phenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory and anti-hyperglycemic (Liu et al., 2012). The contents of total phenolic compounds in the mushroom extracts are shown in Table 1; with the results expressed as milligrams of gallic acid equivalents per gram of dried mushroom (mg GAE/g dw).

The results indicated that the highest amount of total phenolic compounds were found in most mushroom extracts when using WE except for *V. volvacea* where the highest amount was determined using 50 percent EE. In addition, using WE with *L. edodes* presented the highest contents of total phenolics (36.19 mg GAE/g dw) with the five species studied.

These results were quite different from those reported by Fu and Shieh (2002) who indicated that the highest level of total phenolic contents found using 95 percent EE with *V. volvacea* was 0.73 mg/g and *L. edodes* showed a low total phenolic contents of 0.49 mg/g Cheung et al. (2003) reported that the methanolic extract of *V. volvacea* and *L. edodes* showed higher total phenolic contents (15.0 mg GAE/g dw and 4.79 mg GAE/g dw, respectively) than did WE with these two types of mushroom. The results show that the total phenolic compounds found in the five mushroom

Table 1
Total phenolic compounds and total flavonoid contents of five edible mushrooms using three different extractants.[a]

Type of mushrooms	Total phenolic compounds (mg GAE/g dw)			Total flavonoid contents (mg QE/g dw)		
	Water	50% (v/v) Ethanol	Diethyl ether	Water	50% (v/v) Ethanol	Diethyl ether
Lentinus edodes	$36.19 \pm 0.59^{A,a}$	$24.25 \pm 0.49^{B,b}$	$10.46 \pm 1.71^{A,c}$	$3.75 \pm 0.28^{B,a}$	$1.64 \pm 0.36^{C,b}$	$0.58 \pm 0.19^{AB,c}$
Volvariella volvacea	$22.97 \pm 0.29^{B,b}$	$27.89 \pm 0.23^{A,a}$	$1.99 \pm 1.08^{E,c}$	$7.29 \pm 0.21^{A,b}$	$9.05 \pm 0.89^{A,a}$	$0.84 \pm 0.44^{AB,c}$
Pleurotus eous	$20.31 \pm 0.56^{C,a}$	$14.03 \pm 0.74^{C,b}$	$8.63 \pm 0.86^{C,c}$	$2.61 \pm 1.11^{C,a}$	$1.51 \pm 0.98^{C,b}$	$0.94 \pm 0.62^{A,c}$
Pleurotus sajor-caju	$16.46 \pm 0.67^{D,a}$	$12.34 \pm 0.49^{D,b}$	$9.39 \pm 0.99^{B,c}$	$2.29 \pm 0.99^{D,a}$	$1.06 \pm 0.21^{D,b}$	$0.24 \pm 0.13^{C,c}$
Auricularia auricula	$2.90 \pm 0.40^{E,a}$	$2.75 \pm 0.05^{E,a}$	$2.17 \pm 0.40^{D,b}$	$1.62 \pm 0.68^{E,b}$	$3.13 \pm 1.51^{B,a}$	$0.83 \pm 1.05^{AB,c}$

v/v = volume per volume.
[a] Data are expressed as means ± S.D. of triplicate measurements. Means with different upper case letters (A–E) in the same column are significantly different ($p < 0.05$). Means with different lower case letters (a–c) in the same row are significantly different ($p < 0.05$).

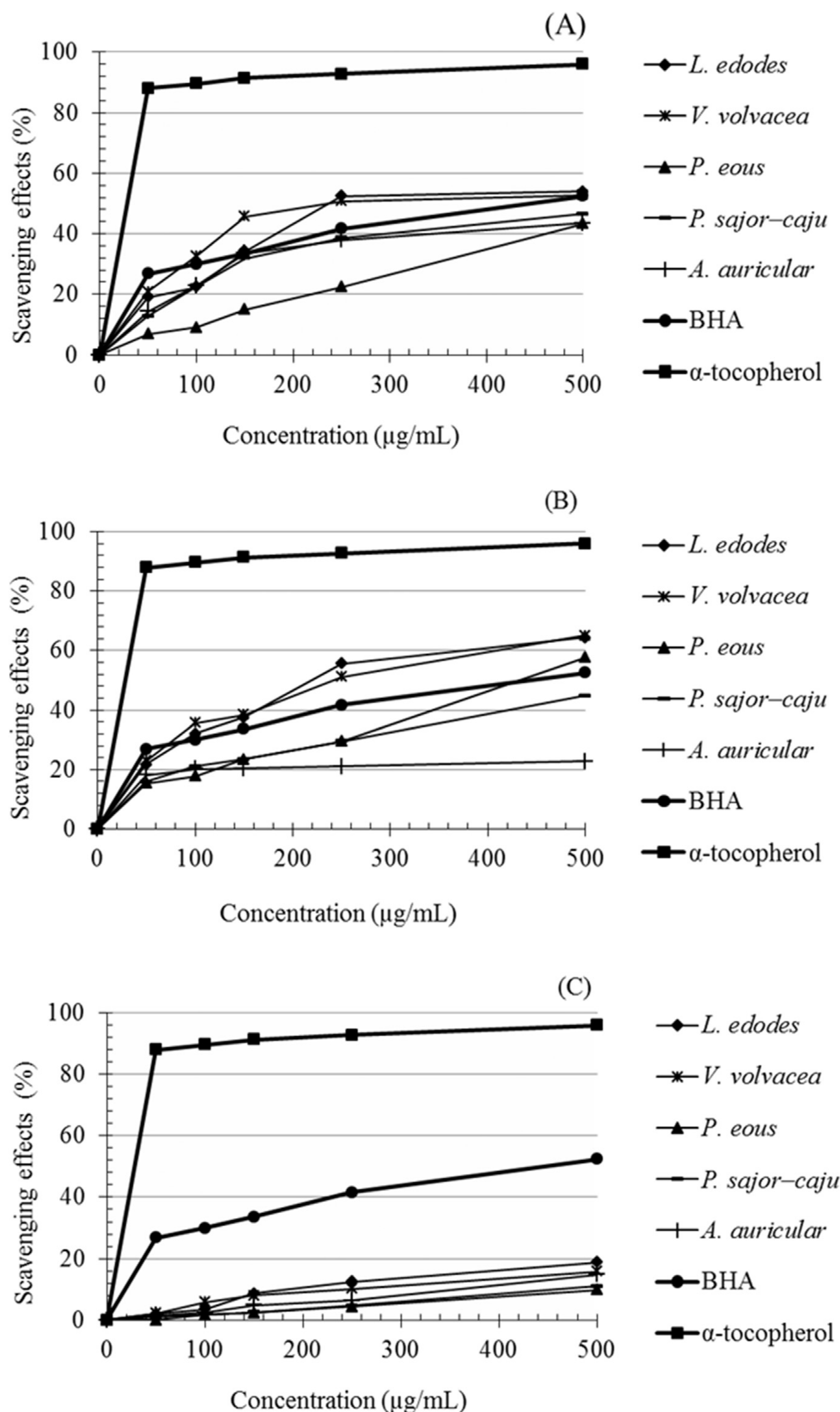

Fig. 1. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity of extracts from five edible mushrooms using: (A) water; (B) 50% volume per volume ethanol; (C) diethyl ether; compared to the positive control of butylated hydroxyanisole (BHA) and α-tocopherol.

extracts included a high polarity compound because when using WE they gave the highest amount in accord with the study by Vaskovsky et al. (1998) who reported that mushrooms contain more polar constituents which dissolved with more polar extractant. The higher contents of total phenolics found in the mushroom extracts could be considered indicative of higher antioxidant ability.

Total flavonoid content

Flavonoids are naturally occurring phenolic compounds in plants that represent the most common and widely distributed group of plant phenolics and the major flavonoid classes include anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol and isoflavones (Morel et al., 1994). Flavonoids have

Table 2
Reducing power of extracts from five edible mushrooms using three different extractants.[a]

Extractant	Type of mushroom	Absorbance of reducing ability (700 nm)				
		Concentration (μg/mL)				
		50	100	150	250	500
Water	*L. edodes*	0.03 ± 0.00^{De}	0.04 ± 0.00^{Fd}	0.06 ± 0.00^{Ec}	0.11 ± 0.00^{Db}	0.18 ± 0.00^{Ea}
	V. volvacea	0.06 ± 0.01^{Cd}	0.07 ± 0.00^{Dd}	0.11 ± 0.00^{Dc}	0.18 ± 0.01^{Cb}	0.28 ± 0.00^{Da}
	P. eous	0.02 ± 0.00^{Ed}	0.03 ± 0.00^{Gd}	0.03 ± 0.00^{Gc}	0.05 ± 0.00^{Fb}	0.10 ± 0.00^{Fa}
	P. sajor-caju	0.04 ± 0.00^{De}	0.07 ± 0.01^{Cd}	0.11 ± 0.00^{Cc}	0.18 ± 0.00^{Cb}	0.37 ± 0.00^{Ca}
Positive control	*A. auricula*	0.02 ± 0.00^{Ed}	0.04 ± 0.00^{Ed}	0.06 ± 0.00^{Fc}	0.09 ± 0.00^{Eb}	0.17 ± 0.00^{Ea}
	BHA	0.27 ± 0.00^{Ae}	0.51 ± 0.00^{Ad}	0.66 ± 0.00^{Ac}	0.71 ± 0.00^{Ab}	0.86 ± 0.00^{Ba}
	α-tocopherol	0.12 ± 0.00^{Be}	0.28 ± 0.00^{Bd}	0.38 ± 0.00^{Bc}	0.66 ± 0.00^{Bb}	1.12 ± 0.00^{Aa}
50% (v/v) ethanol	*L. edodes*	0.01 ± 0.00^{Ce}	0.02 ± 0.00^{Dd}	0.04 ± 0.00^{Cc}	0.06 ± 0.00^{Db}	0.10 ± 0.00^{Ea}
	V. volvacea	0.02 ± 0.00^{Ce}	0.03 ± 0.00^{Cd}	0.05 ± 0.00^{Cc}	0.09 ± 0.00^{Cb}	0.18 ± 0.00^{Ca}
	P. eous	0.01 ± 0.00^{Cd}	0.01 ± 0.00^{Dd}	0.02 ± 0.00^{Dc}	0.05 ± 0.00^{Db}	0.11 ± 0.00^{Da}
	P. sajor-caju	0.02 ± 0.00^{Cd}	0.03 ± 0.00^{Cd}	0.05 ± 0.00^{Cc}	0.10 ± 0.01^{Cb}	0.15 ± 0.00^{Da}
Positive control	*A. auricula*	0.01 ± 0.00^{Cc}	0.02 ± 0.00^{Dc}	0.04 ± 0.00^{Cc}	0.06 ± 0.00^{Db}	0.10 ± 0.00^{Ea}
	BHA	0.27 ± 0.00^{Ae}	0.51 ± 0.00^{Ad}	0.66 ± 0.00^{Ac}	0.71 ± 0.00^{Ab}	0.86 ± 0.00^{Ba}
	α-tocopherol	0.12 ± 0.00^{Be}	0.28 ± 0.00^{Bd}	0.38 ± 0.00^{Bc}	0.66 ± 0.00^{Bb}	1.12 ± 0.00^{Aa}
Diethyl ether	*L. edodes*	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Cd}	0.01 ± 0.00^{Dc}	0.03 ± 0.00^{Db}	0.05 ± 0.00^{Da}
	V. volvacea	0.00 ± 0.00^{Cb}	0.01 ± 0.00^{Cb}	0.02 ± 0.00^{Db}	0.04 ± 0.00^{Ca}	0.09 ± 0.00^{Ca}
	P. eous	0.00 ± 0.00^{Cd}	0.00 ± 0.00^{Cc}	0.01 ± 0.00^{Dc}	0.02 ± 0.00^{Db}	0.05 ± 0.01^{Da}
	P. sajor-caju	0.00 ± 0.00^{Ce}	0.01 ± 0.01^{Cd}	0.03 ± 0.00^{Cc}	0.05 ± 0.00^{Cb}	0.09 ± 0.01^{Ca}
Positive control	*A. auricula*	0.00 ± 0.01^{Ce}	0.01 ± 0.01^{Cd}	0.02 ± 0.00^{Dc}	0.03 ± 0.00^{Db}	0.05 ± 0.01^{Da}
	BHA	0.27 ± 0.00^{Ae}	0.51 ± 0.00^{Ad}	0.66 ± 0.00^{Ac}	0.71 ± 0.00^{Ab}	0.86 ± 0.00^{Ba}
	α-tocopherol	0.12 ± 0.00^{Be}	0.28 ± 0.00^{Bd}	0.38 ± 0.00^{Bc}	0.66 ± 0.00^{Bb}	1.12 ± 0.00^{Aa}

v/v = volume per volume.
[a] Data are expressed as mean ± S.D. of triplicate measurements. Means with different upper case letters (A–E) in the same column are significantly different ($p < 0.05$). Means with different lower case letters (a–c) in same row are significantly different ($p < 0.05$).

been known to possess strong antioxidant properties in order to inhibit lipid peroxidation, to scavenge free radicals and to chelate ferrous ions (Morel et al., 1994). The total flavonoid contents of the studied mushrooms are shown in Table 1; the data were calculated as milligrams of quercetin equivalents per gram of dried mushroom (mg QE/g dw). The total flavonoid contents varied in the ranges 1.62–7.29 mg QE/g dw, 1.06–9.05 mg QE/g dw and 0.24–0.94 mg QE/g dw for WE, 50 percent EE and DE, respectively. The results indicated that the total flavonoid contents were greatest in *V. volvacea* when using WE and 50 percent EE (7.29 mg QE/g dw and 9.05 mg QE/g dw, respectively) but DE presented the highest content in *P. eous* with 0.94 mg QE/g dw.

The results indicated that the total flavonoid content varies depending on the mushroom species. The results were similar to those reported by Palacios et al. (2011) who indicated that *Lactarius deliciosus* had a higher amount of total flavonoids among eight edible mushrooms. Furthermore, a previous study by Barros et al. (2008) also reported different values of the total flavonoid contents in five edible mushroom species of which *Agaricus sivaticus* had a higher content than the other species. In addition, the content of total flavonoid extracted varies depending on the type of extractant, as polar extractants can dissolve more flavonoid contents. The results showed that WE, 50 percent EE and DE with *L. edodes* extracts presented significantly different ($p < 0.05$) total flavonoid contents with 3.75 mg QE/g dw, 1.64 mg QE/g dw and 0.58 mg QE/g dw, respectively. The results were similar to those reported by Jayakumar et al. (2009) where using EE with *Pleurotus ostreatus* presented different total flavonoid contents when compared with the methanolic extract of *P. ostreatus* which was reported by Palacios et al. (2011). However, the methanolic extract of *Agaricus bisporus* (Palacios et al., 2011) showed a similar content of total flavonoids when compared with the methanolic extract of *A. bisporus* (Barros et al., 2008).

Antioxidant activities in mushroom extracts

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity

DPPH is a stable, free radical and accepts an electron or hydrogen radical to become a stable, diamagnetic molecule, produces a purple solution in methanol and becomes pale when it reacts with antioxidant molecules and the DPPH radical which results in the scavenging of the radical by hydrogen donation (Elmastasa et al., 2007). A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract. The results of the DPPH radical-scavenging activity of the five edible mushroom extracts, BHA and α-tocopherol were depicted as the percentage increase with the increased concentrations, as shown in Fig. 1. The results indicated that *L. edodes* presented the strongest DPPH radical-scavenging activity (53.90%, 64.34% and 18.79% at 500 μg/mL for WE, 50% EE and DE, respectively) for all extractants when compared to the other mushrooms studied. However, compared to the positive control, 50 percent EE with *L. edodes* had lower activity than α-tocopherol (95.99%) but was still higher than BHA (52.44%) at 500 μg/mL.

Strong DPPH radical-scavenging activity was shown in the mushroom extracts which contained high total phenolic and flavonoid contents as these had a high hydrogen-donating capacity to scavenge DPPH radicals. The 50 percent EE with *L. edodes* and *V. volvacea* produced the highest DPPH radical-scavenging activity and though not significant ($p > 0.05$) they were found to contain high levels of total phenolic compounds and total flavonoid contents which in turn lead to strong DPPH radical-scavenging activity due to the ability to donate electrons to scavenge DPPH radicals. This could be explained by the DPPH radical-scavenging activity being related to the content of total phenolic compounds and total flavonoid contents found in the extracts. The current study had similar results to those reported by Cheung et al. (2003) who found that at a concentration of 9 mg/mL, WE with *L. edodes* presented higher DPPH radical-scavenging

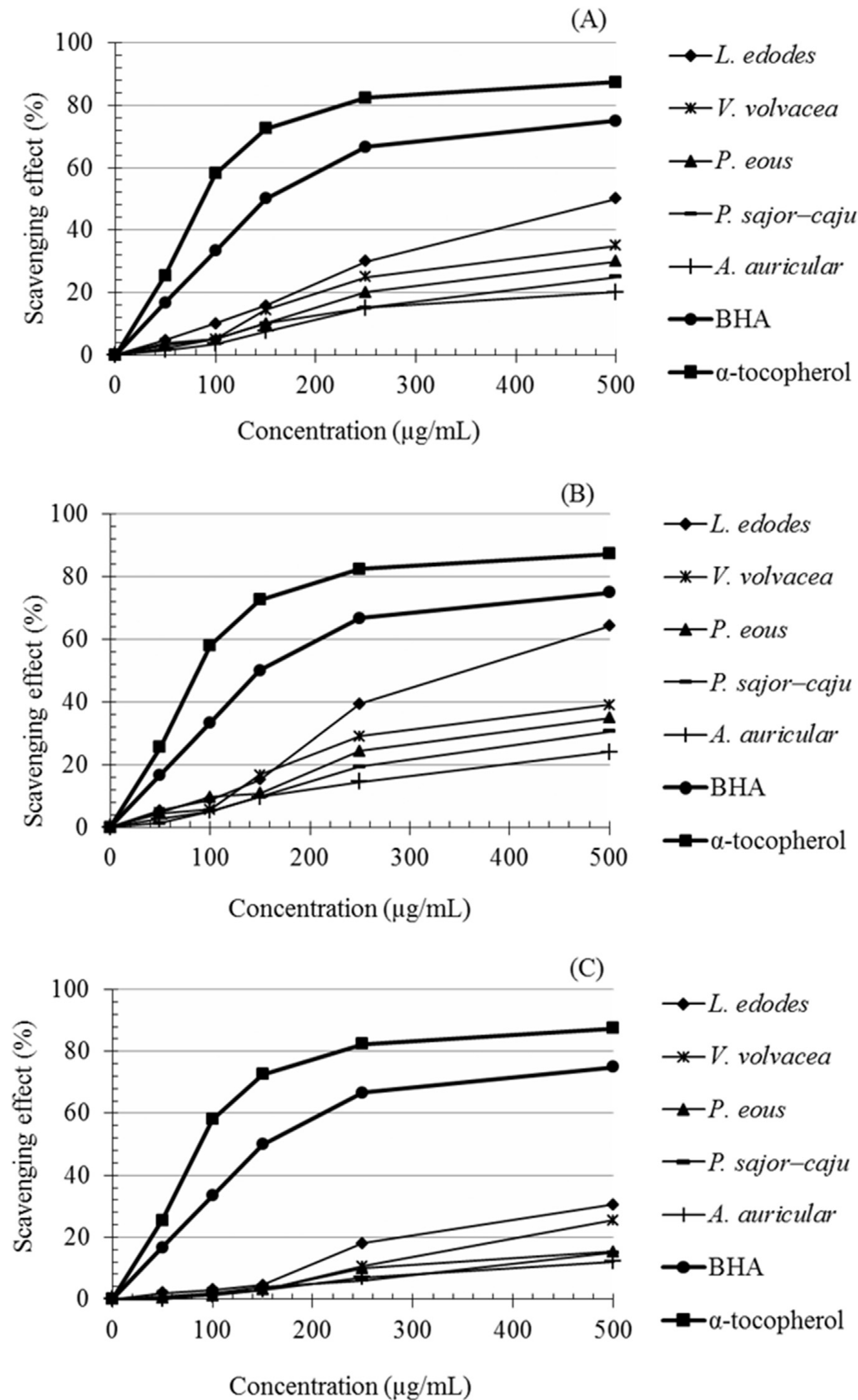

Fig. 2. Superoxide radical-scavenging activity of extracts from five edible mushrooms using (A) water; (B) 50% volume per volume ethanol; (C) diethyl ether; compared to positive control of butylated hydroxyanisole (BHA) and α-tocopherol.

activity (40.4%) than the WE with *V. volvacea* (37.9%). However, the current study had different results to those reported by Lv et al. (2009) where the extract of *L. edodes* presented lower DPPH radical-scavenging activity than the extract of *A. bisporus* for all extractants (water, 70% ethanol, acetone). In addition, Carneiro et al. (2013) also reported that a methanolic extract with *L. edodes* showed lower DPPH radical-scavenging activity ($EC_{50} = 26.32$ mg/mL) than *Agaricus blazei* ($EC_{50} = 6.77$ mg/mL).

Reducing power

The reducing power assay is based on the ability of compounds in the mushroom extracts to reduce the yellow ferric form of iron to the blue ferrous form by donating an electron. A higher absorbance indicates a higher reducing power (Gordon, 1990). The reducing power of mushroom extracts was found to be similar for all extractants used, with the values increasing

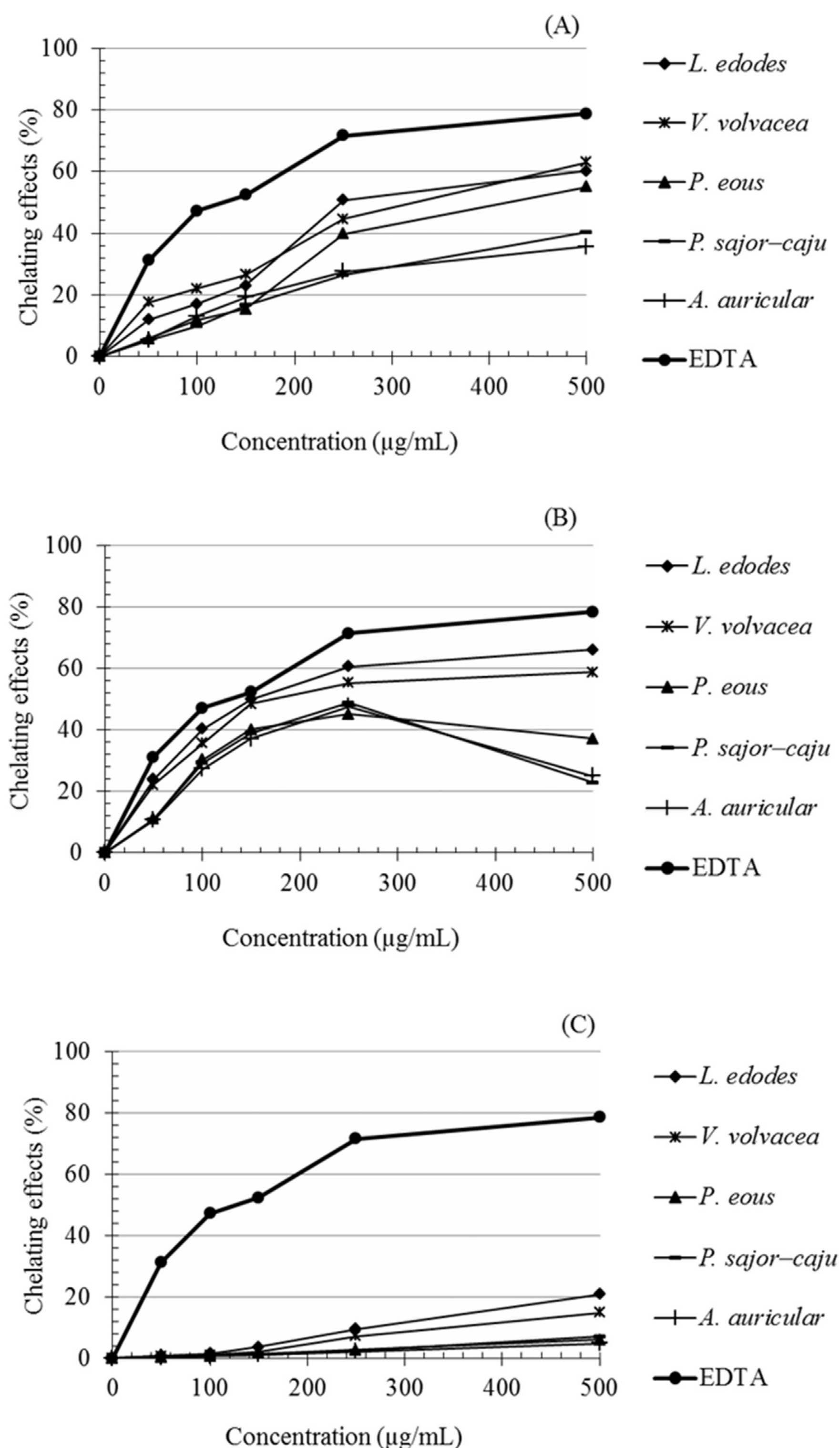

Fig. 3. Chelating effect on ferrous ions of extracts from five edible mushrooms using: (A) water; (B) 50% volume per volume ethanol; (C) diethyl ether; compared to the positive control of ethylenediaminetetraacetic acid (EDTA).

with increasing concentrations of the extracts, but it was lower than with BHA and α-tocopherol (Table 2). With WE, among the studies species of mushroom, *P. sajor-caju* was found to have the highest reducing power (0.37 at 500 μg/mL). EE and DE had quite low reducing power for all mushroom extracts compared to water extraction.

The high reducing power of mushroom extracts might have been due to the high total phenolic and flavonoid contents that break the free radical chain by donating an electron to stabilize and terminate radical chain reactions. The results showed that WE with *P. sajor-caju* had a good source of total phenolic (16.46 mg GAE/g dw) and flavonoid (2.29 mg QE/g dw) contents which led to a high

reducing power of 0.37 at 500 μg/mL. There was no report on the reducing power of *P. sajor-caju*. However, reports by Jayakumar et al. (2009) indicated that EE with *P. ostreatus* resulted in a reducing power of 1.367 which was higher than BHT (1.192) at 10 mg/mL.

Superoxide anion radical-scavenging activity

The superoxide anion radical (O_2^-) is a reactive oxygen species and is one of the various radicals that can contribute to lipid oxidation in biological systems (Gordon, 1990). The scavenging activity of O_2^- is measured using the riboflavin-NBT light system where the photochemically reduced riboflavins generate O_2^- which then reduces NBT, resulting in the formation of blue formazan. When the mushroom extracts were added to the reaction mixture which generated O_2^-, the ability of the compound in the mushroom extracts to scavenge O_2^- thereby inhibited NBT reduction with the formation of blue formazan being inhibited (Halliwell and Gutteridge, 1984). The percentage of scavenging of O_2^- increased with an increasing concentration of the mushroom extracts. The results of the superoxide radical-scavenging activity of the five edible mushroom extracts using different extractants is shown in Fig. 2; *L. edodes* presented the strongest superoxide radical-scavenging activities with 50.00 percent, 64.17 percent and 30.50 percent at 500 μg/mL for WE, 50 percent EE and DE, respectively, which were lower than BHA (75.00%) and α-tocopherol (91.97%).

The strong superoxide radical-scavenging activity could have resulted from the high amount of total phenolic and flavonoid contents found in *L. edodes* compared to other mushroom species that are able to scavenge O_2^- due to their electron-donating properties. A previous study by Jayakumar et al. (2009) supported the results that EE with *P. ostreatus* produced high total phenolic contents and the high contents of the major flavonoids classes included flavonol (rutin) with 31.2 g per 100 g and flavones (chrysin) with 40.0 g per 100 g which also produced strong superoxide radical-scavenging activity (60.02% at 10 mg/mL). Elmastasa et al. (2007) reported that ME with *P. ostreatus* contained little total phenolic contents which was reflected in the low superoxide radical-scavenging activity (87% at 50 μg/mL).

Chelating effect on ferrous ions

Transition metals such as the ferrous ion (Fe^{2+}) are good promoters of free radical reactions because of their single electron transfer during their change in oxidation state, with Fe^{2+} being the most powerful pro-oxidant among various species of metal ions (Jadhav et al., 1996). In addition, transition metals assist the catalytic decomposition of hydroperoxide which appears to be the major source of free radicals. Chelating agents act as secondary antioxidants because they stabilize transition metals in living systems, and are important in retarding the radical degradation and inhibiting the generation of radicals (Gordon, 1990). In the current assay, the chelating activity of the mushroom extracts was determined using a ferrozine assay. Ferrozine can form complexes with Fe^{2+} and the mushroom extracts interfered with the formation of Fe^{2+} and the ferrozine complex, which demonstrated that the mushroom extracts have chelating activity and can capture Fe^{2+} before ferrozine with the result that the red color of the complex is decreased. The rate of red color reduction can be evaluated as the chelating activity of the coexisting chelating agents (Yamaguchi et al., 2000). The results of the chelating effects of five edible mushroom extracts and EDTA on ferrous ions were determined as a percentage that increased with increased concentrations as shown in Fig. 3. The results showed that 50 percent EE with *L. edodes* exhibited the highest chelating effect because this combination had high total phenolic and flavonoid contents which led to strong chelating effects that were 66.28 percent, which was lower than for EDTA (78.64%) at 500 μg/mL.

Jayakumar et al. (2009) supported the current results by reporting that EE with *P. ostreatus* included high amounts of total phenolic compounds and they found high amounts of the major flavonoids classes—flavonol (rutin), 31.2 g per 100 g; and flavones (chrysin) with 40.0 g per 100 g—which also promoted strong chelating effects (60.68% at 10 mg/mL). Elmastasa et al. (2007) reported that the methanolic extract of *P. ostreatus* included little total phenolic content and also had low chelating effects (62.5% at 100 μg/mL). WE gave a similar trend of chelating activity to that of 50 percent EE for all mushroom extracts except *A. auricular* and *P. sajor-caju* and the highest chelating activity was with *V. volvacea*. However, when using DE as an extractant, all mushroom extracts were found to have very low percentage inhibition, and this was lower than with EDTA.

Extractants used to extract five edible mushroom samples and types of mushroom samples clearly influenced their antioxidant constitution which led to divergent antioxidant effects. Among the extractants, 50 percent (v/v) ethanol proved to be the most suitable for antioxidant extraction from the mushroom samples. *L. edodes* demonstrated high levels of antioxidant components including a perceptible amount of total phenolics and flavonoids that possessed strong DPPH radical-scavenging assay, superoxide anion radical-scavenging assay and a chelating effect on ferrous ions. Thus, *L. edodes* could serve as an easily accessible item of food rich in natural antioxidants and the 50 percent (v/v) ethanolic extract of *L. edodes* could probably be used for the development of a food supplement and as an additive or ingredient in the fish and fishery products industries.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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