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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 nitro-blue 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):

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

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