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RESEARCH NOTE

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Antioxidant Activities and Total Phenolics of Ethanol Extracts from Several Edible Mushrooms Produced in Korea

Young Min Choi[1], Ja Bi Ku[1], Hoo Bong Chang[2] and Junsoo Lee[1]*

[1]*Department of Food Science and Technology and Research Center for Bioresource and Health, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea*

[2]*Chungbuk Provincial Rural Development Administration, Chungbuk 363-880, Korea*

Abstract Eight edible mushrooms grown in Korea were extracted with ethanol at room temperature for 24 hr. The extracts were investigated for their antioxidant activities as measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activities. Among the mushroom extracts evaluated in this study, the ethanolic extracts from *Ganoderma lucidum* and *Pleurotus eryngii* showed the greatest potential antioxidant activity, by producing 85 and 88% inhibition in DPPH radical scavenging method and 219 and 165 mg ascorbic acid equivalent antioxidant capacity (AEAC), respectively. Total phenolics and total flavonoids in the ethanolic extracts were determined by spectrophotometric method. Positive correlations were found between total phenolic contents in the extracts and their antioxidant activities, suggesting that phenolic contents in the mushrooms extracts are mainly responsible for their antioxidant activities.

Keywords: antioxidant activities, phenolics, flavonoids, mushrooms

Introduction

The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (1). They are also associated with the activity of free radicals because oxidative damage to DNA, proteins and other macromolecules accumulates with age and has been postulated to be a major type of endogenous damage leading to aging (2). The consumption of plant foods, such as fruits, vegetables, red wines and juices, provides protection against various diseases, including cancer, and cardio and cerebrovascular diseases (3). This protection can be explained by the capacity of antioxidants in the plant foods to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids (4).

Synthetic antioxidants have long been used in foods to prevent and/or delay lipid oxidation. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylated hydroquinone (TBHQ), which are applied in fat and oily foods to prevent oxidative deterioration (5). However, BHA and BHT were found to be anticarcinogenic as well as carcinogenic in experimental animals (6). Therefore, recent studies on the potential applications, for stabilizing foods against oxidation, of natural antioxidants from spices and herbs, have received much attention (7). Phenolics are one of the major groups of non-essential dietary components that have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (8).

*Corresponding author: Tel: 82-43-261-2566; Fax: 82-43-271-4412
E-mail: junsoo@chungbuk.ac.kr
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Mushrooms have long been consumed as a food for their flavor and texture and used as a medicine for their pharmaceutical activities in Korea. Now they are drawing much attention as a functional food as well as an important source of biologically active compounds of medicinal value (9, 10). Furthermore, mushroom phenolic compounds have been found to be an excellent antioxidant (11). Therefore, in this report, the ethanolic extracts of several edible mushrooms produced in Korea were evaluated for their antioxidant activities and their phenolic contents.

Materials and Methods

Extraction Mushroom samples (*Pholiota adiposa, Ganoderma lucidum, Hericium erinaceum, Lyophyllum ulmarium, Lentinula edodes, Grifola frondosa, Pleurotus ostreatus, Pleurotus eryngii*) were donated from Chungbuk Provincial Rural Development Administration. Mushroom powders (10 g) were extracted with 200 mL of 99% ethanol in a shaker for 24 hr. The mixture was further extracted with Polytron® homogenizer for 15 min at medium speed and filtered through filter paper (TOYO, No. 2). The extraction solvents were removed using a rotary evaporator (EYELA, Japan) at 40°C under vacuum and the residues were weighed to calculate the percentage extraction yield obtained from the dry weight divided by the total weight (Table 1). The residue was redissolved into 50 mL of ethanol to be tested.

Measurement of free radical scavenging activity using DPPH radicals The free radical scavenging activity of the ethanolic extracts from mushrooms was analyzed by using 1,1-diphenyl-2-picrilhydrazyl (DPPH) assay (12). Briefly, aliquots (0.2 mL) of the sample extracts were mixed with 0.8 mL of 0.4 mM ethanolic solution of DPPH radical. The absorbance at 520 nm was measured against a

blank of pure ethanol after 10 min of each reaction. All determinations were performed in triplicate. The percent inhibition of the DPPH radical by the sample extracts was calculated by the following formula:

$$\% \text{ inhibition} = \{(A_b - A_s)/A_b\} \times 100$$

where A_b is the absorbance of the blank and A_s is the absorbance of the sample extracts.

Measurement of total antioxidant capacity using ABTS radicals The scavenging activity of the extracts from mushrooms on 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was measured according to the method of Robert *et al.* (13) with some modifications. Briefly, ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution and the mixture was left to stand for overnight in a dark place at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4-1.5 at 414 nm (molar extinction coefficient $\varepsilon = 3.6 \times 10^4$ $mol^{-1}cm^{-1}$) (14). Diluted ABTS radical cation solution (1 mL) was added to 50 μL of extract, ascorbic acid standard solution, or distilled water. After 90 min, the absorbance was measured at 414 nm using a spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). The ABTS radical cation scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as the milligrams of ascorbic acid equivalents per 100 g of sample (15). AEAC was calculated by the following equation:

$$AEAC = (\Delta A_{sample} / \Delta A_{aa}) \times C_{aa} \times V \times (100 / W_{sample})$$

where ΔA_{sample} is the change of absorbance in the presence of the sample extracts, ΔA_{aa} is the change of absorbance after the addition of ascorbic acid standard solution, C_{aa} is the concentration of ascorbic acid standard solution (mg/mL), V is the volume of sample extracts (mL) and W_{sample} is the weight of sample used for extraction (g). All extracts were analyzed in triplicate.

Determination of total phenolic content The total phenolic content in the ethanolic extracts of mushrooms was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method with some modifications (16). The results were calibrated against gallic acid and expressed as mg gallic acid/100 g mushroom.

Determination of total flavonoid content Total flavonoid content in the extracts was determined according to the procedures described by María *et al.* (17). Briefly, 0.1 mL of the sample extracts was mixed with 0.9 mL of 80% aqueous ethanol. An aliquot of 0.5 mL was added to a glass test tube containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1.0 M aqueous potassium acetate and 4.3 mL of 80% ethanol. The tubes were incubated for 40 min at room temperature and then read at 415 nm. Total flavonoid content was calculated using quercetin as the standard.

Results and Discussion

Yield of extraction The study by Cheung and Cheung (18) reported that a relatively higher yield was obtained from two mushrooms, *Lentinus edodes* and *Volvariella volvacea*, when using methanol compared to water as the extraction solvent. In addition, Moure *et al.* (19) reported that the total phenolic content obtained with ethanol was three times higher than that with acetone. Therefore, ethanol was used as an extraction solvent in this study. Ethanol has a similar polarity with methanol, yet is safer and is permitted to be used in the food industry in Korea. Following the extraction with ethanol, the mushrooms gave a yield of 3.9-19.6% (Table 1).

Antioxidant activities Free radical scavenging is one of the well-known mechanisms by which antioxidants inhibit lipid oxidation (11). The methods of scavenging DPPH and ABTS radicals are widely used to evaluate the antioxidant activity of specific compounds or extracts. The scavenging activities of the ethanolic extracts from the mushrooms on DPPH and ABTS radicals are shown in Figs. 1 and 2, respectively. In general, the scavenging activities of the ethanolic from *Ganoderma lucidum* and *Pleurotus eryngii* on DPPH and ABTS radicals were excellent. The scavenging activities of ethanolic extracts from the mushrooms on DPPH and ABTS radicals ranged from 14.8 to 88.1% and from 29.8 to 218.8 mg AEAC per 100 g sample, respectively.

Total phenolic contents in ethanolic mushroom extracts It has been reported that total phenolics were the major naturally occurring antioxidant components found in methanolic extracts from mushrooms (20). Therefore, it is

Table 1. Content of total phenolics and flavonoids in the ethanol extracts of mushrooms and extraction yields[1)]

Mushrooms	Total polyphenolics		Total flavonoids		Yield
	mg/100g sample	mg/g residue	mg/100g sample	mg/g residue	(%)
Pholiota adiposa	60.92	5.82	nd[2)]	nd	10.47
Hericium erinaceum	113.85	5.82	4.91	0.25	19.33
Pleurotus ostreatus	92.28	12.57	4.27	0.58	7.34
Lyophyllum ulmarium	67.40	8.65	6.31	0.81	7.79
Ganoderma lucidum	153.94	39.47	38.03	9.75	3.90
Grifola frondosa	53.90	6.99	36.66	4.75	7.71
Pleurotus eryngii	150.71	21.59	8.20	1.17	6.98
Lentinus edodes	20.21	2.07	3.68	0.38	9.78

[1)]All samples were assayed in triplicate.
[2)]Not detected

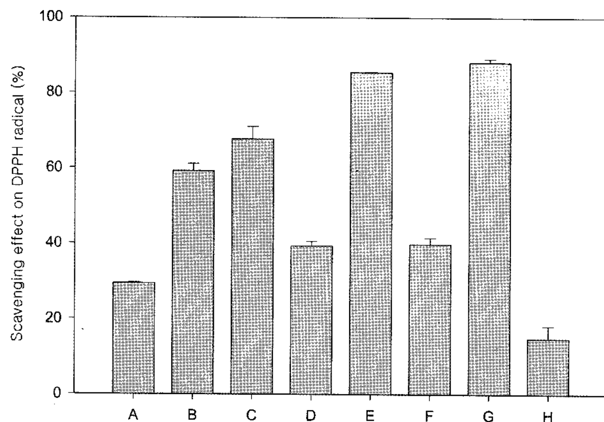

Fig. 1. Scavenging activity of the ethanolic extracts from mushrooms on DPPH radical. Each value represents the mean of triplicate measurements of analyzed samples. (A) *Pholiota adiposa*, (B) *Hericium erinaceum*, (C) *Pleurotus ostreatus*, (D) *Lyophyllum ulmarium*, (E) *Ganoderma lucidum*, (F) *Grifola frondosa*, (G) *Pleurotus eryngii*, (H) *Lentinus edodes*

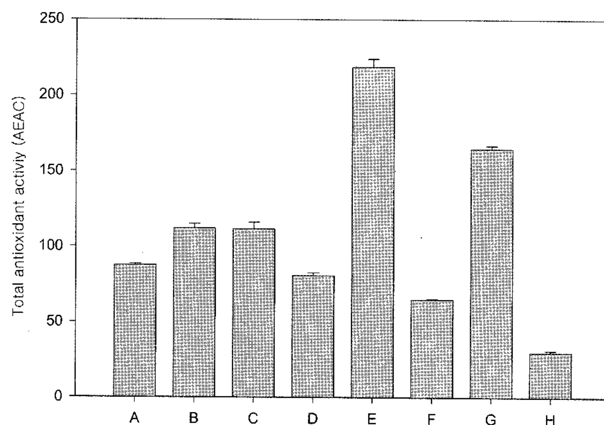

Fig. 2. Total antioxidant activity of the ethanolic extracts from mushrooms on ABTS radical. Each value represents the mean of triplicate measurements of analyzed samples (AEAC: mg ascorbic acid equivalent antioxidant capacity). (A) *Pholiota adiposa*, (B) *Hericium erinaceum*, (C) *Pleurotus ostreatus*, (D) *Lyophyllum ulmarium*, (E) *Ganoderma lucidum*, (F) *Grifola frondosa*, (G) *Pleurotus eryngii*, (H) *Lentinus edodes*

important to quantify the total phenolic contents and to consider their contributions to antioxidant activity. The contents of total phenolics and flavonoids ranged from 20.2 to 153.9 mg per 100 g sample and from 0.0 to 38.0 mg per 100 g sample, respectively. The phenolic contents of the ethanolic extracts were in the order of *Ganoderma lucidum* and *Pleurotus eryngii* > *Hericium erinaceum* > *Pleurotus ostreatus* > *Lyophyllum ulmarium* > *Pholiota adipose* > *Grifola frondosa* > *Lentinus edodes* (Table 1). On the other hand, the order of phenolic contents in extract residue, was *Ganoderma lucidum* > *Pleurotus eryngii* > *Pleurotus ostreatus* > *Lyophyllum ulmarium* > *Grifola frondosa* > *Hericium erinaceum* ≈ *Pholiota adipose* > *Lentinus edodes*. This result may be explained by the different amounts of soluble sugars and sugar alcohols in the extracts other than phenolic compounds (21).

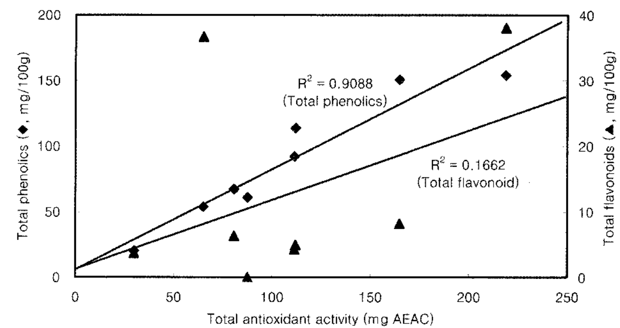

Fig. 3. Linear correlation of total phenolics and flavonoids with respect to total antioxidant activity (mg AEAC) of mushrooms.

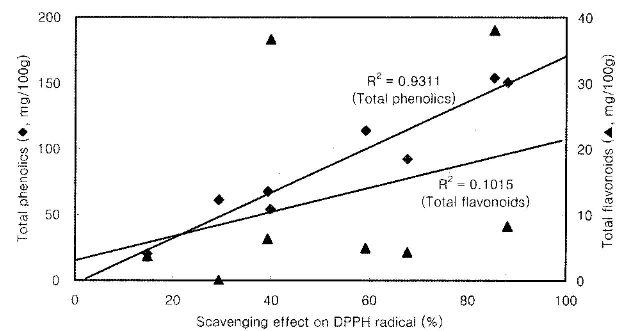

Fig. 4. Linear correlation of total phenolics and flavonoids with respect to scavenging activity on DPPH radicals of mushrooms.

Correlation between antioxidant activities and total phenolics The scavenging activities of ethanolic extracts from the commercial mushrooms on DPPH and ABTS radicals increased with increasing total phenolic content. A stronger and more positive correlation was found between antioxidant activity and total phenolic content than between antioxidant activity and total flavonoid content (Figs. 3 and 4). The higher total phenolic contents in *Ganoderma lucidum* and *Pleurotus eryngii* than in the other mushrooms might be the key component accounting for the better antioxidant activities as measured by DPPH and ABTS. It has been reported that the antioxidant activity of plant material was well correlated with the phenolic compound content (22). Based on the study by Cheung *et al.* (11), the methanol and water extracts from two mushroom varieties (*L. edodes* and *V. volvacea*) were found to have higher antioxidative activities with larger amounts of phenolics than those from ethyl acetate and ether extracts. They suggested scavenging of free radicals, possibly through the hydrogen-donating capacity of phenolics, as a potential mechanism for the antioxidant activity of the mushroom extracts. In conclusion, we have confirmed that the ethanolic extracts of several mushrooms contain potent antioxidant activities, possibly due to their polyphenolic compounds, even though other antioxidants are probably present in the extracts.

Acknowledgments

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