Chemistry of Natural Compounds, Vol. 48, No. 1, March, 2012 [Russian original No. 1, January–February, 2012]

PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITY OF THREE EDIBLE WILD MUSHROOMS FROM TRABZON, TURKEY

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UDC 547.972

Although more than 2000 species of mushrooms exist in nature today, about 25–20 species are widely accepted as food and a few have been evaluated commercially [1, 2]. The biological functions of many wild mushrooms in nature are not known. Today, mushrooms are recognized as a nutritious food as well as an important source of biologically active compounds of medicinal value. Mushrooms include many secondary metabolites, including phenolic compounds, steroids, and terpenes [3–5]. Some wild mushrooms have been found to be medically important for their therapeutic effects such as antitumor [6, 7], antifungal, antiproliferative [8], and free-radical scavenging and antioxidant properties [9]. Turkey, a paradise of wild mushrooms, has suitable conditions for cultivation. In recent years, wild mushrooms are becoming more and more important in our diet for their medicinal benefits. Therefore, our research group intends to go on studying edible mushrooms, documenting their nutraceutical potential and making the information available for better management and conservation of this natural resource and their habitats. In particular, more detailed data on phenolic compositions have not been reported on *C. geotropa*, *P. cornucopiae*, and *P. ostreatus*.

Analysis of phenolic compounds in the mushroom species has been carried out by high-performance liquid chromatography. Most of the phenolic acids have absorption maxima in the UV absorption spectra at wavelengths of 280 and 315 nm and were identified by comparison of retention times (peak normalization, PN). Seventeen phenolic acids were analyzed and fourteen compounds were determined: gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid, benzoic acid, *o*-coumaric acid, abscisic acid, *trans*-cinnamic acid, quercetin, catechin, epicatechin, and rutin, either high or low or not in aqueous extracts of the edible wild mushrooms. Taxonomic findings of the analyzed mushrooms are given in Table 1. HPLC chromatograms of seventeen standards of phenolic compounds include thirteen phenolic acids and four flavonoids. The amounts of the phenolic acids as mg/100 g DW are presented in Table 2. The phenolic chromatograms of the three mushroom species, *C. geotropa*, *P. cornucopiae,* and *P. ostreatus,* are different from each other. Seventeen phenolic compounds were analyzed and about 5–6 individual phenolic compounds were determined in each mushroom. Protocatechuic acid, *trans*-cinnamic acid, and catechin are common among the mushrooms. The three flavonoids quercetin, epicatechin, and rutin were not detected in any of the mushrooms. In addition, the five phenolic acids, gallic acid, chlorogenic acid, caffeic acid, ferulic acid, and benzoic acid were not detected among the samples. Similarly to our results, it was reported [1] that no flavonoids were detected in the sixteen Portuguese wild mushroom samples, but diverse phenolic acids, namely protocatechuic, *p*-hydroxybenzoic, and *p*-coumaric acids, and two vanillic acid isomers, were found. *p*-Hydroxybenzoic acid was the main phenolic acid in *C. geotropa* and was not found in *P. cornucopiae* and *P. ostreatus.* Vanillic acid was found in high concentration only in *P. ostreatus*. However, in another study *p*-hydroxybenzoic acid was found in the majority of the samples, being the most abundant compound in *A. sylvicola* (Vittad.) Lev. (238.7 mg/kg) [1]. The phenolic content and composition of fruits and vegetables depend on genetic and environmental factors as well as postharvest processing conditions. Plant phenolics are the largest class of plant secondary metabolites, which, in many cases, serve as plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [10]. Many studies of plant phenolic compounds have been carried out concerning responses to various stresses [11, 12].

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0009-3130/12/4801-0137 ©2012 Springer Science+Business Media, Inc.

TABLE 1. Taxonomic Findings of Analyzed Mushrooms

Herbarium code	SES 2592	SES 2565	SES 2601
Scientific name	*Clitocybe geotropa* (Bull. ex DC.) Quel.	*Pleurotus cornucopiae* (Paulet) Rolland	*Pleurotus ostreatus* (Jacq.) P. Kumm.
Family	Tricholomataceae	Pleurotaceae	Pleurotaceae
Collection site	Macka-Trabzon (40° 50′ 46.37″ K) (39° 37′ 51.05″ D)	Macka-Trabzon (40° 50′ 54.86″ K) (39° 37′ 32.86″ D)	Sogutlu-Trabzon (41° 00′ 32.15″ K) (39° 36′ 34.74″ D)
Collection date and altitude	08.11.2008 2235 feet	02.11.2008 2574 feet	20.11.2008 155 feet
Habitat	On grass near hornbeam	On stumps and trunks of oak	On stumps and trunks of poplar
Dimensions of the mushroom	Pileus: 15 cm wide Stipe: 12 × 2 cm	Pileus: 10 cm wide Stipe: 15 × 2 cm	Pileus: 15 cm wide Stipe: 10 × 2 cm
Cap color	Pale yellowish buff	Cream-ochre brown	Gray-lilac to violet-brown
Dimensions of the spores	6–7 × 5–6 microns	7–10 × 3–5 microns	6.5–9 × 2.5–3.5 microns
Dimensions of the basidia	42–45 × 7–9 microns	25–35 × 7.5–8 microns	20–35 × 4.5–7 microns

TABLE 2. Identity Peaks of Phenolic Standards in the Chromatogram and Determination of Content of the Mushroom by HPLC (μg/100g DW)*

Compound	RT, min	Relative area, % 280 nm	*Clitocybe geotropa*	*Pleurotus cornucopiae*	*Pleurotus ostreatus*
Protocatechuic acid	5.77	3.842	200.98	640.05	261.4
p-Hydoxybenzoic acid	10.35	4.966	2888.71	Nd.	Nd.
Catechin	12.06	2.124	411.71	763.38	164.9
Vanillic acid	14.13	2.692	Nd.	Nd.	540.7
Syringic acid	16.08	2.474	Nd.	165.48	–
p-Coumaric acid	18.61	0.517	524.46	Nd.	10.4
o-Coumaric acid	22.29	1.687	23.77	Nd.	Nd.
Absisic acid	26.46	2.138	75.61	24.11	–
t-Cinnamic acid	29.53	4.554	1320.26	360.60	21.7

Nd.: not detected.

*Gallic acid, chlorogenic acid, caffeic acid, epicatechin, rutin, ferulic acid, benzoic acid, and quercetin were not detected among the samples.

TABLE 3. Antioxidant Capacity of the Mushrooms

Samples	Total phenolic compounds, mg GAE/g DW	FRAP (μmol Trolox/100 g DW)
Clitocybe geotropa	7.07 ± 0.002	193.54 ± 0.50
Pleurotus cornucopiae	3.32 ± 0.001	117.96 ± 0.86
Pleurotus ostreatus	2.74 ± 0.002	44.11 ± 0.52

In this study, we have also measured antioxidant capacity to evaluate their biological effects. The antioxidant properties of the mushrooms were analyzed in terms of total polyphenols and ferric-reducing/antioxidant power (FRAP). Although several methods have been developed in recent years to determine antioxidant capacity of biological samples, total phenolic content and FRAP methods are used to reflect the total antioxidant capacity of several plant samples [13, 14]. Total phenolic content was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/100 g mushroom. The results of total phenolic content and FRAP activity of methanolic mushroom samples are given in Table 3. Total phenolic contents were found to be 2.74–7.07 mg gallic acid/g DW of methanolic mushroom extract by the Folin–Ciocalteu method. Total phenolic contents of seven mushroom species from the Black Sea region were reported as between 7.7–26.0 mg/g of methanolic extract [9]. Among the three mushroom extracts, the methanolic extract from *C. geotropa* showed the highest phenolic contents. The total phenolic content in Portuguese wild mushrooms from different species was found [3] to be between 33–356 mg/kg. The total phenolic content was reported to be 203 mg/100 g in Chaga mushroom [15].

The reducing power test is commonly used to determine the total antioxidant capacity of plant materials. This procedure involves the reduction of ferric tripyridyltriazine (Fe-(III)-TPTZ) complex to a blue colored Fe (II)-TPTZ by the sample′s antioxidants. The reduction of ferric iron in FRAP reagent will result in the formation of a blue product (ferrous – TPTZ complex) with absorbance at 593 nm. The reducing power measured for the mushroom types showed a concentration-dependent pattern. The increased absorbance is an indication of higher reducing power in this method. Among samples, *C. geotropa* showed higher antioxidant activity than the other two species. A high positive correlation was found between phenolic content and FRAP values of melon samples ($r^2 = 0.92$). This positive correlation matches with the data reported by other researches on mushrooms [1, 9, 17]. Several studies have reported a positive correlation between the content of total phenolic compounds and the antioxidant capacity in other plants [9, 15, 16].

This is the first detailed report concerning the phenolic composition of *C. geotropa*, *P. cornucopiae*, and *P. ostreatus*, edible wild mushrooms collected in Trabzon. They contain a diverse species of phenolic substance. The amount and type of phenolic agents depend on the species of mushrooms, especially *C. geotropa* mushroom whose extracts could be a potential source of natural antioxidant, and the consumption of these mushrooms might serve to protect health and fight against oxidative stress. Actually, mushrooms possess a crucial phenolic compound that will be much more valuable in the future than it is today.

Chemicals and Instrumentation. Standards (purity > 99.0%) were supplied as follows: gallic acid, protocathechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, benzoic acid, *o*-coumaric acid, *trans*-cinnamic acid, absisic acid, catechin, rutin, quercetin, and propylparaben as internal standard (IS) from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Methanol, acetic acid, and acetonitrile were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-*s*-triazine), and Folin–Ciocalteau′s phenol reagent were from Fluka Chemie GmbH (Switzerland). Polytetrafluoroethylene membranes (porosity 0.2 μm) for the filtration of the extracts were obtained from Sartorius (Goettingen, Germany). All phenolic standards were dissolved in HPLC grade methanol at 100 mg/mL concentrations and diluted with 30% methanol (2.5, 5, 10, 20, 25, 50, and 100 mg/mL) to determine calibrations with HPLC. Propylparaben was used as internal standard, whose concentration was adjusted to 10 ppm [2].

HPLC (Shimadzu LC-UV) analysis of phenolic compounds was performed on a reverse-phase Zorbax Eclipse XDB-C18 reverse phase column (150 mm × 4.6 mm i.d., 5 μm particle size; Agilent) using a gradient program with two solvent systems [A: 2% acetic acid in water; B: 80% acetonitrile–water (1:1); at a constant solvent flow rate of 1.0 mL/min (Villers et al., 2004]. Injection volume was 50 μL and column temperature was 30°C. The signals were detected at 280 and 315 nm by UV-VIS detection. An ATI-Unicam UV-2 UV-VIS spectrophotometer (Cambridge, U.K.) was used in all absorbance measurements. All solutions were prepared with deionized water purified in an Elgacan C104 (Elga, England) filtration system.

Samples. Samples of the three different edible wild mushroom species were collected from Trabzon (Turkey) during 2008 (Table 1). In the field, the surrounding vegetation and general properties of the species were noted. After harvesting, the mushrooms were immediately transferred to the biology laboratory. Spore prints were made in order to detect the spore mass, color, and the spores to be used for microscopic measurements. The specimens were dried at room temperature for later analyses and were deposited in a private herbarium (SES) at the Fatih Education Faculty of Karadeniz Technical University. Taxonomic identification was done according to Sesli [18], and dried materials were taken to the biochemistry laboratory for antioxidant activity and phenolic substance analysis.

Preparation of Extracts. About 5–10 g of dried mushroom samples were weighed, and 70 mL methanol was added to each sample; then each mixture was extracted in an ultrasonic bath (Elma® Transsonic Digital, Germany) at 60°C in 3 hours. Solid particles were removed using filter paper, and the filtrate was centrifuged at 4000 × rpm 5 min. The crude methanol extract was hydrolyzed with 2 N HCI at constant temperature (90°C) for 2 h (IKA®-Werke-USA). The acidic mixture was first extracted with 50 mL ethyl acetate and then with 50 mL diethyl ether. The organic solvent was evaporated to dryness under reduced pressure in a rotary evaporator at 40°C. The residue was weighed and dissolved in methanol for HPLC analysis or to test for antioxidant activity.

Determination of Antioxidant Capacity. Total phenolic contents were determined by the Folin–Ciocalteau procedure [19] using gallic acid as standard. Briefly, 0.1 mL of various concentrations of gallic acid and methanolic samples (1 mg/mL) were diluted with 5.0 mL distilled water. Then 0.5 mL of 0.2 N Folin–Ciocalteu reagents was added, and the contents were vortexed. After 3 min incubation, 1.5 mL of Na_2CO_3 (2%) solution was added, and, after vortexing, the mixture was incubated for 2 h at 20°C with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents per g of 100 g DW by using a standard graph.

The antioxidant activity of the samples was determined by FRAP assays. The antioxidant method is based on measurement of the iron-reducing capacities of the mushroom. The working FRAP reagent was prepared as required by mixing 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mmol/L TPTZ solution in 40 mmol/L HCl and 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution [20]. Then 100 μL of the sample was mixed with 3 mL of freshly prepared FRAP reagent.The reaction mixture was incubated at 37°C for 4 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 hour instead of 4 minutes. A calibration curve was plotted using an aqueous solution of ferrous sulfate $FeSO_4 \cdot 7H_2O$ with concentrations in the range 100–1000 μM ($r^2 = 0.98$). The result was compared with Trolox® standard antioxidant, and FRAP values were expressed as wet weight of the mushroom samples based on millimoles of ferrous equivalent Fe (II) per g of sample.

Statistical Analysis. Results are presented as mean values of two replicates. Data were tested using SPSS for Windows Release 10 (version 9.0 for Windows 98, SPSS, Chicago, Illinois, USA). Pearson correlation was used to reveal differences and relations. Differences were considered statistically significant at $p < 0.05$.

ACKNOWLEDGMENT

This study was supported by the Research Fund of Karadeniz Technical University (Project No: 2004.111.002.08).

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