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Chemical Compositions and Antioxidant Activities of 16 Wild Edible Mushroom Species Grown in Anatolia

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Abstract: In this study, chemical compositions and antioxidant activities of 16 wild edible mushrooms (*Agrocybe cylindracea*, *Amanita ceciliae*, *Armillaria mellea*, *Boletus reticulatus*, *Cantharellus cibarius*, *Chlorophyllum rhacodes*, *Coprinus comatus*, *Flammulina velutipes* var. *velutipes*, *Lactarius deliciosus*, *Lactarius salmonicolor*, *Pleurotus ostreatus*, *Polyporus squamosus*, *Rhizopogon roseolus*, *Russula anthracina*, *Suillus collinitus* and *Tricholoma myomyces*) were investigated. Antioxidant properties of methanol extracts were studied by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Among the mushroom extract *Amanita ceciliae* and *Pleurotus ostreatus* (96.16 %) showed the most potent radical scavenging activities at 4.51 and 2.72 mg mL^{-1}, respectively. The lowest scavenging activity was exhibited by *C. rhacodes* (70.46%) at 2.35 mg mL^{-1}.

Key words: Antioxidant activity, chemical content, edible wild mushroom, Anatolia

INTRODUCTION

The members of fungal kingdom are present almost everywhere. They are important for continuation of life because they are able to biodegrade the substrate (Manzi *et al*., 2001). Mushrooms have been used as food and food-flavoring material for centuries (Tsai *et al*., 2007; Shin *et al*., 2007). Mushrooms are widely distributed all over the world and some of them have been used as drug in ethno-medicine. Many edible mushrooms are reputed to possess due to properties of antioxidant, antimicrobial and anticancer (Tambekar *et al*., 2006; Aryantha *et al*., 2010).

Oxidation is essential to aerobic organisms for the production of energy to fuel biological processes. However, the oxygen-derived free radicals is involved in the onset of many diseases such as cancer and atherosclerosis as well as in degenerative processes associated with aging (Turkoglu *et al*., 2007; Thetsrimuang *et al*., 2011). Almost all aerobic organisms are well protected against free radicals by enzymes such as superoxide dismutase or compounds such as tocopherols and glutathione (Elmastas *et al*., 2005; Zongo *et al*., 2010). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing and stress, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant-containing foods may be used to help the human body to protect against oxidative damage (Cazzi *et al*., 1997; Okwulehie *et al*., 2007).

Many species of fruits, vegetables, cereals and seeds have been investigated for antioxidant activity (Halliwell and Gutteridge, 2003). Natural antioxidants are being studied for their capacity to protect cells from damage brought on by reactive oxygen species (Niki *et al*., 1994). Mushrooms accumulate secondary metabolites, including phenolic compounds, terpenes and alkaloids (Buigut, 2002). Mushrooms are appreciated for their chemical and nutritional properties. Mushrooms have also been reported as therapeutic foods that are useful in preventing diseases such as hypertension, hyperglycemia and cancer. These functional characteristics are mainly due to their chemical content (Manzi *et al*., 2001; Chenghom *et al*., 2010). Mushrooms are considered as source of proteins, vitamins, lipids, carbohydrates and minerals (Jiskani, 2001). The essential amino acids, water-soluble vitamins and essential minerals are present (Gulcin *et al*., 2002). Wild edible mushrooms are becoming more and more important in our diet for their pharmacological properties (Halliwell and Gutteridge, 2003).

Although, there are many studies on cultivated and wild mushrooms in the northern hemisphere, there is little information available about antioxidant properties and proximate chemical composition of wild mushrooms collected from different parts of Anatolia. Our objective

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was to evaluate the proximate chemical content and antioxidant activities of methanol extracts of fruit bodies of 16 wild mushrooms by free radical scavenging method.

MATERIALS AND METHODS

Mushrooms: In this study, 16 wild edible mushroom species (*Agrocybe cylindracea* (DC.) Maire, *Amanita ceciliae* (Berk and Broome) Bas, *Armillaria mellea* (Vahl.) P. Kumm., *Boletus reticulatus* Schaeff., *Cantharellus cibarius* Fr., *Chlorophyllum rhacodes* (Vittad.) Vellinga, *Coprinus comatus* (O.F. Müll.) Pers., *Flammulina velutipes* var. *velutipes* (Curtis) Singer, *Lactarius deliciosus* (L.) Gray, *Lactarius salmonicolor* R. Heim and Leclair, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Polyporus squamosus* (Huds.) Fr., *Rhizopogon roseolus* (Corda) Th. Fr., *Russula anthracina* Romagn., *Suillus collinitus* (Fr.) Kuntze and *Tricholoma myomyces* (Pers.) (J.E. Lange) were collected from different parts of Anatolia and were analyzed for their chemical content and antioxidant activities. Origin, fungarium number and families of these macrofungi were given in Table 1. All of the analyzed mushrooms were identified as edible macrofungi belonging to class *Basidiomycetes*. All mushroom samples were deposited in the Ankara University, Department of Biology, Turkey.

Extraction process: A fine dried mushroom sample (1 g) was continuously extracted with methanol in a Soxhlet apparatus for 24 h. The methanolic extract was evaporated to dryness at 45°C and redissolved in methanol and stored at 4°C prior to further use (Barros *et al*., 2007).

Proximate analysis assay: The water amount and total carbohydrates of mushroom samples were determined according to AOAC (2006). Total protein was determined by the Kjeldahl method (AOAC, 2006). Protein was calculated using the general factor of 6.25. The weight of fat extracted from 5 g of mushroom sample was determined to calculate the lipid content. Diethyl ether was used as an extraction solvent where the extraction was performed for 4 h. Two grams of sample, in a porcelain container, was ignited and incinerated in the muffle furnace at about 550°C until a grayish white ash was obtained (AOAC, 2006).

Free-radical scavenging assay: The capacity to scavenge the "stable" free radical DPPH was monitored according to the method of Barros *et al.* (2007). Various concentrations of methanolic extracts from mushrooms (2 mL) were mixed with 2 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol L^{-1}). The mixture was shaken vigorously and left to stand for 30 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The Radical-Scavenging Activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ RSA} = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100$$

where, A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution (Ramkumar *et al*., 2010). Also, extract amounts of the samples were determined and concentrations were calculated. The assays were carried out in triplicate and the results expressed as mean values±standard deviations. Butylated hydroxytoluene (BHT) was used as standard.

Table 1: Geographic distribution of edible mushroom species

Species	Families	Coordinate	Altitude (m)	Fungarium No.
A. cylindracea	Strophariaceae	N 39° 56'-E 32° 49'	860	AKATA 1037
A. ceciliae	Amanitaceae	N 40° 53'-E 39° 50'	850	AKATA 3037
A. mellea	Physalacriaceae	N 40° 35'-E 31° 16'	1420	AKATA 2936
B. reticulatus	Boletaceae	N 41° 03'-E 33° 41'	1880	AKATA 1091
C. cibarius	Cantharellaceae	N 40° 53'-E 39° 50'	850	AKATA 3011
C. rhacodes	Agaricaceae	N 41° 09'-E 33° 50'	1580	AKATA 2005
C. comatus	Agaricaceae	N 39° 56'-E 32° 49'	860	AKATA 2113
F. velutipes	Physalacriaceae	N 39° 56'-E 32° 49'	860	AKATA 2127
L. deliciosus	Russulaceae	N 40° 36'-E 31° 17'	1340	AKATA 2434
L. salmonicolor	Russulaceae	N 40° 36'-E 31° 17'	1350	AKATA 2413
P. ostreatus	Pleurotaceae	N 39° 56'-E 32° 49'	860	AKATA 3093
P. squamosus	Polyporaceae	N 39° 56'-E 32° 49'	860	AKATA 3091
R. roseolus	Rhizopogonaceae	N 40° 35'-E 31° 15'	1530	AKATA 3024
R. anthracina	Russulaceae	N 41° 06'-E 33° 44'	1400	AKATA 1184
S. collinitus	Suillaceae	N 41° 04'-E 33° 44'	1780	AKATA 1068
T myomyces	Tricholomataceae	N 41° 08'-E 33° 50'	1200	AKATA 1561

Statistical analysis: The data presented are the averages of the results of three replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Extraction yields and free radical scavenging activity: The yields of methanol extracts of wild mushrooms are given in Table 2. The methanol extracts of fruit bodies were subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method (Barros *et al*., 2007). Free radical scavenging values of fruit bodies extracts as percentage are shown in Table 2.

Methanol extracts of *P. ostreatus* and *A. mellea* showed the strongest radical scavenging effect (96.16%) at 2.72 and 4.51 mg mL^{-1}, respectively. This activity was followed by *A. cylindracea* (95.79%) and *C. cibarius* (95.64%), respectively (Table 2). The lowest scavenging activity was exhibited by *C. rhacodes* (70.46%). However, the scavenging effect for BHT was 98.24% at 3.0 mg mL^{-1}.

In previous studies, the antioxidant activities of methanolic extracts of several commercial and medicinal mushrooms have been reported (Yang *et al*., 2002; Mau *et al*., 2004). Those studies claimed that the methanolic extracts of mushroom species showed high antioxidant activity on the lipid peroxidation.

Barros *et al*. (2007) found that methanolic extracts of *Leucopaxillus giganteus*, *Sarcodon imbricatus* and *Agaricus arvensis* scavenged 100.00, 80.00 and 68.30% of DPPH radicals at 5.0 mg mL^{-1}, respectively. At 1.50 mg mL^{-1}, the methanolic extracts of *Boletus edulis*, *Xerocomus chrysenteron*, *Suillus collinitus* and *Lactarius deterrimus* scavenged 94.66, 89.61, 88.27 and 27.73%, respectively (Sarikurkcu *et al*., 2008). In present study, scavenging activity of *S. collinitus* was determined at 71.94% at 2.91 mg mL^{-1}. According to Gezer *et al*. (2006), the scavenging effect of *Ramaria flava* was 73.30%.

Table 2: Extraction yields and antioxidant activity values of wild edible mushrooms

Species	Extract concentration (mg mL^{-1})	Extraction yields (%)	RSA (%)
Polyporus squamosus	3.46	17.3	95.35±0.10
Pleurotus ostreatus	2.72	13.6	96.16±0.42
Amanita ceciliae	4.65	23.2	95.35±0.10
Lactarius salmonicolor	2.90	14.5	94.17±0.31
F. velutipes var. *velutipes*	4.45	22.2	95.27±0.00
Russula anthracina	2.31	11.5	90.62±0.73
Agrocybe cylindracea	2.82	14.1	95.79±0.10
Boletus reticulatus	3.17	15.8	94.83±0.84
Tricholoma myomyces	2.97	14.8	84.93±0.42
Cantharellus cibarius	3.34	16.7	95.64±0.10
Armillaria mellea	4.51	22.5	96.16±0.00
Suillus collinitus	2.91	14.5	71.94±1.04
Lactarius deliciosus	2.56	12.7	92.02±0.21
Rhizopogon roseolus	2.61	13.0	88.11±0.52
Coprinus comatus	5.09	25.4	94.61±0.10
Chlorophyllum rhacodes	2.35	11.7	70.46±0.21
BHT	3.00		98.24

RSA: Radical scavenging activity

Gaafar *et al*. (2010) reported that *Pleurotus ostreatus* can improve the antioxidant status during ageing and minimize the occurrence of age-associated disorders associated with involvement of free radicals. Total lipids, triglycerides and total cholesterol reduced in rats supplemented with 10% dried *P. ostreatus* at 6.85%, 34.00% and 19.13%, respectively. Also, some liver enzymes values [Aspartate Amino Transferase (AST), alanine amino transferase (ALT) and Alkaline Phosphatase (AP)] of aging rats decreased at 37.78, 35.57 and 19.55% with 10% dried *P. ostreatus* (Gaafar *et al*., 2010).

Yang *et al*. (2007) found that Am-1 which is one of the saccharides of *A. mellea* has antioxidant property. Am-1 is a glucopyranose (containing glucuronic acid) and mainly linked by β (1-3) and β (1-6) glucosidic linkage (Yang *et al*., 2007). The carbohydrate content of mushrooms represents the bulk of fruiting bodies accounting for 30 to 65% on dry weight basis. The mannitol, also called as mushroom sugar constitutes about 80% of the total free sugars, hence it is dominant. Water soluble polysaccharides of mushrooms are antitumor and antioxidant (Wani *et al*., 2010).

According to Fu and Shieh (2002), *F. velutipes* has total phenolics at 0.75 mg g^{-1}. Free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipid oxidation. The antioxidative activity of phenolics is generally governed by their chemical structures, the activity increases with increasing the number of hydroxyl groups and their location in the molecules involved. *F. velutipes* has tyrosine a phenolic amino acid at 7.85% (Ko *et al*., 1995). Thus, another possibility for the antioxidant activity may be attributed to the presence of small amounts of vitamin C in the mushrooms. *F. velutipes* has ascorbic acid at 46 mg/100 g dry matter (Fu and Shieh, 2002).

Proximate analysis assay: Proximate analysis was carried out on 16 wild edible mushroom species. Results of proximate composition are presented in Table 3. *L. salmonicolor* had the highest concentration of protein (46.81%) followed by *T. myomyces* and *S. collinitus* while *A. cylindracea* had the least (13.32%). With respect to moisture content, *A. cylindracea* had the highest value (10.32%) and *C. rhacodes* the least value (7.24%). *B. reticulatus* had the highest carbohydrate (67.18%) and ash was highest in *L. deliciosus* (15.46%). The ether extract (fat) values are between 1.00% (*B. reticulatus*) and 13.32% (*P. squamosus*) (Table 3).

The analytical food value as approximate indices of nutritional quality, it would appear that some of these

Table 3: Proximate composition (%) of 16 wild edible mushrooms

Species	Ash	Fat	Moisture	Protein	Carbohydrate
Polyporus squamosus	7.14	3.98	10.32	13.32	65.24
Pleurotus ostreatus	7.25	3.21	10.31	16.96	62.27
Amanita ceciliae	10.75	5.91	9.34	31.20	42.81
Lactarius salmonicolor	5.64	1.00	8.48	17.70	67.18
F. velutipes var. *velutipes*	10.40	7.33	9.84	22.04	50.40
Russula anthracina	7.71	9.46	7.24	23.66	51.93
Agrocybe cylindracea	7.25	1.50	9.26	18.83	63.16
Boletus reticulatus	15.29	2.89	8.01	36.67	37.14
Tricholoma myomyces	15.46	3.29	9.03	18.40	53.83
Cantharellus cibarius	5.88	1.35	8.43	46.81	37.53
Armillaria mellea	11.24	5.97	8.94	22.44	51.42
Suillus collinitus	10.49	13.32	9.70	20.33	46.17
Lactarius deliciosus	6.58	7.38	7.93	26.57	51.54
Rhizopogon roseolus	9.37	4.31	8.96	20.55	56.81
Coprinus comatus	14.36	4.90	9.59	38.06	33.10
Chlorophyllum rhacodes	3.37	8.17	9.39	46.77	32.30

mushrooms fall between most legumes and meat. In earlier studies, Gruen and Wong (1982) indicated that edible macrofungi were highly nutritional and compared favorably with meat, egg, legumes and milk. Some of the mushrooms are known to possess anticancer and hypocholesterolaemic agents which implies that mushrooms could hold special attraction for and may be recommended for people with high cholesterol ailments.

The protein contents of the mushrooms were close to those reported by Aletor (1995) in which the author obtained for *Termitomyces robustus* (33.80%), *Psathyrella atroumbonata* (32.80%) and *Schizophyllum commune* (27.00%). The author reported 13.90% ash contents for *T. robustus*. Also, Adejumo and Awosanya (2005) reported that 36.80% protein content for *Termitomyces mammiformis* and 22.80% for *Russula vesca*. In the same study, reported 70.90% carbohydrate content for *R. vesca*.

The protein contents of the mushrooms analyzed in this study were lower than those obtained in the previous study Kalyoncu *et al*. (2010) 83.40% for *Sparassis crispa* and 75.56% for *Meripilus giganteus*. Ash contents of these mushrooms were the same with mushrooms in presented study. In generally, lipid contents of mushrooms are low but may contribute towards palatability.

CONCLUSIONS

Antioxidants are chemical compounds that protect cells from the damage caused by unstable molecules known as Reactive Oxygen Species (ROS) or free radicals. ROS are powerful oxidants and those chemical entities that contain unpaired electrons. They are capable of randomly damaging cells, viz. lipids, proteins, DNA, sugars and are involved in mutations and cancers (Wani *et al*., 2010). The antioxidants are an important defense of the body against ROS and mushrooms which are rich sources of antioxidants (Mau *et al*., 2004).

Antioxidant properties of edible mushrooms are related to low-molecular weight compounds, in particular to the phenolic fractions. Therefore, a wide range of these beneficial phenolic compounds could be natural substrates of oxidative enzymes, such as peroxidases which are present in high levels in mushrooms (Gursoy *et al*., 2009).

On the basis of the results it is suggested that the extract of wild mushroom species evaluated here could be of use as an easily accessible source of antioxidant for the nourishment. However, at present, the active components in the mushroom extract responsible for the observed antioxidant activity are unknown. Therefore, further work could be done on the isolation and purification of the active components from the mushrooms for showing the mode of action of them. As far as our literature survey could ascertain, there is no information about the mushroom species presented here. From this point of view, this study could be assumed as the first report on these wild species and it can be concluded that, since these wild mushroom samples have high free radical scavenging activity, they can be used health beneficial antioxidant supplements.

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