

Antioxidant Properties of Wild Edible Mushrooms

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Abstract

The methanolic extracts of dried wild edible mushroom were analyzed for antioxidant activity in different assays, namely, ferric antioxidant reducing power (FRAP), scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and total phenolic content. Among the twenty four mushroom extracts, the methanolic extracts from *Leccinum scabrum* showed the most potent radical scavenging activity showing 97.96%. The EC50 of *Pleurotus dryinus* and *Lactarius piperatus* methanolic extracts were 24.71 and 24.12 mg/ml, respectively. Total phenolics in the methanolic extracts were the highest in *Boletus edulis*. On the other hand, dry matter and ascorbic acid were determined in twenty four dried wild edible mushrooms. The amounts of ascorbic acid and total phenolic compounds found in the mushroom extracts were determined very low concentrations. Results from the PCA showed that principal components (PC) 1 and 2 described about 79.588 % of the total variation of sample. Therefore, edible mushrooms may have potential as natural antioxidants.

Keywords: Ascorbic acid; Total phenolic; Antioxidant activity; Wild edible mushroom

Introduction

Wild-growing mushrooms have a worldwide distribution and have been a popular delicacy in many countries. In fact, since ancient times mushrooms have been consumed by humans as a part of the normal diet and. They have a highly desirable taste and aroma, being also consumed for their texture: they add flavor and texture to a meal [1].

Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Among the antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging, metal chelation enzyme modulation activities and inhibition of LDL oxidation, among others [2,3]. The term polyphenol refers to a complex group of compounds that includes in their structure an aromatic ring bearing one or more hydroxyl groups. They comprise simple phenols such as phenolic acids and derivatives, as well as complex structures such as flavones, flavonoids or anthocyanins, among others [4-6].

Some common edible mushrooms have currently been found to possess antioxidant activity, which is well correlated with their total phenolic content [7]. Moreover, in the last few years, an increasing interest in the consumption of mushrooms has arisen, due to their elevated polyphenol concentration, which correlates with an elevated antioxidant activity. Several studies analyzing the total phenols and antioxidant activity of fresh and cooked wild and commercial mushrooms have been published [6,8-14]. However, as far as we know, characterization of species grown in different regions of Turkey has not been reported. The objective of this study was to evaluate the antioxidant properties of extracts from twenty four mushrooms from the East Blacksea region of Turkey. Their antioxidant activity was evaluated through the reducing power determination and radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Bioactive compounds such as total phenolic content and ascorbic acid were also determined.

Material and Methods

Material

Twenty-four wild edible mushrooms samples were harvested from the East Black sea region of Turkey and were authenticated by Dr. Ali Keleş, Department of Biology, Faculty of Science and Arts, Yüzüncü Yıl University, Van, Turkey. Mushrooms as fruiting bodies (pileus + stipe) were dried at room temperature. They were stored at the Yüzüncü Yıl University Faculty of Art and Science Microbiology Herbarium Laboratory.

Extract preparation: A fine dried mushroom powder (20 mesh) sample (5 g) was extracted with 80% of methanol (50 mL) for 24h at 4 °C. The mixture was vortexed (Vortex, DAIHAN VM-10 Vortex, SK) for 5 min. Then, the extracts were filtered through Whatman No. 4 filter paper.

Methods

Dry matter: Dry matter content of dried mushroom was determined according to Association of Official Analytical Chemists methods [15]. The dry matter content was determined by drying in an oven at 105 °C for 24 h.

Ferric antioxidant reducing power (FRAP): Methanolic extracts were mixed with 0.95 ml of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-striazine, in 40 mM HCl and 20 mM FeCl₃ in the ratio 10:1:1), and absorbance was measured at 593 nm. FeSO₄ was used as a standard, and total antioxidant activity was expressed as μmol g⁻¹ FRAP [16].

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Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals: The scavenging activity of the methanol extracts from mushrooms on DPPH radicals was measured according to the method of Nakajima et al. [17] with some modifications reported by Chiou et al. [18] 1 ml of DPPH solution (6×10^{-5} M in methanol) was added to a test tube with 50 μ L of the diluted extracts (concentrations 2.5–50 mg L⁻¹). Methanol was used instead of the mushroom sample as a control. The reaction mixture was vortex mixed at room temperature and the absorbance was measured after 30 min at 515 nm with a spectrophotometer. The obtained data were used to determine the quantity of mushrooms required to scavenge 50% of DPPH (EC₅₀). The percent of reduction of DPPH was calculated according to the following equation:

$$\% \text{ DPPH reduction} = (A_c - A_s / A_c) \times 100 \dots \dots \dots \text{Eq. (1)}$$

Where;

A_s is the absorbance of sample after the time necessary to reach the plateau (30 min), A_c is the absorbance of control.

These values were plotted against quantity of mushrooms to obtain the mushrooms amount necessary to decrease the initial DPPH concentration by 50% (EC₅₀) using an exponential curve.

Determination of antioxidant components: Total phenolic content was analyzed using Folin-Ciocalteu reagent [19]. Briefly, 0.5 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagents. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction was kept in the dark for 60 min, after which the absorbance was read at 760 nm a spectrophotometer. Gallic acid was used to calculate the standard curve (50-500 mg/mL). The results were expressed as mg of gallic acid (GAEs) equivalents per kg of extract.

Ascorbic acid content was determined using the 2,6-dichlorophenol-indophenol spectrophotometric method [20]. For ascorbic acid determination, the samples were extracted with metaphosphoric acid (4.5%) for 6 hours at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichlorophenolindophenol (9 ml) and the absorbance was immediately measured at 520 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020-0.120 mg/ml).

Statistical analysis

The PCA was carried out of SPSS base 10.0 [21]. Varimax was applied in order to ensure that the resulting factors were uncorrelated.

Results and Discussion

Ascorbic acid content

Table 1 shows the ascorbic acid concentration in the mushroom extracts. The ascorbic acid was found in small amounts (n.d–249.33 mg/kg), which is in agreement with other authors [9]. This antioxidant was determined in different mushrooms but ascorbic acid was not detected in all mushrooms by spectrophotometry. *Boletus pseudosulphureus* extracts showed the highest ascorbic acid content (249.33 mg/kg); the amount found in *Boletus edulis* (49, 33 mg/kg) was lower than the content found in *Suillus luteus* extracts (82.67 mg/kg). The highest content of ascorbic acid in the *Boletus pseudosulphureus* extracts might account for the better results found for their antioxidant activity. In fact, it had been reported that the antioxidant activity of plant materials

is well correlated with the content of total phenolic content and ascorbic acid [22].

Total phenolic content

Total polyphenols were the major naturally occurring antioxidant components found in the methanolic extracts from wild edible mushrooms. The total phenolic content, expressed as mg of GAEs/kg of dry mushroom, is shown in Table 1. The amount of phenolic compounds in the methanol extracts from the two mushrooms (*Boletus edulis* and *Boletus pseudosulphureus*) was the highest (12775.56–11375.56 mg GAEs/kg of dry mushroom), respectively. Followed by *Boletus erythropus* and *Macrolepiota procera* (9931, 11-8886,67 mg GAEs/kg of dry mushroom), respectively. By summation of the phenolic contents in the methanol solvent extracts, the total phenolic contents was 4020.0 g of GAEs/kg of dry mushroom for *Hydnum repandum* and 12775.33 mg of GAEs/kg of dry mushroom for *Boletus edulis* (Table 1). Between the two mushrooms, the total phenolic content of *Boletus edulis* was almost thirty-three times higher than that of *Hydnum repandum*. The yield of total phenolic compounds extracted from these two mushrooms was higher than that obtained from *Agaricus bisporus* (5.40-0.85 mg of GAEs/kg dry mushroom) [23]. Also, total phenolic contents of ten mushrooms were higher than *Agaricus bisporus* (4020.0 mg/kg) in this study.

Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups [24]. The phenolic compounds may contribute directly to the antioxidative action [25]. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [26]. The highest content of total phenols in the *Boletus edulis* and *Boletus pseudosulphureus* extracts might account for the better results found for their antioxidant activity. It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds. Polyphenols, such as BHT (butylated hydroxytoluene) and gallate, are known to be effective antioxidants [22,27]. So, it is important to consider the effect of the total phenolic content on the antioxidant activity of mushroom extracts.

Ascorbic acid was found in small amounts (n.d–249.33 mg/kg) only six mushrooms in this study. Therefore, polyphenols/phenolics might be responsible for the antioxidant properties studied. The highest content of total polyphenols in *Boletus edulis* might be the key components accounting for the better results found in antioxidant activity, reducing power, scavenging abilities as compared to other mushrooms. Generally, *Boletus edulis* was better in antioxidant activity, reducing power, and scavenging abilities and higher in the content of total polyphenols.

The phenolic compounds may contribute directly to antioxidative action [25]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables [28]. Also, numerous studies have conclusively showed that consumption of foods high in phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, because they act as antioxidants [27,29,30]. Therefore, edible mushrooms may have potential as natural antioxidants in food.

This result indicates that polyphenols may be the main antioxidant compounds found in mushrooms, in agreement with several authors [12-14]. However it is important to evaluate the type of phenol present

in mushroom and its individual contribution to the total antioxidant capacity. In this regards, studies are in progress evaluating other phenols and antioxidants present in mushrooms.

Scavenging activity of DPPH radical

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The radical scavenging activity (RSA) of mushroom extracts was tested against the DPPH. DPPH, a stable free radical with a characteristic sorption at 515 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical-scavenging. Free radical-scavenging capacities of the tracts were measured by DPPH assay. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time. In Table 1, the scavenging activity of the DPPH radical due to its reduction by different mushrooms are illustrated.

The methanol extract of *Suillus luteus* and *Boletus edulis* showed the highest scavenging activity (97.96, 93.18% at 25mg/ml), respectively, but *Hydnum repandum* was much lower than that of all mushrooms in this study (10.17%). Apparently, the scavenging abilities of *Boletus ssp.* They were found higher effectively than those of the other mushrooms in this study (Table 1).

The scavenging effects of methanolic extracts from mushroom species and standards on the DPPH radical decreased in the order of *Lepista nuda*, *Russula delica*, *Polyporus squamosus*, *Pleurotus ostreatus*, *Agaricus bisporus*, *Verpa conica*, *Boletus badius* and were, at the concentration of 180 mg/mL, 91.3, 86.1, 82.8, 81.3, 77.5, 75.7 and 68.7, respectively [6]. In this study, DPPH radical decreased in the order of *Pleurotus ostreatus*, *Lepista nuda*, *Agaricus bisporus*, *Polyporus squamosus*, *Russula delica* and were different from above study, at the concentration of 25 mg/mL, 86.35, 85.61, 67.86, 43.30 and 37.10, respectively. Plant tissue antioxidant capacity is clearly associated with the activity of “free radical scavenging enzymes” (superoxide dismutase, catalase, peroxidase, etc.) and with the contents of antioxidant substances, mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid. It is evident that there is an increasing demand to evaluate the antioxidant properties of direct plant extract [6].

Huang et al. [31] also found that the methanolic extract from *Agaricus blazei* showed a high scavenging ability of 97.1% at 2.5 mg/ml. Tsai et al. [32] mentioned that the methanolic extract from *Agrocybe cylindracea* strain B scavenged DPPH radicals by 93.8% at 5 mg/ml. Scavenging abilities of *C. comatus* were 84.5% at 5 mg/ml and 96.0% at 20 mg/ml [33] and *P. citrinopilatus* showed a scavenging ability of 94.9% at 5 mg/ml [34]. However, *H. Marmoreus* scavenged DPPH radicals by 59.7% at 5 mg/ml and 93.2% at 20 mg/ml [35].

Obviously, the extracts contained antioxidant components, which

	Dry matter, %	Ascorbic acid, mg/kg	Total phenolics, mg/kg	FRAP, µmol/g	DPPH, % ¹	EC 50 mg/ml
<i>Agaricus bisporus</i>	94.55	n.d	4020.00	12171.43	67.86	19.51
<i>Chlororhizum rhacodes</i>	93.75	n.d	4353.33	17885.71	80.64	11.18
<i>Macrolepiota procera var. procera</i>	93.26	n.d	8886.67	7457.14	90.07	7.91
<i>Amanita rubescens var. rubescens</i>	94.05	29.33	5708.89	31814.29	91.31	11.35
<i>Pleurotus dryinus</i>	92.68	20.22	2353.33	11600.00	50.74	24.71
<i>Armillaria ostoyae</i>	94.05	<20	2908.89	5028.57	42.31	-
<i>Pleurotus ostreatus</i>	93.20	n.d	2686.67	2385.71	86.35	11.07
<i>Polyporus squamosus</i>	92.54	<20	4531.11	2242.86	43.30	-
<i>Boletus edulis</i>	93.53	49.33	12775.56	52957.14	93.18	3.95
<i>Boletus pseudosulphureus</i>	95.17	249.33	11375.56	47528.57	90.82	7.88
<i>Leccinum scabrum</i>	93.71	n.d	3175.56	23814.29	74.19	18.74
<i>Suillus luteus</i>	93.85	82.67	5064.44	58528.57	97.96	4.76
<i>Lepista nuda</i>	94.82	n.d	4175.56	12171.43	85.61	16.2
<i>Lepista personata</i>	94.04	n.d	4220.00	8314.29	89.33	16.91
<i>Hydnum repandum</i>	93.32	n.d	420.00	145.50	10.17	-
<i>Lactarius deliciosus</i>	94.17	<20	2708.89	2671.43	47.27	-
<i>Lactarius piperatus</i>	92.13	n.d	3442.22	3528.57	52.60	24.12
<i>Lactarius salmonicolor</i>	93.05	27.11	3242.22	4242.86	46.15	-
<i>Lactarius volemus</i>	92.77	<20	2331.11	3171.43	62.28	21.37
<i>Russula delica</i>	95.18	n.d	2020.00	1160.00	37.10	-
<i>Russula integra var.integra</i>	95.54	n.d	4508.89	1210.00	33.62	-
<i>Russula nigricans</i>	93.89	<20	4664.44	23600.00	78.16	19.40
<i>Russula vinosa</i>	91.25	n.d	3064.44	1985.71	72.21	21.26
<i>Boletus erythropus var. erythropus</i>	93.59	n.d	9931.11	62771.43	90.32	9.26

n.d: not detected; ¹ 25 mg/ml reducing power,

Table 1: The results of dry matter and antioxidant capacity of wild edible mushrooms collected from the East Blacksea region of Turkey.

could react rapidly with DPPH radicals, and reduce most DPPH radical molecules. This result reveals that the extracts are a free radical inhibitor or scavenger, acting possibly as primary antioxidants. Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction [36-38]. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction [33]. These results indicated that methanolic extracts of mushroom species have a noticeable effect on scavenging free radical.

Ferric antioxidant reducing power (FRAP)

In the present work, antioxidant activity was measured by the FRAP method, which measures the capacity of an antioxidant to reduce a Fe^{3+} -TPTZ complex to Fe^{2+} -TPTZ. In this way, a higher Fe^{3+} -TPTZ reduction means a higher antioxidant activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [39]. Table 1 indicates the reductive capabilities of methanolic extract of mushroom species. The extracts of *Boletus erythropus var. erythropus* possess the highest activity compared to other extracts. Among methanolic extracts from twenty four wild edible mushrooms, the reducing power of *Boletus erythropus var. erythropus* and *Suillus luteus* were 62771.43 and 58528.57 $\mu\text{mol/g}$, respectively (Table 1). From the FRAP values of *Boletus erythropus var. erythropus* mushroom extracts in this study, it could be noted that *Boletus erythropus var. erythropus* had a higher reducing power due to the fact that it contained higher total phenolic content 21 mushrooms in all mushrooms (Table 1). When the antioxidant activity values of the wild mushrooms determined by the FRAP method where compared with other fruits, it was observed that mushrooms presented higher antioxidant activity than those reported for peaches, which ranged from 0.84 to 1.2 mmol $\text{Fe}^{2+}/100$ g FW, but lower than those for strawberries (19.3-24.4 mmol $\text{Fe}^{2+}/100$ g DW) [40].

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [41-43].

EC50

The antioxidant properties assayed herein were summarized in Table 1 and the results were normalized and expressed as EC50 values (mg various extracts per ml) for comparison. Effectiveness of antioxidant properties inversely correlated with their EC50 values. In Table 1, we present the EC50 values for reducing power and DPPH scavenging effects obtained from each mushroom methanolic extract.

Overall, *Suillus luteus* revealed better antioxidant properties than *Lactarius piperatus* (lower EC50 values), which is in agreement with the higher content of phenols found in the first species. This was much more evident in EC50 values for DPPH scavenging effect (4.76 mg/ml for *Suillus luteus* versus 24.12 mg/ml for *Lactarius piperatus*). Searching wild sources may bring new natural products into the food industry with safer and better antioxidants that provide good protection against the oxidative damage, which occurs both in the body and our daily foods. Therefore, new wild edible mushrooms, as natural sources, could be introduced for this purpose. As far as our literature survey could ascertain, little information was available on the *in vitro* antioxidative activities of Turkey wild mushrooms.

EC50 values in scavenging ability on DPPH radicals were 4.76, 3.95, and 11.35 mg/ml for methanolic extracts from *Suillus luteus*, *Boletus edulis* and *Amanita rubescens var. rubescens*, respectively. Although *Armillaria ostoyae*, *Polyporus squamosus*, *Hydnum repandum*, *Lactarius deliciosus*, *Lactarius salmonicolor*, *Russula delica* and *Russula integra var. integra* were had DPPH, EC50 not found in this study.

It can be seen that EC50 values of all extracts were below 25 mg/ml on the basis of dry sample in antioxidant properties assayed (Table 1). Although BHA and α -tocopherol were good in inhibitory ability on lipid oxidation, reducing power and scavenging ability on DPPH radicals and EDTA was excellent for chelating ferrous ions, they are additives and used or present in mg levels in foods. However, *Suillus luteus*, *Boletus edulis*, *Amanita rubescens var. rubescens*, *Boletus pseudosulphureus* and *Boletus erythropus var. erythropus* could be used in g levels as food or a food ingredient. Therefore, these mushrooms might serve as possible protective agents in human diets to help human reduce oxidative damage.

The results of PCA of the mean values of (total phenol, DPPH, FRAP and ascorbic acid) component 2 (EC50) of mushrooms samples are depicted in a 2-dimensional plot (Figure 1).

Results from the PCA showed that principal components (PC) 1 and 2 described about 79.588% of the total variation of sample: 54.820% PC1 and 24.768% PC2. Principal component 1 was heavily loaded on total phenol, DPPH, FRAP and ascorbic acid, whereas component 2 was loaded on EC50. The PCA analysis showed that total phenol, DPPH, FRAP and ascorbic acid were positively correlated to each other., Results also showed that there is low significant relationship among total phenol, DPPH, FRAP, ascorbic acid and EC50 (Figure 1). In this study, no significant correlation was found between the amounts of EC50 and the total phenol, DPPH, FRAP and ascorbic acid in the mushroom samples in Figure 1. We found a high positive significant correlation between total phenol and FRAP.

A relationship between the reducing power and DPPH-scavenging activity was found, indicating that the mechanisms of action of the extracts for the antioxidant activity may be identical, being related to the content of total phenols. Though other antioxidants were probably present in these mushroom extracts, the amounts of ascorbic acid

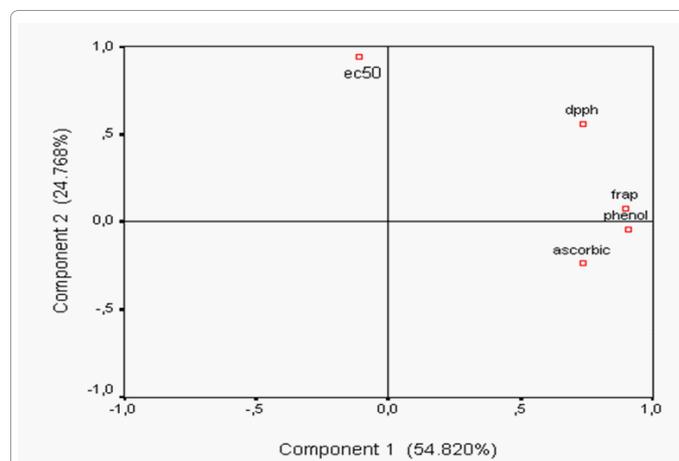


Figure 1: Principal component analysis biplot of total phenol, FRAP, ascorbic acid and EC50 of mushrooms. (DPPH= Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radicals, FRAP= Ferric Antioxidant Reducing Power).

found in this mushroom extracts were very low, which emphasises the idea that phenolic compounds could make a significant contribution to the mushrooms' antioxidant activity.

Conclusions

According to the results of this study, it is clearly indicated that the methanolic extract of mushroom species has significant antioxidant activity against various antioxidant systems *in vitro*; moreover, the mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The various antioxidant mechanisms of the mushroom species extract may be attributed to strong hydrogen-donating ability, a metal-chelating ability, and their effectiveness as good scavengers of superoxide and free radicals. Phenolic compounds seem to be the main components responsible for the antioxidant activity of all the mushroom species extracts. From the wild mushrooms studied, *Boletus* may be an interesting group due to their high total phenol concentration, antioxidant activity and ascorbic acid. In general, a correlation between higher antioxidant activity and larger amount of total phenolics was found in the mushroom extracts. Though other antioxidants were probably present in these mushroom extracts, total phenolic compounds could make a significant contribution to the antioxidant activity in these extracts. Having established the antioxidant activity in these mushroom extracts, the chemical characteristics of the antioxidative components in these extracts will be further investigated. It revealed that in addition to these antioxidant activities, other factors contribute in part to the antioxidant properties of wild edible mushrooms. It is recommended *Boletus* for use in foods as natural antioxidants or extracts. To study the antioxidant mechanisms by some other potential antioxidant components, the fractionation of the methanolic extract and further identification are in progress.

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