Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 4, October-December 2012, p. 1647-1654

ANTIOXIDANT PROPERTIES OF WILD EDIBLE MUSHROOM *PLEUROTUS ERYNGII* COLLECTED FROM TUNCELI PROVINCE OF TURKEY

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Antioxidants are our first line of defense against free radical damage, and are critical for maintaining health. The need for antioxidants becomes even more critical with increased exposure to free radicals. In the past few years there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Mushrooms have been not only used as food materials with their unique flavor and texture, but also recognized as an important source of biologically active compound of medicinal value. In our study, to determine the antioxidant properties of the methanolic extracts of *Pleurotus eryngii* strains collected from different region of Tunceli, radical scavenging activity, total phenolic contents, reducing power, metal chelating activity and total antioxidant status (TAS) were analyzed. The analysis of phenolic compounds was performed by HPLC. In particular, the methanolic extract of *P. eryngii* collected from city center revealed the highest DPPH radical scavenging activity and reducing power, while the highest total phenolics and total antioxidant status was determined in *P. eryngii* collected from Pülümür. *P. eryngii* collected from Ovacık showed the highest chelating effects. Concentration of phenolic compounds found in three wild edible mushrooms. Kaempferol and catechin were not determined in musrooms species. The highest rutin levels were determined in ovacık. Resveratrol was determined only in Pulumur. The studied wild mushrooms might be beneficial to protect human body against oxidative damage.

(Received September 17, 2012; Accepted October 19, 2012)

Keywords: Pleurotus eryngii, total phenolic contents, Total antioxidant status, Free radical scavenging capacity, Reducing power, Metal chelating activity, Tunceli

1. Introduction

Natural products, in the form of pure compounds or extracts with antioxidant activity, may help the endogenous defense system of the body [1]. Antioxidants obtained through diet are taking on major significance as possible protector agents to diminish oxidative damage, and wild mushrooms might be used as nutraceuticals or directly eaten in the diet to maintain good health [2]. As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, have received much attention [3]. Antioxidant supplements or antioxidant-containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration [4]. The antioxidants contained in foods, especially vegetables, are phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid [4,5]. that are important protective agents for human health [6]. Wild or cultivated mushrooms have long been a popular part of the human diet because of their

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agreeable sensory qualities. In addition to their nutritious value, they have been used in traditional medicine in many countries [7]. Generally, mushrooms are rich in dietary fiber, minerals, vitamins and low in fat [8]. Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Phenolic compounds are one of the most widely distributed plant secondary products. The ability of these compounds to act as antioxidants have been well established [9]. Polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers [10]. Also, a mushroom phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic [11]. Many of the biological functions, such as anticancer, antiviral, 63 immunopotentiating, and hypolipidemic activities, are considered to be attributed to 64 their free radical scavenging and antioxidant activity. Therefore, the secondary 65 compounds with antioxidant activities such as phenolics and flavonoids in mushrooms 66 are of great interest as possible protective agents to help human health.

In our study, to determine the antioxidant properties of the *P. eryngii* strains collected from different region of Tunceli, DPPH radical scavenging activity, total phenolic contents and total antioxidant status (TAS), Reducing power, Metal chelating activity were determined. Phenolic compounds were determined.

2. Experimental

2.1 Samples

P. eryngii was collected from different regions (city center, Ovacik and Pulumur provinces) of Tunceli, Turkey in May 2012 (Figure.1).

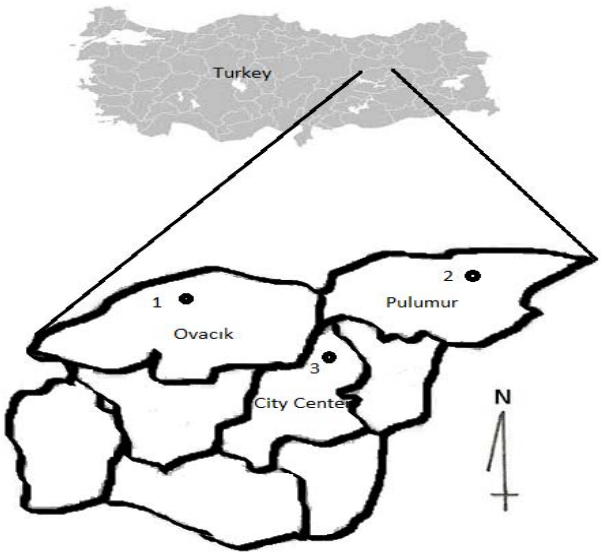

Fig. 1. Location of the sampling area, Tunceli from Turkey: 1. Ovacik, 2. Pulumur, 3. City center.

2.2 Sample preparation

Fresh mushroom material was washed with tap water, air dried and then chopped into small fragments, which was shade-dried and reduced to a coarse powder in a mortar and pestle. The aerial parts of the mushroom samples (2 g) were extracted with 20 ml methanol (MetOH). The organic solvents were evaporated to dryness under vacuum at low temperature using a rotary

evaporator. The dried extracts were dissolved in methanol to a final concentration of 25 mg/ml and used as such for the phenolic compounds and antioxidant testing [12]

2.3 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of the mushroom extracts was measured and compared with the activity of butylated hydroxy anisol (BHA) for radical-scavenging ability using the stable radical DPPH [13]. The free-radical scavenging activities of extracts and BHA (used as a standard) were measured by decrease in the absorbance of methanol solution of DPPH.
The 0.1 mM solution of DPPH in methanol was prepared and 1.5 mL of this solution was added to 3.5 mL of extract solution in water at different concentrations (50-500 μg mL-1). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Radical scavenging activity } = ([\ A_0 - A_1\ ]\ A_0\ ) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample and standards.

2.4 Reducing power

Reducing power of musrooms were determined by the method of Oyaizu (1986)[14]. Each concentration of the methanolic extracts (1.0 mL) was added to 1 mL of potassium ferricyanide (10 mg/mL), and the mixture was incubated at 50°C for 20 min. After incubation, a 1-ml of trichloroacetic acid (100 mg/mL) was added to the mixture and then the mixture was centrifuged at 13,400 ´ g for 5 min. The supernatant (1.0 mL) was mixed with 1.0 ml of distilled water and 0.1 mL of ferric chloride (1.0 mg/mL), and then its absorbance was measured at 700 nm.

2.5 Chelating effects on ferrous ions

The chelation of ferrous ions by extracts was estimated by method of Dinis et al. (Dinis et al., 1994)[15]. Briefly, 50 μl of 2 mM $FeCl_2$ was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm.

The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as [(A_0-A_s)/ A_s] x 100, where A_0 was the absorbance of the control, and As was the absorbance of the extract/ standard. Na_2EDTA was used as positive control.

2.6 Total phenolic contents

The Singleton et al. (1999) method, using Folin-Ciocalteu reagent, was used to determine the total phenolic content. Each plant extract was prepared at a concentration of 1 mg mL-1. The absorbances of all samples were measured at 760 nm against a methanol blank using a spectrophotometer (Shimadzu UV 1800). The standard calibration curve was plotted using gallic acid. The mean of three readings was used and the results expressed as g of Gallic Acid Equivalents (GAE) per 100 g of lyophilised extract [16].

2.7 Total Antioxidant Status (TAS)

Total antioxidant status was determined by using Rel assay diagnostics tas assay kit (Lot.RL024) by Multiscan FC (Thermo). Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant levels of the sample. The assay is calibrated with a stable antioxidant Standard solution which is traditionally named as Trolox Equivalent that is a vitamine E analog.

2.8 Apparatus and HPLC conditions

The HPLC system used was Shimadzu Prominence HPLC, equipped with a degasser DGU-20A5, a binary pump LC-20AT, an autosampler SIL-20AHT, a column oven CTO-10ASVP and a diode array detector SPD-M20A. The column used was a Kromasil 100-5C18 (150x4.6 mm, 5μm), operated at 25 ^{o}C. An isocratic mode was used and mobile phase was 1% acetic acid in methanol/water/acetonitrile (46:46:8 v/v/v). The flow rate was set to 1 mL/min. The injected volume was 10 μL. Kaempferol, rutin, resveratrol and catechin were used as standart. Identification and quantitative analysis were done by comparison with standarts. HPLC-DAD analysis was carried out in the range between 200 and 500 nm, setting the detector at 265 nm for identification of kaempferol, at 254 nm for rutin, at 306 nm for resveratrol and at 280 nm for catechin.

3. Results and discussion

3.1 Scavenging activity of DPPH radical

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells [17,18]. This oxidative stress may be related to many disorders, such as cancer, atherosclerosis, diabetes and liver cirrhosis [19]. 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 [20]. Barros et al., 2007 evaluated the antioxidant activities of three Portuguese wild edible mushroom species, Leucopaxillus giganteus, Sarcodon imbricatus, and Agaricus arvensis. They screened methanolic extracts for their reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity [21] . In Table 1, the scavenging activity of the DPPH radical due to its reduction by different mushroom isolated from Tunceli is illustrated. The most strong DPPH radical scavenging activity was found in the mushroom isolated from city center (%39.13) (Table 1).

3.2 Total phenolic content

Among the protective actions in biological systems, phenolic compounds exhibit antioxidant activity. Thus, phenolics can be classified as free radical inhibitors (chain breaker), peroxide decomposers, metal inactivators or oxygen scavengers [22]. Numerous studies have showed the consumption of foods high in phenolics can reduce the risk of heart disease by slowing the progression of atherosclerosis due to their antioxidative properties [23, 24]. Antioxidant properties of methanolic extracts three species medicinal mushrooms that (*G. lucidum, G. tsugae and C. versicolor*) in Taiwan and results showed that *G. lucidum* and *G.tsugae* were higher in antioxidant activity, reducing power, scavenging and chelating abilities, which was attributed to their total phenolic content [25].

There are large numbers of studies reporting determination of total phenols in mushrooms by Folin-Ciocalteu's assay [2]. The total phenolic content, expressed as mg of GAEs/g of dry mushroom, is shown in Table 1. The highest amount of phenolic compounds was found in *Pleurotus* eryngii isolated from Pulumur (Table 1). The total phenolic contents was 32.21 mg of GAEs/g of dry mushroom isolated from pulumur, 30.20 mg of GAEs/g of dry mushroom for ovacik and 29.49 mg of GAEs/g of dry mushroom isolated from city center (Table 1).

3.3 Reducing power

The antioxidant activity has been reported to be concomitant with the development of reducing capacity [26]. Therefore, reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [27]. Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain by donating a hydrogen atom or a single electron [28]. The electron donation group, especially hydroxyl group located at o- or p-positions of the compounds, makes the compound polar and therefore reducing power is increased [29]. The reducing power of the ethanolic extract of *P.*

ostreatus was found to steadily increase in direct proportion to the increasing concentration of the extract [30]. Reis et al., 2012 investigated Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms. The antioxidant activity was evaluated through reducing power (Folin–Ciocalteu and Ferricyanide/ Prussian blue assays), free radical scavenging activity (DPPH assay) and lipid peroxidation inhibition. The analysis of phenolic compounds was performed by HPLC/PAD. Overall, in this study, A. bisporus (brown) was the mushroom species with highest reducing power and antioxidant potential [31]. In our study, the highest reducing power was found in *P. eringii* collected from city center (Table 1).

3.4 Metal chelating effect of *P. erygnii*

Transition metals are believed to serve as the catalysts for the initial formation of radicals. Chelating agents, on the other hand may stabilize transition metals in living systems and inhibit generation of free radicals, consequently reducing free radical mediated damage [32]. Methanolic extracts of medicinal mushrooms such as *G. lucidum, G. lucidum antler, G. tsugae and C. versicolor* have been found to chelate ferrous ions [25]. In our study, the methanolic extract of *P. eryngii* collected from ovacık revealed the highest chelating effects (%12.58) (Table 1).

Table 1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Reducing power, Chelating effects on ferrous ions, Total phenolic contents (TPC), Total Antioxidant Status of wild edible mushroom Pleurotus eryngii collected from different region of Tunceli (Turkey)

	Location of the sampling area of *P. eryngii*		
Antioxidant parameters	**1 Pulumur**	**2 Ovacik**	**3 City center**
Chelating effects (%)	8.56±2.90^{b}	17.29±8.75^{a}	12.58±1.01^{ab}
Reducing power (%)	33.86±2.31^{ab}	21.33±10.64^{b}	37.20±0.61^{a}
Total phenolics*	32.21±0.92^{a}	30.20±2.40^{a}	29.49±0.74^{a}
Scavenging activity of DPPH radical (%)	37.36±5.10^{ab}	25.08±0.62^{b}	39.13±5.71^{a}
Total Antioxidan Status (TAS)**	1.93±0.02^{a}	1.54±0.34^{a}	1.35±0.06^{a}

The results are expressed as mean ± SE (n = 3). In each line different letters mean significant differences between results ($p < 0.05$). * Total phenolics; mg of GAEs/g of dry mushroom. **Total Antioxidan Status (TAS); mmolTrolox Equiv./L.

3.5 Antioxidant Components

It has been described that the main phenolic compounds found in mushrooms are phenolic acids [2]. Catechin was described in other mushrooms such as Russula delica [33] Also antioxidant, antibacterial activity of catechin was reported by many authors [33]. Kaempferol is a natural flavonoid isolated from tea, mushrooms, kale, broccoli, and other plant sources [34]. Kaempferol has been reported to have several health-promoting effects. For example, kaempferol possesses antioxidative and anti-inflammatory properties [35] and exhibits antitumor activity [36]. Using HPLC the phenolic compounds like gallic acid, caffeic acid, catechin, epicatechin and rutin were detected and quantified in latvian wıld edible mushroom *Boletus edulıs* by Kuka and Cakste [37]. The phenolic composition of A.bisporus methanolic extracts was analysed by HPLC and found to contain rutin, gallic acid, caffeic acid and catechin which contibuted to its antioxidant activity [38]. Palacios et al., (2011) evaluated total phenolic and flavonoid contents occurring in eight types of edible mushrooms (*Agaricus bisporus, Boletus edulis, Calocybe gambosa, Cantharellus cibarius, Craterellus cornucopioides, Hygrophorus marzuolus, Lactarius deliciosus*

and Pleurotus ostreatus). Flavonoids, such as myricetin and catechin were also detected in the mushrooms studied [39].

In present study, concentration of phenolic compounds found in three wild edible mushrooms (Table 2). Kaempferol and catechin were not detected whereas Resveratrol was found in small amounts (1.1 mg/kg) in *P. eryngii* collected from Pulumur. Rutin level was 4.4 mg/kg in *P. eryngii* collected from Pulumur and 9.4 mg/kg of dry mushroom for ovacik.

3.6 Total antioxidant status

Studies in antioxidant status of fruits, vegetables and mushrooms an have begun to take the spotlight in recent research. The concentrations of 11 phenols and 5 furans were measured in 12 categories of distilled spirits by HPLC methodology, together with the total antioxidant status (TAS) of the same beverages. Highest TAS values were given by armagnac, cognac, and bourbon whiskey, all three of which tended toward the highest concentrations of phenols [40]. In order to identify wild fruits possessing high nutraceutical potential, the antioxidant activities of 56 wild fruits from South China were systematically evaluated. The antioxidant capacities of the extracts were evaluated using the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays, and their total phenolic contents were measured by the Folin-Ciocalteu method. A significant correlation between the FRAP value and the TEAC value suggested that antioxidant components in these wild fruits were capable of reducing oxidants and scavenging free radicals. A high correlation between antioxidant capacity and total phenolic content indicated that phenolic compounds could be the main contributors to the measured antioxidant activity [41] . In our study, highest TAS value (1.93 mmolTrolox Equiv./L) was found in *P.eringii* isolated from Pulumur which tended toward the highest concentrations of total phenolics.

Table 2. Concentration of phenolic compounds found in three wild edible mushrooms. Results are expressed as mg of phenolics per kg of dried mushroom and the standard deviation is indicated in brackets.

	Concentrations of phenolic compounds (mg/kg)*			
Location of the sampling area	**Kaempferol**	**Rutin**	**Resveratrol**	**Catechin**
Pulumur	ND**	4.4±0.54	1.1±0.04	ND
Ovacik	ND	9.4±1.22	ND	ND
City center	ND	ND	ND	ND

**ND: Not Determined, *Means±SE, (n = 3)

4. Conclusion

Based on the results obtained, the methanolic extract of *P. eryngii* collected from city center revealed the highest DPPH radical scavenging activity and reducing power, while the highest total phenolics and total antioxidant status was determined in *P. eryngii* collected from Pulumur. *P. eryngii* collected from Ovacik showed the highest chelating effects. Concentration of phenolic compounds found in three wild edible mushrooms. Kaempferol and catechin were not determined in musrooms species. The highest rutin levels were determined in ovacık. Resveratrol was determined in only Pulumur.

Therefore, consumption of wild mushrooms might be beneficial to protect human body against oxidative damage. With the established antioxidant activity of these mushroom extracts,

the chemical characteristics of the antioxidative components in the extracts should be further investigated.

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