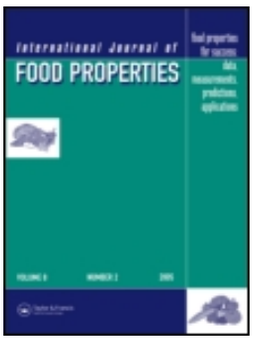

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ANTIOXIDANT PROPERTIES OF CULTURED MYCELIA FROM FOUR *PLEUROTUS* SPECIES PRODUCED IN SUBMERGED MEDIUM

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The ethanolic extracts of dried cultured mycelia of* Pleurotus ostreatus, Pleurotus eryngii, Pleurotus florida*, *and* Pleurotus sajor-caju *were analyzed for antioxidant activity in different systems. Tests used are as follows: reducing power, free radical scavenging, superoxide anion radical scavenging, total antioxidant activity, metal chelating activitiy, etc.; total phenolic content was determined. The percentage inhibition of* P. ostreatus, P. eryngii, P. florida, *and* P. sajor-caju *at 20 mg/mL concentration on peroxidation in a β-carotene–linoleic acid system was 57.19, 60.68, 62.12, and 58.81%, respectively. The reducing power of* P. eryngii *was higher than the other samples, and its value was 0.86 at 10 mg/mL concentration.* P. ostreatus *and* P. sajor-caju *proved to be better at scavenging superoxide anion radicals than the* P. eryngii *and* P. florida*. In the scavenging effect of DPPH radical test,* P. ostreatus *showed the highest activity potential and* P. sajor-caju *showed the strongest metal chelating capacity.

Keywords: Pleurotus ***species, Cultured mycelia, Antioxidant, Submerged medium, Oxidation.***

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to carry out biological processes. Free radicals are produced in normal and/or pathological cell metabolism.[1] However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases, such as cancer, rheumatoid arthritis, cirrhosis, and atherosclerosis, as well as in degenerative processes associated with ageing.[2] Almost all the organisms are well protected against free radical damage by oxidative enzymes, such as superoxide dismutase (SOD), glutathione peroxidise (GSHPx), and catalase (CAT),[3] or chemical compounds, such as tocopherol, ascorbic acid, carotenoids, and polyphenol compounds.[2] Phytochemicals, especially phenolics in fruits and vegetables, are suggested to be the major bioactive compounds for health benefits and are found to be associated

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with the inhibition of atherosclerosis and cancer.[4] Many species of fruits, vegetables, herbs, cereals, sprouts, and seeds have been investigated for antioxidant activity in the past.[5]

Mushrooms have been part of the human diet for thousands of years, involving a large number of edible species. In most countries, there is a well-established consumer acceptance for cultivated mushrooms, probably due to their unique flavor and texture. Edible wild mushrooms are traditionally used in many Asian countries as food and medicine.[6] Recently, mushrooms have become an attractive functional food mainly because of their chemical composition,[1] and this can be explained by the antioxidant capacity of mushrooms to scavenge free radicals, which are responsible for oxidative damage of lipids, proteins, and nucleic acids.

Pleurotus species, the third largest commercially produced mushroom in the world,[7] are found growing naturally on rotten wood material. The growing increase in consumption of oyster mushroom is largely due to its taste and medicinal and nutritional properties.[7] *Pleurotus* species have been used by human cultures all over the world for their nutritional value, medicinal properties, and other beneficial effects. *Pleurotus* species are a good source of dietary fiber and other valuable nutrients. They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumor growth and inflammation, have hypoglycemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities.[6] Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes, and steroids.[8] The antioxidants present in mushrooms are of great interest as protective agents to help the human body reduce oxidative damage without any interference. They are known as functional foods and as a source of physiologically beneficial components.[9]

Although there are many studies on cultivated and wild edible mushrooms in the northern hemisphere, there is little knowledge available about antioxidant properties of wild edible mushrooms of Turkey. As far as our literature survey could ascertain, there is no report in the literature on cultured mycelia of these mushroom species collected from the southeast of Turkey. Therefore, data given for the antioxidant statue of cultured mycelia of wild mushrooms here could be regarded as the first report on this topic. Our objective was to evaluate the antioxidant properties of ethanol extract of cultured mycelia from four wild oyster mushroom species (*Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus florida*, and *Pleurotus sajor-caju*), with the antioxidant tests, such as total antioxidant activity, superoxide anion scavenging activity, reducing power, free radical scavenging activity, metal chelating activity, and determination of total phenolic compounds.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), atocopherol, 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), butylated hydroxyanisole (BHA), and trichloraceticacid (TCA) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate and butylated hydroxytoluene (BHT) were purchased from E. Merck (Darmstadt, Germany). All other chemicals were analytical grade and obtained from either Sigma-Aldrich or Merck.

Preparation of Cultured Mycelia of *Pleurotus* Species

Cultured mycelia of *Pleurotus* species were obtained via submerged culture by using the samples originally isolated from fresh specimens collected in the southeast of Turkey. During the experimental work, the isolates were kept on petri dishes on Malt Extract Agar (MEA, Fluka, Buchs, Switzerland) at 25 ± 1°C, and they were re-inoculated every 3 weeks to maintain their viability and activity as described by Dundar et al.[10] Isolates were grown in a malt extract liquid medium. Mycelia was cultured in 500-mL flasks, each of which contains 100 mL of medium inoculated with 1 cm^2 cuts of a 7-day-old culture from MEA, and each of which is kept in a growth chamber at 25 ± 1°C with no agitation. In this growth condition, the mycelia of mushrooms developed a solid pellicle aggregate and this aggregate was harvested after 20 days of growth. The mycelial pellets were removed aseptically through filtration and washed three times with RO (reverse osmosis) water and then air-dried in an oven at 40°C before analysis.

Mushroom Mycelia Extraction

For ethanol extraction, 5 g of dried cultured mycelia of mushroom samples were weighed and ground into a fine powder in a mill, then mixed with 100 mL of ethanol at room temperature at 150 rpm for 24 h. The residue was re-extracted under the same conditions until the extraction solvents became colorless. The extract obtained was filtered over Whatman No. 1 paper and the filtrate was collected, then ethyl alcohol was removed using a rotary evaporator (Laborata 4001, Serial no. 069800367, Heidolph WB, Schwabach, Germany) at 40°C to obtain dry extract. The dried extract was used directly for analyses of total phenols or dissolved in ethanol up to a concentration of 5 mg/mL and stored at 4°C for further antioxidant analyses.

Total Antioxidant Activitiy by the β-Carotene–Linoleic Acid Method

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation.[11] A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 μL of linoleic acid and 200 mg of Tween 40 were added to this solution. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added and it was shaken vigorously. Finally, 4.6 mL of this reaction mixture was dispersed to test tubes and 0.4 mL of various concentrations (0.5, 2.5, 5.0, and 10.0 mg/mL) of the extracts in ethanol was added and the emulsion system was incubated for up to 2 h at 50°C. The same procedure was applied for BHT, a-tocopherol, quercetin, and blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared. The bleaching rate (R) of β-carotene was calculated according to Eq. (1):

$$R = \ln(a/b)/t, \tag{1}$$

where ln = natural log, a = absorbance at time 0, b = absorbance at time t (30, 60, 90, 120 min) (Cheung et al., 2003). The antioxidant activity (AA) was calculated in terms of percentage inhibition related to the control using Eq. (2):

$$AA = [(R_{control}\text{-}R_{sample})/R_{control}] \times 100. \quad (2)$$

Antioxidative activities of the extracts were compared with those of BHT, a-tocopherol, and quercetin at 0.5 mg/mL and also compared with blank consisting of only 0.4 mL ethanol.

Determination of Reducing Power

The reducing power of ethanolic extracts dried cultured mycelia was determined according to the method of Oyaizu.[12] Various concentrations of ethanolic extract (1, 2, 5, and 10 mg/mL) in 1 mL of ethanol were mixed with phosphate buffer (2.5 mL,0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ was added. This mixture was incubated at 50°C for 20 min, and 2.5 mL of trichloroacetic acid (10%) was added to the mixture, and finally centrifugated for 10 min at 1000 g (MSE Mistral 2000, Serial no: S693/ 02/444, Sanyo Gallenkamp PLC, Leicestershine, UK). The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm.

Superoxide Anion Radical Scavenging Activity

Superoxide radicals of ethanolic extract of cultured mycelia were determined according to the method of Zhishen et al.[13] All solutions were 0.05 M in phosphate buffer (pH 7.8). The photo-induced reactions were performed in an aluminium foil-lined box with two 20 W fluorescent lamps. The distance between reactant and lamp was adjusted until the intensity of illumination reached 4000 lx. The total volume of the reactant was 5 mL and the concentrations of riboflavin, methionine, and nitro blue tetrazolium (NBT) were 3×10^{-6}, 1×10^{-2}, and 1×10^{-4} M, respectively. The reactant was illuminated at 25°C for 25 min. The photochemically reduced riboflavins generated superoxide anion radicals, thus reducing NBT to form blue formazan. The un-illuminated reaction mixture was used as a blank. Absorbance (A) was measured at 560 nm. Ethanolic extracts of mushroom species and standards were added to the reaction mixture, in which superoxide anion was scavenged, thereby inhibiting the NBT reduction. Absorbance (A_1) was measured and decrease in superoxide radical was represented by $A - A_1$. The degree of scavenging was calculated by the following equation:

$$\%\ \text{Scavenging} = [(A\text{-}A_1)/A] \times 100. \quad (3)$$

Free Radical Scavenging Activity

The free radical scavenging activities of mushroom species were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois.[14] Briefly, 0.1 mM solution of DPPH in methyl alcohol was prepared and 1 mL of this solution was added to 3 mL of ethanolic extract of cultured mycelia mushroom species at different concentrations (1.0, 2.0, 5.0, and 10 mg/mL). The mixture was shaken vigorously and left standing at room temperature for 30 min. Then, the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture would indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect} = [(A_0\text{-}A_1)/A] \times 100, \quad (4)$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of cultured mycelia extract.

Chelating Effect on Ferrous Ions

The chelation of ferrous ions of mushroom species was studied through the method of Dinis et al.[15] Briefly, different concentrations of ethanolic extract of mushrooms species (2, 5, and 10 mg/mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by adding 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. All the tests and analyses were carried out in triplicate and then averaged. The percentage inhibition of ferrozine–Fe^{2+} complex formation is given with this formula:

$$\% \text{ Inhibition} = [(A_0\text{-}A_1)/A] \times 100, \quad (5)$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of mushroom species extract and standards. The control did not contain $FeCl_2$ or ferrozine, complex formation molecules.[16]

Determination of Total Phenolics

Total phenolic compounds of ethanolic extract of cultured mycelia were determined with Folin–Ciocalteau reagent according to the method of Slinkard and Singleton[17] using gallic acid as a standard phenolic compound. Briefly, 1 mL of extract solution (containing 1 mg extracts) was transferred into a volumetric flask diluted with distilled water (46 mL); 1 mL of Folin–Ciocalteau reagent was added and the content of the flask was mixed. After the process of mixing (3 min), 3 mL of Na_2CO_3 (2%) was added to the mixture and it was left to be shaken intermittently for 2 h. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the diluted extract was determined as microgram of gallic acid equivalent by using an equation that was obtained from the standard gallic acid graph ($R_2 = 0.995$):

$$\text{Absorbance} = 0.001 \times \text{Total phenols[gallic acid equivalent } (\mu g)] - 0.0154. \quad (6)$$

RESULTS AND DISCUSSION

Total Antioxidant Activity Determination by the β-Carotene–Linoleic Acid Method

Using the β-carotene–linoleic acid method, ethanolic extract of mycelia of *P. ostreatus*, *P. eryngii*, *P. florida*, and *P. sajor-caju* showed different patterns of total antioxidant activities (Table 1). As the concentration increased, the antioxidant activity of extracts increased. At 10 mg/mL concentrations, *P. florida* showed the highest linoleic acid thus preventing capacity against the oxidative stress available in the medium. Antioxidant activity of this mushroom was found to be 62.12% and this is closely followed by *P. eryngii*

Table 1 Antioxidant activity (%) of the ethanolic extracts of cultured mycelia and standards in β-carotene–linoleic acid test system.[a]

Cultured mycelia and standards	Sample concentration (mg/mL)			
	0.5	2.5	5.0	10.0
P. eryngii	45.23 ± 0.02	52.12 ± 0.01	54.25 ± 0.02	60.68 ± 0.78
P. ostreatus	38.05 ± 0.01	43.11 ± 0.00	51.23 ± 0.01	57.19 ± 0.05
P. florida	47.03 ± 0.00	52.09 ± 0.03	57.20 ± 0.02	62.12 ± 0.41
P. sajor-caju	34.04 ± 0.01	41.08 ± 0.01	50.21 ± 0.04	58.81 ± 0.90
Trolox	58.34 ± 0.01	63.12 ± 0.01	69.25 ± 0.02	74.73 ± 0.01
BHT	47.93 ± 0.02	52.11 ± 0.00	58.16 ± 0.02	62.45 ± 0.56
BHA	49.50 ± 0.01	53.29 ± 0.03	60.54 ± 0.02	65.62 ± 0.61
α-tocopherol	27.63 ± 0.00	33.78 ± 0.01	40.76 ± 0.02	47.44 ± 0.06

[a]Values expressed are means ± S.D. of three parallel measurements.

(60.68%), *P. sajor-caju* (58.81%), and *P. ostreatus* (57.19%). In this study, the percentage inhibitions of positive controls were 74.73, 65.62, 62.45, and 47.44% for trolox, BHA, BHT, and α-tocopherol, respectively. Lo[18] found that the antioxidant activity of the ethanolic extracts from the basidiocarp of the *P. ostreatus* and *P. eryngii* was 58.60 and 64.50%, respectively, at 5 mg/mL concentration. Jayakumar et al.[19] mentioned that *P. ostreatus* showed 56.20% antioxidant activity. Total antioxidant activity of methanolic extract of the basidiocarp of *P. ostreatus* was found to be 98.3% by Elmastas et al.[1] From these findings, we conclude that our ethanolic extract of cultured mycelia species showed moderate antioxidant activity on the lipid peroxidation and can be used as a natural antioxidant agent instead of the synthetic antioxidants. The differences between the values obtained from the study of the antioxidant activity may be due to the diversity of mushroom species used in this study.[20]

Determination of Reducing Power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity of putative antioxidants has been attributed to various mechanisms, among which prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging.[21] In the present study, assay of reducing activity was based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples. The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm.[12] Table 2 shows the reducing power of cultured mycelia ethanolic extracts as a function of their concentration. As concentration increased (1–10 mg/mL), the reducing power of the ethanolic extracts from the cultured mycelia increased. At 10.0 mg/mL concentration, the absorbance values were higher than 0.73 for all of the extracts. According to these results, the most active mushroom was *P. eryngii* with an absorbance value of 0.86. At the concentration value of 10 mg/mL, this mushroom was followed by *P. ostreatus*, which was 0.81, *P. florida*, 0.75, and *P. sajor-caju* 0.74. Lo[18] mentioned that at 20 mg/mL, reducing powers of ethanolic extracts of basidiocarps of *P. eryngii* and *P. ostreatus* were 0.75 and 0.61, respectively. Jayakumar et al.[19] reported that the ethanolic extract from *P. ostreatus* showed a reducing power of 1.367 at 10 mg/mL. BHT, BHA, and α-tocopherol exhibited 0.51, 0.49, and 0.99 activities

Table 2 Reducing power of cultured mycelia and standards.[a]

Cultured mycelia and standards	Sample concentration (mg/mL)			
	1.0	2.0	5.0	10.0
P. eryngii	0.05 ± 0.02	0.12 ± 0.01	0.25 ± 0.02	0.86 ± 0.23
P. ostreatus	0.05 ± 0.01	0.11 ± 0.00	0.23 ± 0.01	0.81 ± 0.34
P. florida	0.03 ± 0.00	0.09 ± 0.03	0.20 ± 0.02	0.75 ± 0.23
P. sajor-caju	0.04 ± 0.01	0.08 ± 0.01	0.21 ± 0.04	0.74 ± 0.03
BHT	0.06 ± 0.03	0.15 ± 0.01	0.30 ± 0.02	0.51 ± 0.12
α-tocopherol	0.14 ± 0.01	0.22 ± 0.00	0.53 ± 0.00	0.99 ± 0.21
BHA	0.09 ± 0.00	0.14 ± 0.01	0.17 ± 0.07	0.49 ± 0.16

[a]Values expressed are means ± S.D. of three parallel measurements.

at 10 mg/mL, respectively. Except for α-tocopherol, reducing powers of all of the ethanolic extracts of cultured mycelia were superior to the BHT and BHA. It was clearly stated by the researchers[1,18] that the synthetic antioxidants, such as BHT, BHA, and TBHQ, have mutagenic activity. Therefore, the antioxidant capacity of natural resources could be used as an alternative to these compounds mentioned.

Scavenging Effect on Superoxide Anion Radicals

As it was presented in Table 3, the superoxide radical-scavenging activity of the ethanolic extracts of the cultured mycelia of *P. ostreatus*, *P. florida*, *P.sajor-caju*, and *P. eryngii* was measured by the riboflavin-light system *in vitro*. The superoxide radical is known to be very harmful to cellular components since it is a precursor of more reactive oxygen species.[22] Photochemical reduction of flavin generates superoxide anion radicals, which could reduce NBT, resulting in the formation of blue formazan.[23] In the present study, cultured mycelia extracts were found to be notable scavengers of superoxide radicals generated in the riboflavin-NBT light system. As in other tests, superoxide radical-scavenging activity of ethanolic extracts from cultured mycelia increased with increasing concentrations (1–10 mg/mL). The extracts in a concentration of 10 mg/mL inhibited the formation of blue formazan and the percentages of inhibitions were 76.14 for *P. ostreatus*, 74.77 for *P. florida*, 75.91 for *P. sajor-caju*, and 67.92 for *P. eryngii*. Etahnolic extract from *P. ostreatus*, *P. florida*, and *P. sajor-caju* proved to be better at scavenging superoxide anion radicals than *P. eryngii* at scavenging superoxide anion radicals This may be explained by

Table 3 Superoxide anion radical scavenging effect (%) of cultured mycelia and standards.[a]

Cultured mycelia and standards	Sample concentration (mg/mL)			
	1.0	2.0	5.0	10.0
P. eryngii	12.67 ± 0.09	30.14 ± 0.12	48.29 ± 0.32	67.92 ± 0.23
P. ostreatus	17.69 ± 0.08	27.17 ± 0.09	51.94 ± 0.076	76.14 ± 0.34
P. florida	16.21 ± 0.01	30.25 ± 0.11	44.18 ± 0.10	74.77 ± 0.76
P. sajor-caju	18.72 ± 0.02	25.34 ± 0.15	52.74 ± 0.87	75.91 ± 0.62
BHA	91.10 ± 0.06	91.89 ± 0.08	94.86 ± 0.76	95.21 ± 0.29
α-tocopherol	91.89 ± 0.05	92.69 ± 0.02	94.75 ± 0.23	96.35 ± 0.85

[a]Values expressed are means ± S.D. of three parallel measurements.

the interaction of the different flavonoids in these extracts.[13] BHA and α-tocopherol were used as positive controls for comparison and displayed excellent activity and they were 95.21 and 96.35%, respectively. All of the tested concentration of standards showed higher scavenging activity than ethanolic extracts of cultured mycelia. Jayakumar et al.[19] found that the superoxide anion radical scavenging activity of ethanolic extract of *P. ostreatus* basidiocarp was 60.02% and Elmastas et al.[1] found it to be 87%.

Free Radical Scavenging Activity

The model of scavenging the stable DPPH radical is a method that is widely used to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability.[24] DPPH is a stable free radical and accepts an electron or hydrogen radical in order to become a stable diamagnetic molecule.[25] The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The maximum absorption of a stable DPPH radical in ethyl alcohol was at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidants because of the reaction between antioxidant molecules and the radical, thus resulting in the scavenging of the radical by hydrogen donation. A discoloration from purple to yellow is visually noticeable. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants.[26] Scavenging abilities of cultured mycelia ethanolic extracts sharply increased from 16.0 to 61.97%, from 16.74 to 68.01%, from 16.74 to 62.82%, and from 19.92 to 71.29% at 1.0 to 10 mg/mL for *P. florida*, *P. eryngii*, *P. sajor-caju*, and *P. ostreatus*, respectively (Table 4). BHA and α-tocopherol exhibited higher radical scavenging activity than cultured mushroom mycelia extracts at all the concentrations and their DPPH radical scavenging activities were found to be 94.70 and 95.76% at 10 mg/mL concentrations, respectively. Elmastas et al.[1] found that the methanolic extract from *P. ostreatus* showed a high scavenging ability of 81.3% at 200 μg/mL. Lo[18] mentioned that the ethanolic extracts from *P. ostreatus* and *P. eryngii* scavenged DPPH radicals with 74.4 and 92.2% at 5 mg/mL, respectively. The findings of researchers mentioned above are higher than ours. These results revealed that ethanolic extracts of cultured mycelia were free radical scavengers, acting possibly as primary antioxidants. Obviously, the extracts contained antioxidant components, which could react rapidly with DPPH radicals, and reduce more DPPH radical molecules. Ethanolic extracts from cultured mycelia might react with free radicals, which are the major initiators of the autoxidation chain of fat, thereby terminating the chain reaction.[27]

Table 4 Scavenging effect (%) of cultured mycelia and standards on 1,1-diphenyl-2-picrylhydrazyl.[a]

	Sample concentration (mg/mL)			
Cultured mycelia and standards	1.0	2.0	5.0	10.0
P. eryngii	16.74 ± 0.24	31.04 ± 1.23	52.01 ± 1.52	68.01 ± 1.39
P. ostreatus	19.92 ± 0.65	32.42 ± 1.98	58.16 ± 2.30	71.29 ± 1.21
P. florida	16.00 ± 0.52	27.44 ± 2.05	46.82 ± 0.61	61.97 ± 1.87
P. sajor-caju	16.74 ± 0.62	28.18 ± 1.01	47.14 ± 1.01	62.82 ± 0.48
BHA	91.31 ± 0.11	92.16 ± 0.54	93.64 ± 1.76	94.70 ± 0.56
α-tocopherol	92.06 ± 0.48	93.11 ± 0.65	94.49 ± 2.87	95.76 ± 2.43

[a]Values expressed are means ± S.D. of three parallel measurements.

Metal Chelating Activity

Transition metals have been proposed as the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in living systems and inhibit a generation of radicals, consequently reducing free radical-induced damage. To better estimate the antioxidant potential of the cultured mycelia extracts, its chelating activity was evaluated against Fe^{2+}. The chelating effects of the ethanol extract of cultured mycelia and standard antioxidants on ferrous ions increased with increasing concentrations (Table 5). In this assay, ethanolic extracts of cultured mycelia and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by the Fenton reaction, and also can accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation.[28] The chelating effects of the cultured mycelia were compared with α-tocopherol and BHT as standards. As can be seen from the Table 5, chelating capacity of the ethanol extracts was increased with the increasing concentration. At all of the concentrations studied (2.0, 5.0, and 10 mg/mL), ethanolic extracts of mycelia showed higher chelating activites than the α-tocopherol and BHT. The chelating effects of ethanolic extracts from cultured mycelia species and standards on the ferrous ions decreased in the order of *P. sajor-caju* > *P. eryngii* > *P. ostreatus* > *P. florida* > α-tocopherol > BHT and values were 69.5, 68.2, 65.6, 57.3, 35.9, and 20.5% at the concentration of 10 mg/mL, respectively. The chelating activity of methanolic extract from *P. ostreatus* was found as 62.5% at 100 μg/mL. Elmastas et al.[1] and Jayakumar et al.[19] reported the activity as 60.68% at 10 mg/mL concentration for the ethanolic extracts of *P. ostreatus* basidiocarp. The percentage chelating activity of ethanolic extracts from *P. eryngii* and *P. ostreatus* basidiocarp at 5 mg/mL was reported as 41.4 and 64.0 by Lo.[18]

Determination of Total Phenolic Compounds

Total phenolic compounds the major natural antioxidant components, found in the ethanolic extracts from cultured mycelia and their contents, were in order of *P. florida* > *P. eryngii* > *P. ostreatus* > *P. sajor-caju* (Table 6). Phenols are important plant constituents due to their hydroxyl groups thus having scavenging ability.[29] The phenolic compounds may contribute directly to the antioxidative action.[26] In addition, it was reported that

Table 5 Chelating effect (%) of cultured mycelia and standards.[a]

	Sample concentration (mg/mL)		
Cultured mycelia and standards	2.0	5.0	10.0
P. eryngii	20.20 ± 1.25	39.90 ± 1.43	68.20 ± 2.98
P. ostreatus	10.60 ± 1.01	35.80 ± 0.92	65.60 ± 1.12
P. florida	6.70 ± 0.65	32.80 ± 1.10	57.30 ± 1.34
P. sajor-caju	20.30 ± 0.87	41.00 ± 0.54	69.50 ± 1.29
Trolox	0.90 ± 0.20	5.70 ± 1.23	12.20 ± 1.27
α-tocopherol	12.10 ± 1.15	23.20 ± 1.43	35.90 ± 1.62
BHT	7.70 ± 1.12	14.60 ± 1.65	20.50 ± 2.56
BHA	10.70 ± 1.20	13.90 ± 0.12	18.90 ± 1.07

[a]Values expressed are means ± S.D. of three parallel measurements.

Table 6 Total phenol content of cultured mycelia.[a]

Cultured mycelia	Total phenol mg/g (dry weight)
P. eryngii	4.45 ± 0.03
P. ostreatus	4.37 ± 0.10
P. florida	4.56 ± 0.15
P. sajor-caju	3.97 ± 0.29

[a]Values expressed are means ± S.D. of three parallel measurements.

phenolic compounds were associated with antioxidant activity and played an important role in stabilizing lipid peroxidation.[26] Polyphenols, such as BHT and gallate, were known to be effective antioxidants.[29] Because of the similarities of total phenolic content of ethanolic extracts from cultured mycelia, they showed close activities in the antioxidant tests taken under study. Numerous studies have conclusively showed that food consumption with high phenolic content can reduce the risk of heart disease by slowing the progression of atherosclerosis, since they act as antioxidants.[30,31] From the study, we conclude that the cultured mycelia of *P. florida*, *P. eryngii*, *P. ostreatus*, and *P. sajor-caju* mushroom species showed effective antioxidant activity as basidiocarps of these mushrooms.[1,17,18]

CONCLUSION

The various antioxidant mechanisms of the ethanolic extracts of cultured mycelia may be attributed to strong hydrogen-donating ability, metal-chelating ability, and their effectiveness as good scavengers of superoxide and free radicals. In the present study, the antioxidant properties of ethanol extracts from cultured mycelia of *P. florida*, *P. eryngii*, *P. ostreatus*, and *P. sajor-caju* were described by using a series of testing systems *in vitro* like the ones mentioned above. According to the results of this study, it is clearly seen that the ethanolic extract of cultured mycelia of *P. florida*, *P. eryngii*, *P. ostreatus*, and *P. sajor-caju* 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 the pharmaceutical industry. Furthermore, it is known that production of mycelia is easier than the production of fungus basidiocarps under laboratory conditions. Therefore, these cultured mycelia species can be produced in a desired level in huge fermentors instead of basidiocarp production of these mushrooms. In order to investigate the antioxidant mechanism of some potential antioxidant molecules, the fractionation and the identification of the ethanolic extract containing the low molecular weight compounds are in progress.

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