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RESEARCH ARTICLE

Antioxidant Activity of Various Extracts from an Edible Mushroom *Pleurotus eous*

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Abstract *Pleurotus eous* is a pink edible oyster mushroom cultivated in Coimbatore. The aim of present study was to evaluate antioxidant potential of ethyl acetate, methanol, and hot water extracts from *P. eous* using various *in vitro* ROS/RNS generated chemical and biological models. Results demonstrated that the graded-dose (2-50 mg/mL) of various extracts markedly scavenged the DPPH, ABTS, and hydroxyl radicals, and showed metal chelating ability, reducing capacity in Fe^{3+}/ferricyanide complex, and ferric reducing antioxidant power assays. In biological models, different extracts were found to inhibit lipid peroxidation in brain and liver homogenate. Total antioxidant activity was high in methanolic extract. Hot water extract showed higher amount of total phenol and methanolic extract showed higher amount of flavonoid as compared to other extracts. Based on EC_{50} values it can be concluded that the various extracts from *P. eous* were good in antioxidant properties with methanol and ethyl acetate extracts being more effective. The results of this study showed that various extracts could serve as natural antioxidants owing to their significant antioxidant activity.

Keywords: *Pleurotus eous*, phenol, flavonoid, antioxidant activity, lipid peroxidation

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Introduction

Mushrooms are worldwide appreciated for their taste and flavor and are consumed both in fresh and processed form. From a nutritional point of view, mushrooms are not rich in protein or fat but they contain appreciable amounts of dietary fiber, particularly important for the regulation of physiological functions in the human organism (1). Studies have demonstrated that the regular consumption of mushrooms or consumption of isolated bioactive constituents present in mushrooms is beneficial to health. The antioxidants present in dietary mushrooms are of great interest as possible protective agents to help the human body reduces oxidative damage without any interference. Mushrooms may thus be considered as functional food or nutraceutical product (2-4).

Mushrooms accumulate a variety of secondary metabolites such as phenolic compounds, polyketides, terpenes, and steroids possibly involved in their medicinal effects and functional values (5). Some common edible mushrooms, which are widely consumed in Asian culture, have currently been found to possess antioxidant activity which is well correlated with their total phenolic content (6).

Edible mushrooms have been widely used as human food for centuries and have been appreciated for texture and flavor as well as some medicinal properties. However, the awareness of mushrooms as being a healthy food and as an important source of biological active substances with medicinal value has only recently emerged. Various activities of mushrooms have been studied which include antibacterial, antifungal, antioxidant, antiviral, antitumour, cytostatic, immunosuppressive, antiallergic, antiatherogenic, hypoglycemic, anti-inflammatory, and hepatoprotective activities (7).

Pleurotus species have been used by human cultures all over the world for their nutritional value, medicinal

properties, and other beneficial effects. Oyster mushrooms 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 (8).

The mushroom *P. eous* has been more often used as a food than for medicinal purposes and this attracted our curiosity to investigate the antioxidant activity of the mushroom. The main objectives of this study were to evaluate the antioxidant potential of the ethyl acetate, methanol, and hot water extracts of *P. eous* by 9 different antioxidant test systems namely DPPH, OH, $ABTS^{-+}$ radicals, reducing power, chelating effect, ferric reducing antioxidant power (FRAP), β-carotene/linoleic acid, phosphomolybdenum assay, and lipid peroxidation inhibition and to determine phenolic and flavonoid contents.

Materials and Methods

Extraction The fruiting bodies of *Pleurotus eous* (pink mushroom) were collected from local farm in Coimbatore. The fruiting bodies were dried at 60ºC to constant weight and then powdered to pass through a 20 mesh sieve and stored in air-tight plastic bags for further analysis.

Four different solvents (petroleum ether, ethyl acetate, methanol, and water) were used to fractionate the soluble compounds from the mushrooms in ascending polarity. Petroleum ether has a defatting property that removes the lipid in mushrooms before fractionation of the extracts. Mushroom powders (10 g) were extracted by using a Soxhlet extractor for 3 h with 100 mL of extractant, including petroleum ether, ethyl acetate, and methanol under reflux conditions. The residues were then extracted by boiling water (100 mL). The organic solvent in the extracts was removed with a rotary evaporator. The water extract was dried in a freeze-drier. As the yield of the petroleum ether was very low, only the ethyl acetate, methanol, and water extracts were analyzed for their antioxidant activities. The dried extract was stored at 4ºC in amber bottles until used within a maximum period of 1 week. Each extracts were dissolved in their respective solvent accordingly, to a concentration of 50 mg/mL and used directly for analysis of antioxidant components and then diluted to prepare a series of concentrations for antioxidant assays.

Chemicals and reagents DPPH, ABTS, β-carotene, and rutin were obtained from Sigma-Aldrich (Bangalore, India). Ferrozine, gallic acid, catechin, linoleic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 2,4,6-tripyridyl-s-triazine (TPTZ), ascorbic acid, Tween 80, and thiobarbituric acid (TBA) were obtained from Himedia (Mumbai, India). Potassium ferricyanide, ferric chloride, trichloroacetic acid (TCA), aluminium chloride, potassium persulphate, ammonium persulphate, ferrous suphate, hydrogen peroxide, sodium salicylate, ammonium molybdate, sodium carbonate, aluminium chloride, sodium nitrate, sodium hydroxide, Folin-Ciocalteu's phenol reagent, ferrous chloride, and solvents were obtained from Merck (Mumbai, India).

Determination of total phenolics The total phenolics in various mushroom extracts were measured according to the method of Singleton and Rossi (9) with some modifications. One mL of the sample was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated Na_2CO_3 (35%) was added to the mixture and it was made up to 10 mL by adding deionised distilled water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured at 725 nm against the reagent blank. Gallic acid was used as the reference standard. The total phenolic content is expressed as mg of gallic acid equivalents (GAE)/g of extract.

Determination of total flavonoids Total flavonoid content was determined as described by Jia *et al*. (10). A portion (0.25 mL) of various extracts was diluted with 1.25 mL of distilled water. A portion (75 µL) of a 5% $NaNO_2$ solution were added and after 6 min 150 µL of a 10% $AlCl_3 \cdot H_2O$ were added and mixed. After 5 min, 0.5 mL of 1 M NaOH was added. The absorbance was measured immediately against the reagent blank at 510 nm. Catechin was used as the reference standard. The total flavonoid content is expressed as mg of catechin equivalents (CAE)/g of extract.

Phosphomolybdenum assay The antioxidant activity of the sample was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (11). An aliquot of 0.1 mL of sample solution was mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped with silver foil and incubated at 95ºC for 90 min. The tubes were cooled to room temperature and the absorbance of aqueous solution was measured at 695 nm against a blank. Gallic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of gallic acid.

DPPH radical scavenging activity The scavenging effect of mushroom extract on DPPH radicals was determined

according to the method of Shimada *et al*. (12). Various concentrations of sample (4 mL) were mixed with 1 mL of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Absorbance of the radical without antioxidant was used as control. The amount of sample necessary to decrease the absorbance of DPPH by 50% (EC_{50}) was calculated graphically. The percentage inhibition was calculated according to the formula: $(A_0-A_1)/A_0]\times100$, where, A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

ABTS radical cation scavenging activity The ABTS radical cation scavenging activity was performed with slight modifications described by Re *et al*. (13). The ABTS radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700±0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 μL of test sample with 1.0 mL of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly after 6 min. The percentage inhibition was calculated according to the formula: $[(A_0-A_1)/A_0]\times100$, where, A_0 was the absorbance of the control, and A_1 was the absorbance of the sample.

Reducing power The reducing power of the mushroom extract was determined by the method of Oyaizu (14). Various concentrations of mushroom extracts (2.5 mL), phosphate buffer (2.5 mL, 0.2 M, pH 6.6), and 1% potassium ferricyanide (2.5 mL) were mixed and incubated at 50°C for 20 min. Ten % TCA (2.5 mL) was added to the mixture. The mixture was centrifuged at 3,750×*g* for 10 min. A portion (2.5 mL) of the supernatant was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. After 10 min of incubation, the absorbance was measured at 700 nm against a blank.

Ferric reducing antioxidant power (FRAP) FRAP assay was measured according to the procedure described by Benzie and Strain (15). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3\cdot6H_2O$, and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37°C. A 900 μL FRAP reagent was mixed with 90 μL water and 30 μL of the extract. The reaction mixture was incubated at 37°C for 30 min and the absorbance was measured at 593 nm.

Ferrous ion chelating ability The ability of the mushroom extract to chelate ferrous ions was estimated by the method of Dinis *et al.* (16). Briefly, 2 mL of various concentrations of the extracts in methanol were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0-A_1)/A_0]\times100$, where, A_0 was the absorbance of the control, and A_1 of the mixture containing the extract or the absorbance of a standard solution.

β-Carotene bleaching assay The antioxidant activity of extracts was evaluated by the β-carotene linoleate model system according to the method of Yae *et al.* (17). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two mL of this solution were pipetted into a 100-mL round-bottom flask. After the chloroform was removed at 40°C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask with vigorous shaking. A 4.8 mL of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of the mushroom extracts. The tubes were shaken and incubated at 50°C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/ initial β-carotene content)×100.

Hydroxyl radical scavenging assay Hydroxyl radical scavenging activity of extracts was assayed by the method of Smirnoff and Cumbes (18). The reaction mixture 3.0 mL contained 1.0 mL of 1.5 mM $FeSO_4$, 0.7 mL of 6 mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate, and varied concentrations of the extracts. After incubation for 1 h at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The scavenging activity of hydroxyl radical effect was calculated as follows: $[1-(A_1-A_2)/A_0]\times100$, where, A_0 is absorbance of the control (without extract), A_1 is the absorbance in the presence of the extract, and A_2 is the absorbance without sodium salicylate.

Lipid peroxidation inhibition assay in rat brain homogenate Lipid peroxidation inhibition was evaluated according to the method of Ng *et al*. (19) with some modifications. Brains of Sprague Dawley (SD) rats were dissected and homogenized in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain homogenate which was centrifuged at 3,000×*g* for 10 min. A 0.1 mL

aliquot of the supernatant was incubated with 0.2 mL of the various extracts in the presence of 0.1 mL of 10 μM $FeSO_4$ and 0.1 mL of 0.1 mM ascorbic acid at 37ºC for 1 h. The reaction was stopped by the addition of 0.5 mL of 28% TCA, followed by 0.38 mL of 2% TBA, and the mixture was heated at 80ºC for 20 min. After centrifugation at 3,000×*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%)=[(A-B/A)]×100, where, A and B were the absorbance of the control and the sample, respectively.

Lipid peroxidation inhibition assay in rat liver homogenate The lipid peroxide formed was estimated by measuring TBA reacting substances (TBARS) by a modified procedure of Okhawa *et al*. (20). Rat liver was processed to get 10% homogenate in cold phosphate buffered saline, pH 7.4. Different concentration of the extracts was added to the liver homogenate. Lipid peroxidation was initiated by adding 100 μL of 15 mM ferrous sulphate solution to 3.0 mL of the tissue homogenate. After 30 min, 100 μL of this reaction mixture was taken in a tube containing 1.5 mL of 10% TCA. After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5 mL of 0.67% TBA in 50% acetic acid. The mixture was heated for 30 min in a boiling water bath. The intensity of the pink colored complex formed was measured at 535 nm.

Statistical analysis All assays were carried out in triplicates and results are expressed as mean±standard deviation (SD). The data were analysed using SPSS software. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used to analyze the differences among scavenging activity and EC_{50} of various extracts for different antioxidant assays with least significance difference (LSD) $p<0.05$ as a level of significance. Experimental results were further analyzed for Pearson correlation coefficient of phenolic, flavonoids with different antioxidant assays.

Results and Discussion

Extraction yield, total phenolic content, total flavonoid content, and total antioxidant activity The extraction yield, total phenolic content, total flavonoid content and total antioxidant activity of various extracts of *P. eous* are presented in Table 1. The yield of the extracts varied from 1.13 to 41.56 %. The yield of various extracts is presented in the following order: hot water > methanol > ethyl acetate. This implies that most of the soluble components in mushrooms were high in polarity. There was significant difference in the extraction yield ($p<0.05$).

The total phenolic content of various mushroom extracts varied from 7.00 to 8.77 mg GAE/g (Table 1). Total phenolic content of various extracts is arranged in the following descending order: hot water > methanol > ethyl acetate ($p<0.05$). Several studies have revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (21).

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants (22). The total flavonoid content of various extracts of *P. eous* varied considerably from 6.38 to 7.79 mg CAE/g extract (Table 1). Total flavonoid content of various extracts is arranged in the following sequence: methanol > hot water > ethyl acetate ($p<0.05$). Flavonoids are the major phenolic compounds present in the mushroom extract.

Total antioxidant capacity assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Different mushroom extracts exhibited various degrees of antioxidant capacity. Total antioxidant activity varied between 1.98-5.84 mg GAE/g extract (Table 1). Methanol extract showed higher activity followed by ethyl acetate and hot water extracts.

DPPH radical scavenging activity The DPPH free radical is a stable free radical, which has been widely

Table 1. Extraction yield, total phenols, total flavonoids, and total antioxidant capacity of various extracts from *Pleurotus eous*

Extract	Extraction yield (%)	Total Phenols (mg GAE/g)	Total flavonoids (mg CAE/g)	Total antioxidant activity (mg GAE/g)
Ethyl acetate	1.13±0.03[a1)]	7.00±0.22[a]	6.38±0.07[a]	3.96±0.06[b]
Methanol	15.32±0.66[b]	7.17±0.22[a]	7.79±0.04[c]	5.84±0.05[c]
Hot water	41.56±1.01[c]	8.77±0.22[b]	7.03±0.07[b]	1.98±0.15[a]

[1)]Values are expressed as mean±SD ($n=3$); Means within the same column not sharing common superscript letters (a-c) differ significantly at 5% level by DMRT.

accepted as a tool for estimating the free radical scavenging activities of antioxidants (23). The DPPH scavenging activities of various extracts were presented in Fig. 1A. The different extracts of *P. eous* on DPPH radicals increased with increase in concentration. The ethyl acetate, methanolic, and hot water extracts of the mushroom were seen to scavenge the stable DPPH radical directly to different extent over a concentration range of 2-10 mg/mL with an inhibition percentage of 7.40-78.40, 19.57-85.19, and 12.42-70.21%, respectively. At 10 mg/mL, the DPPH scavenging activities of methanol extract was more effective followed by ethyl acetate and hot water extract. The commercial antioxidants used were BHT, BHA, and ascorbic acid. In comparison, at 0.2 mg/mL, scavenging abilities of BHA, BHT, and ascorbic acid were 86.88, 98.08, and 98.09%, respectively. The better scavenging ability of the mushroom extract might be due to more hydrogen-donating components extracted from the mushroom. The various extracts contained active substances including phenolic compounds that had a high hydrogen-donating capacity to scavenge DPPH radicals as possible mechanism for their antioxidant activities.

ABTS radical scavenging activity The ABTS radical scavenging test is widely used to determine the antioxidant activity of both hydrophilic and lipophilic compounds. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants (24). At 10-50 mg/mL, scavenging abilities of ethyl acetate, methanolic, and hot water extracts on ABTS radicals increased from 22.87-81.52, 35.58-97.21, and 26.62-90.65%, respectively (Fig. 1B). The scavenging activity was better in methanol extract when compared to hot water and ethyl acetate extract at 50 mg/mL. Therefore, the ABTS radical scavenging activity of various extract indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

Reducing power assay This assay compares antioxidants based on their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion through the donation of an electron, with the resulting ferrous ion (Fe^{2+}) formation monitored spectrophotometrically at 700 nm. A given antioxidant donates electrons to reactive free radicals species, thus promoting the termination of free radical chain reactions. The ability of the antioxidant to reduce Fe^{3+} to its more active Fe^{2+} form might also be indicative of its ability to act as a prooxidant in the system. The different extracts from mushrooms exhibited a dose dependent reducing power activity at various concentrations. The reducing powers of ethyl acetate, methanolic, and hot water extracts of *P. eous* were 0.956, 0.587, and 0.673 at 2 mg/mL and the extracts

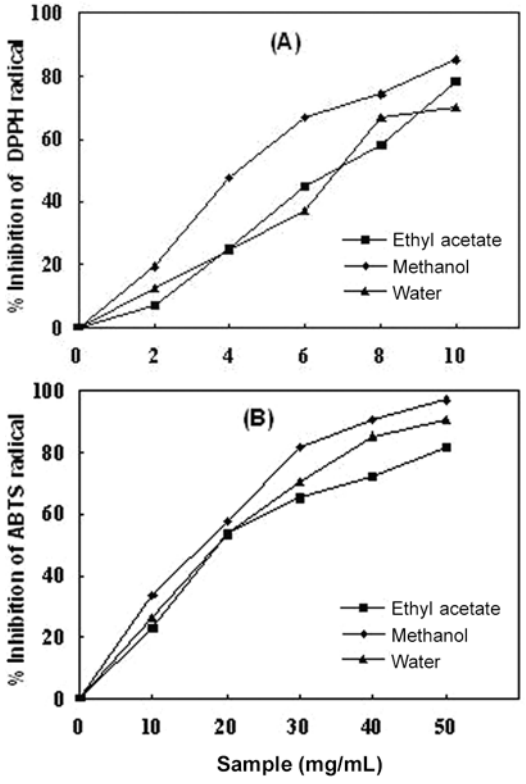

Fig. 1. DPPH (A) and ABTS (B) radical scavenging activity of various extracts from *P. eous*.

showed an excellent reducing power of 1.950, 1.132, and 1.632 at 10 mg/mL, respectively (Fig. 2A). Ethyl acetate extracts revealed highest reducing power followed by hot water and methanol extract at 10 mg/mL. The reducing powers were 1.130, 0.413, and 1.037 for ascorbic acid, BHT and BHA at 0.1 mg/mL, respectively. It was reported that the reducing power of mushrooms might be due to their hydrogen-donating ability (12). Accordingly, *P. eous* might contain higher amounts of reductones, which could react with free radicals to stabilize and block radical chain reactions.

Ferric reducing antioxidant power assay The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Antioxidant potential of mushroom extracts was estimated from the ability to reduce TPTZ-Fe (III) to TPTZ-Fe (II) complex (15). At 2 mg/mL, the ferric reducing power of ethyl acetate extract was 0.635 whereas those of methanol and hot water extracts were 0.250 and 0.597, respectively. The ferric reducing power of ethyl acetate, methanol, and hot water extracts was 1.114, 0.817, and 1.156 at 10 mg/mL, respectively (Fig. 2B). The ferric ion reducing power of hot water extract was more effective than the methanol extract and comparable to the ethyl acetate extract. The results reveal that various extracts possessed hydrogen-donating

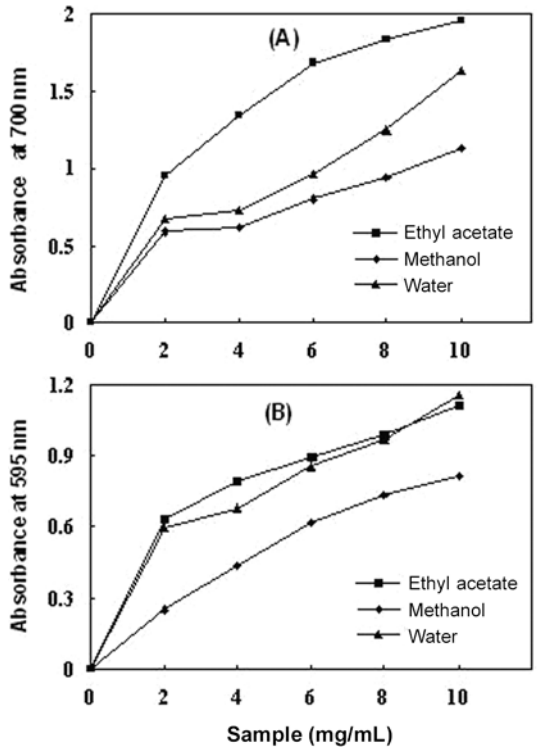

Fig. 2. Reducing power (A) and FRAP (B) assay of various extracts from *P. eous*.

capacity indicating the significant reducing power of the extract.

Ferrous ion chelating ability In this assay, the chelating agents disrupt the ferrozine-Fe^{2+} complex, thus decreasing the red color. The measurement at 550 nm of the rate of color reduction, therefore, allows estimation of the chelating activity. A lower absorbance indicates higher chelating ability. The chelating actions of the various extracts of *P. eous* on ferrous ions increased with their concentrations (Fig. 3). The ethyl acetate, methanolic, and hot water extracts of mushroom were shown to chelate the ferrous ion to different extent over a concentration range of 2-10 mg/mL with an inhibition percentage of 17.59-75.80, 19.97-69.88, and 21.10-63.46%, respectively. The ferrous ion chelating activities were effective in the order: ethyl acetate > methanol > hot water at 10 mg/mL. EDTA showed a high chelating ability of 99.3% at 0.5 mg/mL. Metal chelating activity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (25). The data obtained reveal that various extracts demonstrate an effective capacity for iron binding, suggesting its action as peroxidation inhibitor that may be related to its iron-binding capacity. Since ferrous ions are the most effective prooxidants that promote the oxidation of lipid in food systems (26) the higher chelating effect of mushroom extracts would be beneficial.

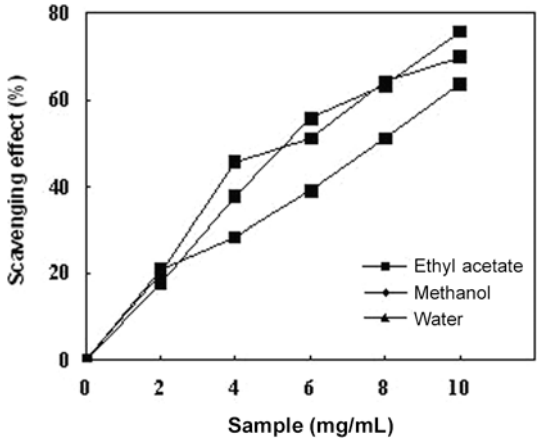

Fig. 3. Chelating activity of various extracts from *P. eous*.

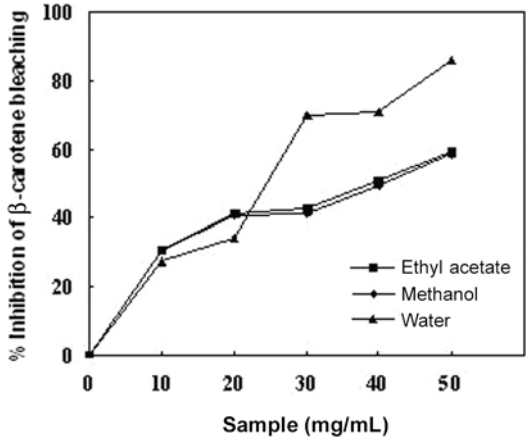

Fig. 4. β-Carotene bleaching activity of various extracts from *P. eous*.

β-Carotene-linoleic acid assay In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (27). The ethyl acetate, methanolic, and hot water extracts of mushroom showed scavenging activity to different extent over a concentration range of 10-50 mg/mL with an inhibition percentage of 30.61-59.23, 30.48-58.52, and 27.27-86.31%, respectively (Fig. 4). Hot water extract was better in their effect on reducing the oxidation of β-carotene than ethyl acetate and methanol at 50 mg/mL, and the degradation rate of β-carotene clearly depends on the antioxidant activity. It is probable that the antioxidative components in the mushroom extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system.

Hydroxy radical (·OH) scavenging activity The ·OH scavenging activity of mushroom extracts was assessed by its ability to compete with salicylic acid for ·OH in the ·OH generating/detecting system. The ethyl acetate, methanolic,

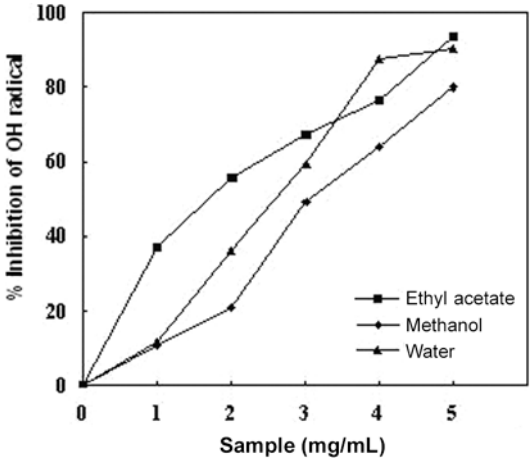

Fig. 5. Hydroxy radical scavenging activity of various extracts from *P. eous*.

and hot water extracts of *P. eous* were shown to scavenge the ·OH directly to different extent over a concentration range of 1-5 mg/mL with an excellent inhibition percentage of 37.31-93.88, 10.93-80.00, and 11.84-90.20%, respectively (Fig. 5). When compared to all the mushroom extracts, the ethyl acetate had the highest ·OH scavenging activity followed by hot water and methanol extract at 5 mg/mL. Hydroxy radicals are known to be capable of abstracting hydrogen atoms from membrane and bring about peroxidic reactions of lipids (28). From this it was expected that the extracts from mushroom would show antioxidant effects against LPO on biomembranes and scavenge the ·OH and superoxide anions at the stage of initiation and termination of peroxy radicals. Overall the scavenging might be due to the active hydrogen donating ability of hydroxy substitutions.

Lipid peroxidation inhibition assay in rat brain and liver homogenate The process of lipid peroxidation is initiated by the abstraction of a hydrogen atom in an unsaturated fatty acyl chain and propagated as a chain reaction (29,30). Therefore, inhibition of lipid peroxidation is of great importance in the disease processes involving free radicals. The production of lipid peroxides by Fe^{2+}-ascorbate system in rat brain homogenate was strongly inhibited by the various mushroom extracts in a dose-dependent manner.

The ethyl acetate, methanolic, and hot water extracts of mushroom were shown to inhibit brain lipid peroxidation in a concentration range of 0.04-0.2 mg/mL with an inhibition percentage of 16.36-75.38, 19.80-75.04, and 7.72-76.54%, respectively (Fig. 6A). The lipid peroxidation inhibition was equally effective in all the extracts at 0.2 mg/mL.

At 2-10 mg/mL, the ethyl acetate, methanol, and hot water extracts of mushroom exhibited lipid peroxidation inhibition in liver homogenate with an inhibition percentage

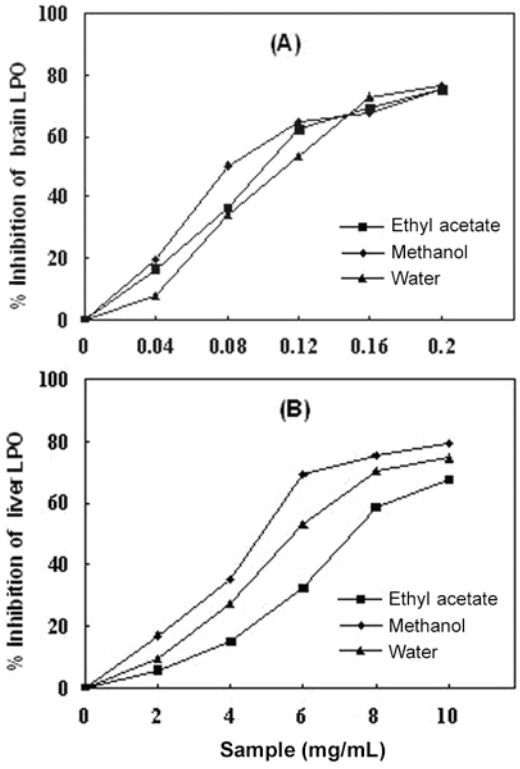

Fig. 6. Lipid peroxidation inhibition in rat brain (A) and liver homogenate (B) of various extracts from *P. eous*.

of 5.65-67.74, 16.92-79.23, and 9.57-74.78%, respectively. The lipid peroxidation inhibition was effective in the order: methanol > hot water > ethyl acetate at 10 mg/mL (Fig. 6B). The extracts exhibited a strong scavenging effect of hydroxyl radical generated from Fe^{2+}-ascorbate system indicating lipid peroxidation inhibiting activity.

EC_{50} values in antioxidant properties The antioxidant properties assayed herein are summarized in Table 2 and the results expressed as EC_{50} values (mg/mL) for comparison.

With regard to the scavenging ability on DPPH radicals, various extracts were effective in the order: methanol > ethyl acetate > hot water. Effectiveness in reducing power was in the descending order: ethyl acetate > methanol > hot water whereas with regard to chelating ability on ferrous ions it was ethyl acetate > methanol > hot water. The ABTS scavenging activities were effective in the order: methanol > ethyl acetate > hot water. The ferric reducing power of mushroom was effective in the order: ethyl acetate > hot water > methanol. The hydroxy radical scavenging activities were effective in the order: ethyl acetate > hot water > methanol. With regard to β-carotene bleaching, the hot water is more effective followed by ethyl acetate and methanol. The descending order of their ability to inhibit lipid peroxidation in liver homogenate were methanol, followed by ethyl acetate and hot water whereas in brain homogenate it was methanol > hot water > ethyl acetate.

Table 2. EC_{50} values[1)] of various extracts from *P. eous* for antioxidant properties

Extracts	DPPH	RP	Chelation	β-Bleaching	ABTS	FRAP	OH	LPO (brain)	LPO (liver)
Ethyl acetate	6.3±0.12[2)]	1.0±0.11	5.2±0.11	40.0±0.18	19.0±1.20	1.5±0.12	1.7±0.09	0.10±0.003	0.74±0.04
Methanol	4.2±0.14	1.3±0.10	5.6±0.13	43.0±0.21	17.0±0.39	4.5±0.26	3.1±0.08	0.08±0.003	0.48±0.01
Hot water	7.0±0.23	1.4±0.15	7.8±0.16	26.0±0.90	19.0±0.57	1.6±0.10	2.6±0.06	0.12±0.004	0.58±0.02

[1)]EC_{50} value was obtained by interpolation from linear regression analysis; RP, reducing power; LPO, lipid peroxidation
[2)]Values are expressed as mean±SD (n=3).

Generally, EC_{50} values of lower than 10 mg/mL indicated that the various extracts were effective in antioxidant properties. Among the antioxidant properties assayed, the methanol extract was effective in scavenging DPPH, ABTS radicals, and lipid peroxidation inhibition in brain and liver homogenate. The ethyl acetate extract was most effective in reducing power, FRAP, in chelating ferrous ions and scavenging hydroxyl radical. The hot water extract inhibited the bleaching of β-carotene.

The results from various antioxidant capacity assays reveal that the antioxidant activity of the mushroom extracts was concentration dependent and all the extracts exhibited strong antioxidant properties. *P. eous* extracts could terminate free radical chain reaction *in vitro*. Though other antioxidants were probably present in the mushroom extracts, phenolic compound in the extracts were responsible for their effective antioxidant properties. Antioxidant activity of the edible mushrooms has significant importance because this activity greatly contributes to their nutraceutical properties, thus enhancing their nutritional value. *P. eous* thus significantly contributes to the protective role in free radical related pathogenesis and could be considered as effective nutraceuticals or functional food.

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