Food Sci. Biotechnol. 25(2): 371-377 (2016)
DOI 10.1007/s10068-016-0052-4

Food Science and Biotechnology

Comparative Study on the Antioxidant Activity of Methanolic and Aqueous Extracts from the Fruiting Bodies of an Edible Mushroom *Pleurotus djamor*

Govindan Sudha*, Amal Janardhanan, Aswini Moorthy, Meena Chinnasamy, Sasikala Gunasekaran, Alamelu Thimmaraju, and Jayanthi Gopalan[1]

Department of Biochemistry, School of Biosciences, Periyar University, Salem 636011, India
[1]*Department of Biochemistry, Government Arts College for Women, Krishnagiri 635002, India*

Received May 23, 2015
Revised September 13, 2015
Accepted September 14, 2015
Published online April 30, 2016

*Corresponding Author
Tel/Fax: +91-9442018284
E-mail: sudhagovindan67@gmail.com

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract *In vitro* antioxidant activities, total phenolic, and flavonoid contents of *Pleurotus djamor* extracts were analyzed based on radical scavenging activities of methanol and aqueous extracts using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), N,N-dimethyl-p-phenylenediamine (DMPD), total Fe^{3+} reducing power, CUPRAC, phosphomolybdenum, metal chelating activity, and lipid peroxidation inhibition assays. Both extract types showed efficient radical scavenging activities against DPPH and DMPD radicals, ferric (Fe^{3+}) and cupric (Cu^{2+}) ion reducing powers, metal chelating activities, and lipid peroxidation inhibition. Total phenolic contents of methanol and aqueous extracts were 2.79 and 5.95 mg of GAE/g, respectively. Flavonoid contents of methanol and aqueous extracts were 6.35 and 5.75 mg of CAE/g, respectively. Consumption of the mushroom *P. djamor* can be beneficial due to antioxidant properties.

Keywords: *Pleurotus djamor,* edible mushroom, antioxidant activity, total phenol, total flavonoid

Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen ions and peroxides (1). ROS including radical species such as hydroxyl ($^{\bullet}OH$) and superoxide radical ($O_2^{-\bullet}$) along with non-radical species hydrogen peroxide (H_2O_2) are generated during normal cellular metabolism. Increased ROS levels cause damage to lipids, proteins, and nucleic acids (2,3) leading to physiological disability that often results in metabolic disorders of inflammation, aging, cancer, and hypertension (4). Normally, cells are protected against ROS-mediated damage via actions of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) antioxidant enzymes (5,6). Ascorbic acid (vitamin C) and tocopherol (vitamin E) are also important cellular antioxidants. Recently, a great deal of attention has been focused on study of natural antioxidants.

Mushrooms have been a perennial component of the human diet since time immemorial. They have been consumed by humans as part of the normal diet but also as a delicacy because of desirable taste and aroma. Research activities during the last two decades had indicated that mushrooms exhibit a number of nutritional, nutraceutical properties and considered as sources of bioactive metabolites (7-9). Mushrooms are rich in proteins and provide all essential amino acids, and low in carbohydrate, fat, and high in fiber contents. Mushrooms can be considered as functional foods or nutraceutical products that contribute to the design of healthy dietary patterns. Mushroom contain large amounts of vitamins, vitamin precursors, variety of minerals, trace elements, and specific β-glucans (10). They also exhibit antioxidant properties which are mainly attributed to phenolic compounds. In addition to bioactive proteins and polysaccharides, mushrooms are important sources of natural antioxidants constituting phenolic compounds, tocopherols, ascorbic acid, and carotenoids. Phenolic compounds found in mushrooms include benzoic acid, gallic acid, catechin, tannic acid, caffeic acid, and resveratrol. Various phenolic compounds have been reported to possess potent antioxidant, antimutagenic, anticancer, antiatherosclerotic, anti-inflammatory, antiviral, and antibacterial activities (11). Some common edible mushrooms that are widely consumed in Asia have been found to exert antioxidant activities that are correlated with total phenolic contents (12). Therefore, there is growing interest in natural and safer antioxidants from natural sources and consumption of antioxidant diet and natural food supplements as part of healthy lifestyle are now being recognized to protect health from oxidative stress.

Edible mushrooms belonging to Genus *Pleurotus* are most widely cultivated in different parts India, Europe, and Africa. *Pleurotus*

species have been used by human cultures all over the world due to nutritional values, medicinal properties, and other beneficial effects. Oyster mushrooms have been used thousands of years as a culinary and medicinal ingredient. They are high in nutrients and contain significant levels of minerals and vitamins. Oyster mushrooms stimulate the immune system, inhibit the proliferation of cancer cells, lowers systemic inflammation, modulate cholesterol levels, prevent the buildup of plaque in the arteries that leads to cardio-vascular diseases, counter the Hepatitis C virus, exert antioxidant, and antibacterial properties (13).

The mushroom *P. djamor* used as an edible mushroom was investigated for antioxidant activities, reports of which are unknown. The aim of this study was evaluation and comparison of the antioxidant activities of methanol and aqueous extracts of *P. djamor* based on scavenging assays *in vitro,* and also determination of total phenolic and flavonoid contents. Different antioxidant compounds may act *in vivo* through different mechanisms and no single analytical method can be used for a full evaluation of the total antioxidant capacity of foods. Hence, several complementary assays were used in this study. Antioxidant activities were accessed using different assays that measured free radical scavenging activities, reducing powers, metal chelating activities, and lipid peroxidation inhibition for applications in food and pharmaceutical industries.

Materials and Methods

Chemicals and reagents 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 4 N,N-dimethyl-p-phenylenediamine (DMPD), and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ferrozine, gallic acid, catechin, ascorbic acid, and thiobarbituric acid (TBA) were obtained from Himedia (Mumbai, India). All other chemicals used were of analytical grade.

Mushrooms The fruiting bodies of *Pleurotus djamor* (MDU-1) were collected from VG mushroom farm, Salem, Tamil Nadu in January of 2015. Fruiting bodies were oven dried at 40±2°C for 48 h and milled into powder through 0.5 mm sieve using a hammer mill (IKAWERKE MF10; IKA-Works, Staufen im Breisgau, Germany), and stored in air-tight plastic bags prior to analysis.

Preparation of extracts Ten g of mushroom powder was subjected to extraction via stirring with 100 mL of methanol or water at 25°C for 24 h under dark conditions. After centrifugation (ultracentrifuge R-8C; Remi laboratory instruments, Thane, India) at 5,000×*g* for 10 min, resulting residues were subjected to re-extraction twice as described above. Supernatants were pooled and combined extracts were evaporated under reduced pressure at 45°C using a vacuum rotary evaporator (Rotavapor R-144; Buchi, Flawil, Switzerland). Dried extracts thus obtained were re-dissolved in methanol or water to a concentration of 50 mg/mL and stored at 4°C in a refrigerator until further use and subjected to *in vitro* assays for evaluation of antioxidant activities. Analyses were carried out in triplicate.

DPPH free radical scavenging activity The scavenging effect of mushroom extracts against DPPH radicals was determined the following method of Shimada *et al*. (14). Different concentrations of 4.0 mL samples of mushroom were mixed with 1.0 mL of a methanol solution containing DPPH radicals, resulting in a final DPPH concentration of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, then the absorbance was measured using UV-Vis spectrophoto-meter 106 (Systronics, Ahmedabad, India) at 517 nm. Percentage inhibition was calculated as: $[(A_0–A_1)/A_0]×100$, where A_0 is the absorbance of a control and A_1 is the absorbance of a sample. Ascorbic acid as a natural antioxidant that is frequently used in food production was included as a positive control.

Hydroxyl radical scavenging assay Hydroxyl radical scavenging activities of extracts were assayed following the method of Smirnoff and Cumbes (15). A 3.0 mL reaction mixture 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 1.0 mL of different sample concentrations. After incubation (incubator Model NEC 401; Remi laboratory instruments) for 1 h at 37°C, the absorbance of the hydroxylated salicylate complex was measured using UV-Vis spectrophotometer at 562 nm. Scavenging activities against hydroxyl radicals were calculated as: $[1–(A_1–A_2)/A_0]x100$, where A_0 is the absorbance of a control lacking the extract, A_1 is the absorbance in the presence of the extract, and A_2 is the absorbance without sodium salicylate. Ascorbic acid was used as a positive control.

Ferric cyanide (Fe^{3+}) reducing antioxidant power assay The reducing power of mushroom extracts was determined following the method of Oyaizu (16). Different concentrations of 2.5 mL of a sample, 2.5 mL of a 0.2 M phosphate buffer at pH 6.6, and 2.5 mL of 1% potassium ferricyanide were mixed and incubated at 50°C for 20 min. Then, 2.5 mL of 10% (w/v) trichloroacetic acid was added to the mixture, followed by centrifugation (ultracentrifuge R-8C; Remi laboratory instruments) at 3,750×*g* for 10 min. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. After 10 min of incubation, at room temperature the absorbance was measured using a UV-Vis spectrophotometer at 700 nm against a blank. Ascorbic acid was used as a positive control.

CUPRAC cupric ion (Cu^{2+}) reducing power assay The cupric ion (Cu^{2+}) reducing power was determined following the method of Apak *et al*. (17) with minor modification of a method reported by Balaydin *et al*. (18). Briefly, 0.25 mL of a 0.01 M copper (II) chloride solution, 0.25 mL of a 7.5 mM ethanol neocuproine solution, and 0.25 mL of a 1 M ammonium acetate buffer solution were added to a test tube, containing different concentration of the sample. Then the total volume was adjusted with distilled water to 2.0 mL and mixed

vigorously. The absorbance against a reagent blank was measured at 450 nm after 30 min using UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicated an increased reduction capability. Ascorbic acid was used as a positive control.

Metal chelating activity against ferrous ions (Fe^{2+}) The ability of mushroom extracts to chelate ferrous ions was estimated following the method of Dinis *et al*. (19). Briefly, 2.0 mL of different sample concentrations in methanol were added to 0.05 mL of a solution of 2 mM $FeCl_2$. The reaction was initiated using addition of 0.2 mL of 5 mM ferrozine. The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically using UV-Vis spectrophotometer at 562 nm. Percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as: $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of a control, and A_1 is the absorbance of a mixture containing the extract. EDTA was used as a positive control.

Lipid peroxidation inhibition assays in egg homogenates A modified thiobarbituric acid reactive species (TBARS) assay reported by Ohkawa *et al*. (20) was used for measurement of the amount of lipid peroxide formed using an egg yolk homogenate as a lipid rich medium, as described by Ruberto *et al*. (21). Malondialdehyde (MDA), a secondary end product of polyunsaturated fatty acid oxidation, reacts with 2 molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm. One-half mL of a 10% (v/v) egg homogenate and 0.1 mL of extract were added to a test tube and made up to 1.0 mL using distilled water. Then, 0.05 mL of 0.07 M $FeSO_4$ was added to induce lipid peroxidation, followed by incubation for 30 min. Then, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 using NaOH), 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulfate, and 0.05 mL of 20% TCA were added and the resulting mixture was vortexed in a vortex mixer (CM 101 Plus; Remi laboratory instruments), then heated at 95°C in a water bath for 60 min. After cooling to room temperature, 5.0 mL of n-butanol was added to each tube, followed by centrifugation at 3,000×*g* for 10 min. The absorbance of the organic upper layer was measured at 532 nm using UV-Vis spectrophotometer. Inhibition of lipid peroxidation (%) was calculated as: $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of a control and A_1 is the absorbance of a sample. Ascorbic acid was used as a positive control.

Phosphomolybdenum assay The antioxidant activity of a sample was evaluated using the phosphomolybdenum method following the procedure of Prieto *et al*. (22). An aliquot of 0.1 mL of a sample solution was mixed with 1.0 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Tubes were capped with silver foil and incubated at 95°C for 90 min. Tubes were then cooled to room temperature and the absorbance of the aqueous solution was measured using UV-Vis spectrophotometer against a blank at 695 nm. Ascorbic acid was used as a standard and total antioxidant activity was expressed as ascorbic acid equivalents (AAE) per g of extract.

Total phenolic content The total phenolic contents of mushroom extracts were measured following the method of Singleton and Rossi (23) with modification. One mL of a sample was mixed with 1.0 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 1.0 mL of 35% saturated sodium carbonate was added and the mixture was made up to 10 mL using addition of deionized water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured using UV-Vis spectrophotometer at 725 nm against a blank. Gallic acid was used as a reference standard. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of extract.

Total flavonoid content The total flavonoid content was determined as described by Jia *et al*. (24). An amount of 0.25 mL of mushroom extract was diluted using 1.25 mL of distilled water. Then, 75 μL of 5% sodium nitrite was added and, after 6 min, 150 μL of 10% aluminium chloride was added, followed by mixing. After 5 min, 0.5 mL of 1 M sodium hydroxide was added. The absorbance was measured using UV-Vis spectrophotometer immediately against a prepared blank at 510 nm. Catechin was used as a reference standard. The total flavonoid content was expressed as mg of catechin equivalents (CAE) per g of extract.

Statistical analysis All assays were carried out in triplicate and results were expressed as mean±standard deviation (SD). Data were analyzed using SPSS software. An analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were used for analysis of differences among scavenging activities and EC_{50} values of extracts for different antioxidant assays with a least significance difference (LSD) of $p<0.05$ as the level of significance. EC_{50} value (mg extract/mL) is the effective concentration at which radicals were scavenged by 50% or at which the absorbance is 0.5 and was obtained by interpolation from linear regression analysis.

Results and Discussion

DPPH radical scavenging activity The stable DPPH radical model is a widely used method for the evaluation of free radical scavenging activity of natural compounds and the absorption maximum of DPPH radical in methanol is 517 nm (25,26). When antioxidants reacts with DPPH free radicals, either an electron or a hydrogen atom is transferred to DPPH which is reduced to DPPH-H thus neutralizing the free radical character (27). The scavenging effects of methanol and aqueous extracts against the DPPH radical were measured and the results are shown in Fig. 1A.

DPPH scavenging activities of methanol and aqueous extracts of *P. djamor* increased with an increase in concentration, resulting in inhibition percentages of 4.13-86.12 and 4.99-66.41% at 1 to 5 mg/

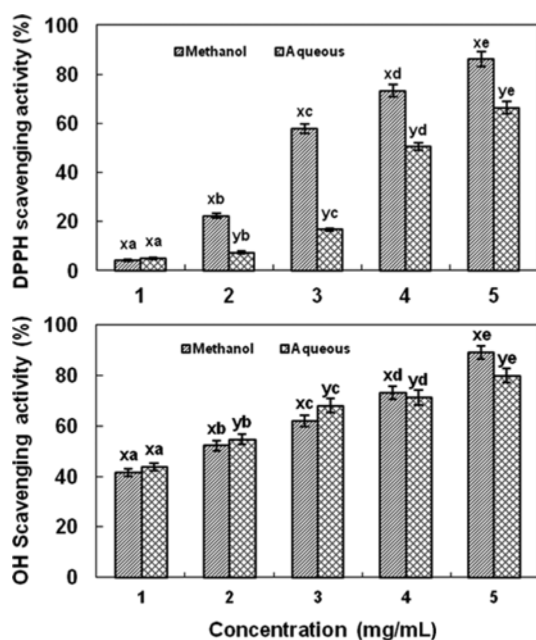

Fig. 1. (A) DPPH radical scavenging activities of methanol and aqueous extracts of *P. djamor.* (B) Hydroxy radical scavenging activities of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference at $p<0.05$ between 2 different extracts of the same concentration. Different letters (a-e) indicate a significant difference at $p<0.05$ between concentrations of the same extract (ANOVA, DMRT).

mL, respectively. These values were lower than the vitamin C (Vc) value of 94.77% at 0.1 mg/mL. There was a significant ($p<0.05$) difference between inhibition percentages for concentrations tested for the same methanol and aqueous extracts. The lowest EC_{50} value that corresponded to the highest scavenging activity of EC_{50}=2.75 mg/mL against DPPH radicals was obtained using a methanol extract. The aqueous extract EC_{50} value was 3.90 mg/mL. A significant ($p<0.05$) difference was found between EC_{50} values of methanol and aqueous extracts. The scavenging ability of mushroom extracts might have been due to more hydrogen donating components extracted from mushrooms. Mushroom extracts contained active substances, including phenolic compounds, with a high potential for hydrogen donation for scavenging of DPPH radicals. *Agaricus bisporus, A. arvensis, A. romagnesii, A. sivaticus,* and *A. silvicola* methanol extracts exhibited DPPH EC_{50} values of 9.61, 15.85, 6.22, 5.37, and 6.39 mg/mL respectively (28). The DPPH free radical scavenging activities of *P. djamor* extracts in this study were superior to the activities of *P. eous* extracts with EC_{50} values of 4.2 mg/mL for methanol and 7.0 mg/mL for aqueous extracts (29).

Hydroxyl radical scavenging activities The hydroxyl radical is the most harmful free radical among ROS for induction of severe damage to carbohydrate, protein, lipid, and DNA biomolecules (30). Thus, removal of hydroxyl radicals is important for antioxidant defense in cells and food systems. The •OH scavenging activity of mushroom extracts was assessed based on an ability to compete with salicylic acid for •OH radicals in an •OH generation/detection system. Scavenging abilities of methanol and aqueous extracts of *P. djamor* showed concentration dependent increases from 41.62 to 89.09%, and from 43.81 to 80.09%, respectively, at 1 to 5 mg/mL (Fig. 1B), However, values were lower than the value for Vc of 96.01% at 0.5 mg/mL. A significant ($p<0.05$) difference in hydroxyl radical scavenging activities was observed between the same concentrations for methanol and aqueous extracts. The scavenging activity against hydroxyl for the aqueous extract of 1.47 mg/mL was better than the value for the methanol extract of 1.83 mg/mL, based on EC_{50} values. A significant ($p<0.05$) difference was observed in EC_{50} values against hydroxyl between methanol and aqueous extracts. Thus, mushroom extracts can be considered as good scavengers of hydroxyl radicals.

Hydroxyl radicals can abstract hydrogen atoms from membranes and cause lipid peroxidation reactions (31). Thus, the mushroom extracts showed antioxidant effects against lipid peroxidation on biomembranes and scavenge hydroxyl radicals and superoxide anions at the initiation and termination stages of peroxy radicals.

Sudha *et al*. (29) reported EC_{50} values of 3.1 and 2.6 mg/mL for hydroxyl scavenging activities of methanol and aqueous extracts from *P. eous*, which were lower than values reported for *P. djamor* in this study. Hot-water extracts of *Agrocybe cylindracea* were effective scavengers of hydroxyl free radicals with an EC_{50} value of 25.37 mg/mL (32).

Reducing power assay In the reducing power assay, antioxidants convert ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions via donation of an electron and the resulting ferrous ion (Fe^{2+}) formation can be measured spectrophotometrically at 700 nm. This donation of electrons to reactive free radical species, promotes termination of free radical chain reactions. The presence of reducers (i.e. antioxidant) reduce Fe^{3+} to the more active Fe^{2+} form might also be indicative of an ability to act as pro-oxidant. Methanol and aqueous extracts of *P. djamor* exhibited a dose-dependent reducing power at 1-5 mg/mL (Fig. 2A). The reducing power values of methanol and aqueous extracts were 0.218 and 0.312 at 1 mg/mL, and 0.911 and 1.142 at 5 mg/mL, both lower than the value for Vc of 1.213 at 1.0 mg/mL. There was a significant ($p<0.05$) difference in reducing power values between concentrations tested for the same methanol and aqueous extracts. Aqueous extracts exhibited the highest reducing power of EC_{50}=1.57 mg/mL. The methanol extract value was EC_{50}=2.81 mg/mL. A significant ($p<0.05$) difference was observed in EC_{50} values between methanol and aqueous extracts. The reducing property of an extract generally depends on the presence of reductones, which exhibits antioxidant action by breaking the free radical chain via hydrogen ion donation and the reductones are believed to react with certain precursors of peroxide, thus preventing formation of peroxides (14). The reducing power of mushroom extracts might be due to hydrogen-donating abilities. EC_{50} values obtained from reducing power assays using methanol extracts of *Agaricus bisporus, A. arvensis, A. romagnesii, A.*

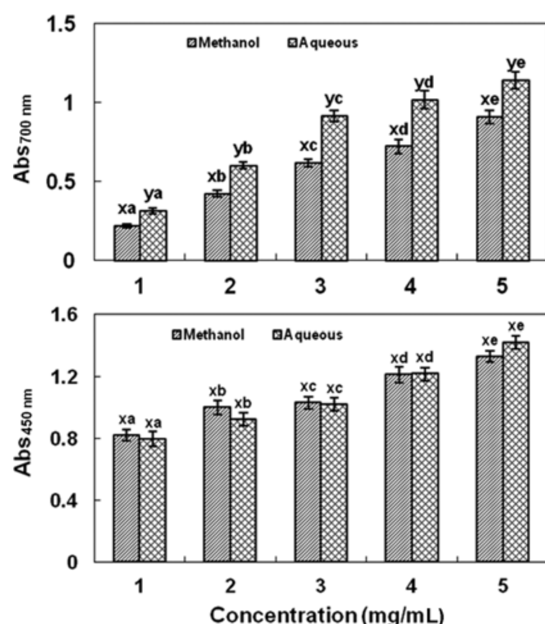

Fig. 2. (A) Reducing power assay results of methanol and aqueous extracts of *P. djamor.* (B) CUPRAC assay results of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference (p<0.05) between 2 different extracts of the same concentration. Different letters (a-e) indicate a significant difference (p<0.05) between concentrations of the same extract (ANOVA, DMRT).

sivaticus, and *A. silvicola* were of 4.49, 2.72, 6.18, 2.08, and 3.24 mg/mL respectively (28). Hot water extracts of mature and immature Ling chih mushrooms exhibited reducing power of 1.08 and 1.04 at 5 mg/mL, respectively (33). The reducing power of hot water extracts of *A. cylindracea* was 0.87 at 5 mg/mL (32).

CUPRAC assay The cupric ion reducing antioxidant power assay is based on the reduction of Cu (II) to Cu (I) by antioxidants in the presence of neocuprine and a rise in the absorbance indicates higher cupric ion reducing power (34,35). The Cu^{2+} reducing activity of *P. djamor* extracts was concentration-dependent (Fig. 2B). At 1 to 5 mg/mL, the cupric ion (Cu^{2+}) reducing abilities of methanol and aqueous extracts of *P. djamor* were between 0.820-1.329 and 0.796-1.420, respectively, both lower than the Vc value of 1.632 at 0.2 mg/mL. No significant (p<0.05) difference was observed in Cu^{2+} reducing abilities between concentrations tested for methanol and aqueous extracts in this study. The EC_{50} values of aqueous (0.60 mg/mL) and methanol (0.61 mg/mL) extracts were similar. The CUPRAC chromogenic redox reaction is carried out at pH (7.0), close to the physiological pH and the method is proved to be efficient for glutathione and thiol-type antioxidants for which ferric reducing antioxidant power (FRAP) method is nonresponsive (36). Noorlidah *et al*. (37) reported that CUPRAC values of hot water extracts of *Pleurotus* species at 1 mg/mL ranged between 0.390-0.530, which were lower than values for methanol and aqueous extracts of *P. djamor* reported in this study.

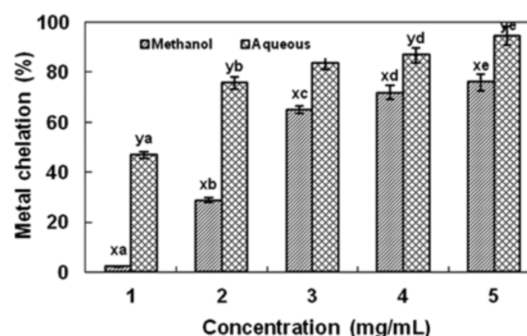

Fig. 3. Chelating ability of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference (p<0.05) between 2 different extracts of the same concentration. Different letters (a-e) indicate a significant difference (p<0.05) between concentrations of the same extract (ANOVA, DMRT).

Ferrous ion chelating ability Chelation of metal ions is often associated with redox active metal catalysis, which prevents generation of ROS (38). In this assay, chelating agents disrupt the ferrozine-Fe^{2+} complex, thus decreasing the red color intensity. Measurement of the rate of color reduction at 550 nm, therefore, allows estimation of the chelation activity. A lower absorbance value indicates a higher chelation ability. The chelating activity of methanol and aqueous extracts of *P. djamor* against ferrous ions was concentration-dependent and there was a significant (p<0.05) difference between values for the concentrations tested for the same methanol and aqueous extracts (Fig. 3). Methanol and aqueous extracts chelated ferrous ions to a different extent over a concentration range of 1 to 5 mg/mL with inhibition percentages of 2.16-75.92 and 46.75-94.52%, respectively, both lower than the value for EDTA of 86.82% at 0.05 mg/mL. Aqueous extracts showed excellent chelating abilities against ferrous ions with an EC_{50} value=1.45 mg/mL, compared with a methanol extract value of EC_{50}=2.55 mg/mL. These difference were significantly (p<0.05) different. EDTA showed a high chelating ability of 86.82% at 0.05 mg/mL. The metal chelating activity is important as the concentration of a catalyzing transition metal is reduced in lipid peroxidation (39).

Mushroom extracts exerted antioxidant activities due to chelation of Fe^{2+} ions to keep the ion in an oxidized form, which can prevent production of free radicals and, thus, oxidative damage. The chelating ability of methanol extracts of mature *Agaricus brasiliensis* at 10 mg/mL was 62% with an EC_{50} value of 5.00 mg/mL (40). Mau *et al*. (41) reported that methanol extracts of *Ganoderma lucidum*, *G. tsugae,* and *Coriolus versicolor* chelated 55.50, 44.80, and 15.20% of ferrous ions, respectively, at 2.4 mg/mL. The chelating ability of hot water extracts of *Agrocybe cylindracea* exhibited an EC_{50} value of >20 mg/mL (32). Antioxidant activities of *Pleurotus eous* extracts resulted in EC_{50} values for chelation of methanol and aqueous extracts of 5.6 and 7.8 mg/mL, respectively (29), in agreement with results reported herein.

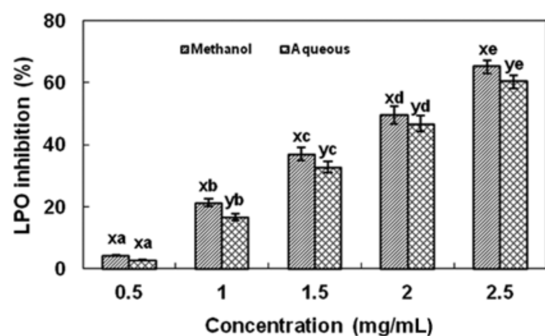

Fig. 4. Lipid peroxidation inhibition of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference (p<0.05) between 2 different extracts of the same concentration. Different letters (a-e) indicate a significant difference (p<0.05) between concentrations of the same extract (ANOVA, DMRT).

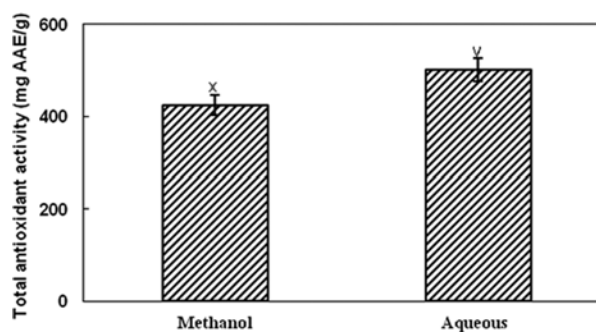

Fig. 5. Total antioxidant activities of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference (p<0.05) between 2 different extracts (ANOVA, DMRT).

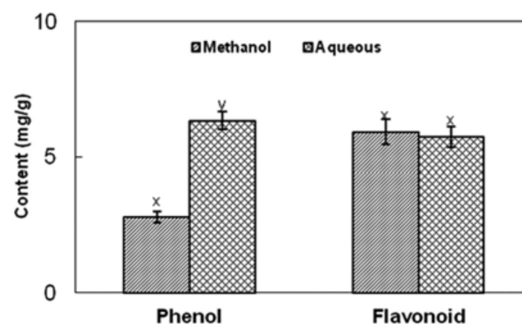

Fig. 6. Total phenolic and flavonoid contents of methanol and aqueous extracts of *P. djamor.* Results are expressed as mean±SD (n=3). Different letters (x-y) indicate a significant difference (p<0.05) between 2 different extracts (ANOVA, DMRT).

Lipid peroxidation inhibition assays in egg homogenates Poly-unsaturated fatty acids (PUFAs) of cell membranes reacts with ROS and induce lipid peroxidation which leads to changes in the permeability and fluidity of the lipid bilayer that alters cellular integrity. It is therefore important to measure lipid peroxidation inhibition as an indicator of potent antioxidant properties. Inhibition of lipid peroxidation was assessed based on the amount of MDA produced. Homogenate phospholipids extracted from egg yolk undergo rapid nonenzymatic peroxidation in the presence of ferrous sulphate. Production of MDA was prevented by methanol and aqueous extracts in a concentration-dependent manner (Fig. 4) and there was a significant (p<0.05) difference between concentrations tested for the same methanol and aqueous extracts. LPO inhibition sharply increased from 4.37 to 65.11% and from 2.80 to 60.32% when the concentration increased from 0.5 to 2.5 mg/mL for methanol and aqueous extracts, respectively, both lower than the Vc value of 68.21% at 0.1 mg/mL. Based on EC_{50} values, lipid peroxide inhibition followed the order of methanol (2.05 mg/mL)>aqueous (2.12 mg/mL) extracts. At a concentration of 10 mg/mL, an ethanol extract of *P. ostreatus* provided 56.20% inhibition of LPO activity with an EC_{50} value of 8 mg/mL (9). The ability of methanol and hot water extracts of *P. eous* to inhibit liver lipid peroxidation resulted in EC_{50} values of 0.48 and 0.58 mg/mL, respectively (29).

Total antioxidant activity based on phosphomolybdenum assays The total antioxidant capacity was measured by phosphomolydenum method which is based on the reduction of Mo (VI) to Mo (V) by the mushroom extract and subsequent formation of green phosphate-Mo (V) complex at acidic pH with maximum absorption at 695 nm. The total antioxidant activity was found to be in the order of aqueous (501.90 mg AAE/g)>methanol (424.35 mg AAE/g) extracts (Fig. 5). Total antioxidant activities of methanol and hot water extracts of *P.eous* were 5.84 and 1.98 mg of GAE/g of extract, respectively (29).

Total phenolic and total flavonoid contents Determinations of phenolic and flavonoid contents are important because antioxidant activities of plant extracts are commonly attributed to the presence of these compounds, particularly flavonoids. These bioactive compounds act as reducing agents, free radical scavengers, metal chelators, and deactivators of singlet oxygen, and/or display simultaneously more than one of these functions (42). Antioxidant properties also interfere with oxidative reactions of chain propagation (43). Total phenolic and total flavonoid contents of methanol and aqueous extracts of *P. djamor* are shown in Fig. 6. The order of the phenolic contents in extracts was aqueous (5.93 mg of GAE/g>methanol (2.79 mg of GAE/g), and the order of flavonoids was methanol (6.35 mg of CAE/g)>aqueous (5.75 mg of CAE/g). A significant (p<0.05) difference in phenolic and no significant (p>0.05) difference in flavonoid contents was observed between extracts. The phenolic and flavonoid contents of methanol extracts of *Agaricus* sp. ranged from 2.23 to 8.95 mg of GAE/g, and from 1.65 to 3.88 mg of CAE/g, respectively (28). Amounts of phenolics in methanol and water extracts of *L. edodes* and *V. volvacea* were 4.79 and 15.0 mg of GAE/g, and 1.33 and 1.34 mg of GAE/g, respectively (11).

Antioxidant activities of both methanol and aqueous extracts of *P. djamor* were concentration-dependent and exhibited moderate to high antioxidant activities in this study. Based on EC_{50} values, methanol extracts of *P. djamor* exhibited excellent DPPH radical

scavenging activities and lipid peroxidation inhibition, perhaps attributable to a high total flavonoid content. An excellent hydroxyl radical scavenging activity, ferric ion reducing power, cupric ion reducing power, ferrous ion chelation, and total antioxidant activity exhibited by aqueous extracts of *P. djamor* may be attributed to a high phenolic content. *P. djamor* is a promising mushroom for large scale industrial cultivation with an excellent antioxidant potential. In addition, *P. djamor* can be used as an easily accessible source of natural antioxidants, and possibly as a food supplement. However, further studies for isolation and identification of bioactive constituents are needed.

Acknowledgments Laboratory facilities and financial support for this project were provided by Periyar University.

Disclosure The authors declare no conflict of interest.

References

1. Si CL, Shen T, Jiang YY, Wu L, Yu GJ, Ren XD, Xu GH, Hu WC. Antioxidant properties and neuroprotective effects of isocampneoside II on hydrogenperoxide-induced oxidative injury in PC12 cells. Food Chem. Toxicol. 59: 145-152 (2013)
2. Kim YG, Sumiyoshi M, Sakanaka M, Kimura Y. Effects of ginseng saponins isolated from red ginseng on ultraviolet B-induced skin aging in hair-less mice. Eur. J. Pharmacol. 602: 148-156 (2009)
3. Ziegler F, Seddiki L, Marion-Letellier R, Lavoinne A, Déchelotte P. Effects of L-glutamine supplementation alone or with antioxidants on hydrogen peroxide-induced injury in human intestinal epithelial cells. E. Spen Eur. E. J. Clin. Nutr. Metab. 6: e211-e216 (2011)
4. Lee SH, Kang MC, Moon SH, Jeon BT, Jeon YJ. Potential use of ultrasound in antioxidant extraction from *Ecklonia cava*. Algae 28: 371-378 (2013)
5. Olsvik PA, Kristensen T, Waagbø R, Rosseland BO, Tollefsen KE, Baeverfjord, G, Berntssen MHG. mRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon (*Salmo salar*) exposed to hyperoxic water during smoltification. Comp. Biochem. Phys. C 141: 314-323 (2005)
6. Verma K, Mehta SK, Shekhawat GS. Nitric oxide (NO) counteracts cadmium induced cytotoxic processes mediated by ROS in *Brassica juncea*: Cross-talk between ROS, NO and antioxidant responses. Biometals 26: 255-269 (2013)
7. Ferreira ICFR, Barros L, Abreu R. Antioxidants in wild mushrooms. Curr. Med. Chem. 16: 1543-1560 (2009)
8. Yaltirak T, Aslim B, Ozturk S, Alli H. Antimicrobial and antioxidant activities of *Russula delica* Fr. Food Chem. Toxicol. 47: 2052-2056 (2009)
9. Jayakumar T, Thomas PA, Geraldine P. *In vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. Innov. Food Sci. Emerg. 10: 228-234 (2009)
10. Kalacį P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem. 113: 9-16 (2009)
11. Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81: 249-255 (2003)
12. Lo KM, Cheung PCK. Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. Food Chem. 89: 533-539 (2005)
13. Gunde-Cimerman N. Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales s.l., Basidiomycetes). Int. J. Med. Mushrooms 1: 69-80 (1999)
14. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autioxidation of soybean oil in cyclodextrin emulsion. J. Agr. Food Chem. 40: 945-948 (1992)
15. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28: 1057-1060 (1989)
16. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet. 44: 307-315 (1996)
17. Apak A, Guclu K, Ozyurek M, Karademir SE, Erca E. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int. J. Food Sci. Nutr. 57: 292-304 (2006)
18. Balaydin HT, Gülçin Í, Menzek A, Göksu S, Şahin E. Synthesis and antioxidant properties of diphenylmethane derivative bromophenols including a natural product. J. Enzym. Inhib. Med. Ch. 25: 685-695 (2010)
19. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169 (1994)
20. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358 (1979)
21. Ruberto G, Baratta MT, Deans SG, Dorman HJD. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med. 66: 687-693 (2000)
22. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269: 337-341 (1999)
23. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphotungstic acid reagents. Am. J. Enol. Viticult. 16: 144-158 (1965)
24. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559 (1998)
25. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28: 25-30 (1995)
26. Villano D, Fernández-Pachón MS, Moyá, ML, Troncoso AM, García-Parrilla MC. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 71: 230-235 (2007)
27. Liu CF, Tseng KC, Chiang SS, Lee BH, Hsu WH, Pan TM. Immunomodulatory and antioxidant potential of *Lactobacillus* exopolysaccharides. J. Sci. Food Agr. 91: 2284-2291 (2011)
28. Barros L, Falcão S, Baptista P, Freire C, Vilas-Boas M, Ferreira ICFR. Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chem. 111: 61-66 (2008)
29. Sudha G, Vadivukkarasi S, Indhu Shree RB, Lakshmanan P. Antioxidant Activity of various extracts from an edible mushroom *Pleurotus eous*. Food Sci. Biotechnol. 21: 661-668 (2012)
30. Li J, Liu Y, Fan L, Ai L, Shan L. Antioxidant activities of polysaccharides from the fruiting bodies of *Zizyphus jujuba cv.* Jinsixiaozao. Carbohyd. Polym. 84: 390-394 (2011)
31. Kitada M, Igarashi K, Hirose S, Kitagawa H. Inhibition by polyamines of lipid peroxide formation in rat liver microsomes. Biochem. Bioph. Res. Co. 87: 388-394 (1979)
32. Tsai SY, Huang SJ, Mau JL. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chem. 98: 670-677 (2006)
33. Mau JL, Lin HC, Song SF. Antioxidant properties of several specialty mushrooms. Food Res. Int. 35: 519-526 (2002)
34. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agr. Food Chem. 52: 7970-7981 (2004)
35. Gülçin İ. Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. J. Enzym. Inhib. Med. Ch. 23: 871-876 (2004)
36. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J. Agr. Food Chem. 53: 1841-1856 (2005)
37. Noorlidah A, SitiMarjiana I, Norhaniza A, Adawiyah SS, Beng FL. Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. Evid-Based Compl. Alt. 2012: 1-12 (2012)
38. Ebrahimzadeh MA, Pourmorad F, Bekhradina AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol. 7: 3188-3192 (2008)
39. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). Lebensm. Wiss U. Technol. 32: 269-277 (1999)
40. Soares AA, de Souza CGM, Daniel FM, Ferrari GP, da Costa SMG, Peralta RM. Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (*Agaricus blazei* Murril) in two stages of maturity. Food Chem. 112: 775-781 (2009)
41. Mau JL, Lin HC, Chen CC. Antioxidant properties of several medicinal mushrooms. J. Agr. Food Chem. 50: 6072-6077 (2002)
42. Gramza A, Khokhar S, Hes M, Jedrusek-Golinska A. Tea extracts influence on catalytical properties of Fe^{2+} in lipids. Pol. J. Environ. Stud. 13: 143-146 (2004)
43. Ruiz-Navajas Y, Viuda-Martos M, Fernández-Lopez J, Zaldivar-Cruz JM, Kuri V, Pérez-Álvarez JA. Antioxidant activity of artisanal honey from Tabasco, México. Int. J. Food Prop. 14: 459-470 (2011)