

Chemical Composition and Antioxidant Activity of a Wild Edible Mushroom *Pleurotus flabellatus*.

Adhiraj Dasgupta¹, Manjula Rai² and Krishnendu Acharya^{1*}

¹Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata, 700019, West Bengal, India.

²Department of Botany, St. Joseph's College, Darjeeling, 734104, West Bengal, India.

*Corres.Author: krish_paper@yahoo.com

Abstract: Ethanolic fraction from edible mushroom, *Pleurotus flabellatus*, was tested for total phenol, flavonoid, α -carotene, lycopene and ascorbic acid and in vitro antioxidant activity in terms of DPPH radical scavenging chelating effect of ferrous ion, reducing power and total antioxidant capacity assay. Findings showed that EC₅₀ values were below 1 mg/ml except DPPH radical scavenging test. The extract exhibited 50% DPPH radical scavenging activity at only 1.8 mg/ml concentration. Estimated putative antioxidant components was in order of ascorbic acid > phenol > flavonoids > α -carotene > lycopene. Result implies that *P. flabellatus* can be a potential source of natural antioxidant which may be used as food supplement to treat various oxidative stress related diseases.

Keywords: Antioxidant activity; Antioxidant components; Chelating effect; *Pleurotus flabellatus*; Reducing power; Scavenging effect.

Introduction

Deviation from normal metabolic processes create an imbalance between pro oxidants and antioxidants in an organism that lead to oxidative stress and invites a number of degenerative diseases such as diabetes, lung diseases, neurological disorders, cardiovascular problems aging, rheumatoid arthritis etc.¹ When the normal protective mechanisms are disrupted by various pathological processes, antioxidant supplements are vital to combat oxidative damage. So, the antioxidants can protect the human body from damage caused by reactive oxygen species (ROS) which are ultimately associated with lipid peroxidation, protein damage and DNA denaturation² and can prevent an individual from these killer diseases. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been reported but they invite many side effects like hepatotoxicity, pneumotoxicity and cancer^{3,4}. Therefore, compounds from natural sources that possess antioxidant potential are being sought and naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most appealing modes of modern therapy. Among them, mushrooms or their derivatives or extracts occupy an elite position to perform this function^{1, 5-9}. Furthermore, the effect of mushroom nutrition in clinical conditions for the treatment of cancer¹⁰⁻¹³, heart ailments^{10, 14}, diabetes¹⁵, inflammation¹⁶, hepatic damage¹⁷⁻¹⁹, microbial pathogens^{20, 21} etc. have attracted great interest in the scientific community. Our objective was to evaluate the antioxidant activity and

free radical scavenging capacity of the ethanolic extract of a wild edible mushroom *Pleurotus flabellatus*, and finally a correlation has been drawn between the antioxidant components with the antioxidant capacity.

Materials and Methods:

Materials:

The mushroom *Pleurotus flabellatus* was collected from the local markets of Darjeeling, West Bengal, India. The fruit bodies were thoroughly cleaned and then dried. 25 g of the dried mushroom sample was extracted with 100 ml ethanol overnight at room temperature and was filtered using Whatman No. 2 filter paper. The residue was then extracted with an additional portion of ethanol under the same conditions. The ethanolic extract was evaporated using a rotary evaporator at 50° C for dryness. The dried extract was resolubilized in ethanol to obtain various concentrations of EfraPf.

Chemicals:

BHT (butylated hydroxytoluene), L-ascorbic acid, quercetin, gallic acid, EDTA (ethylenediaminetetraacetic acid), potassium ferricyanide, ferrous chloride, Folin-Ciocalteu reagent, NBT (nitroblue tetrazolium), DPPH (1,1-diphenyl 1-2-picrylhydrazyl), TCA (trichloroacetic acid), ammonium molybdate and methionine were purchased from Sigma chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Determination of total phenols:

Total phenols in the extract were measured using Folin-Ciocalteu reagent²². 1ml of ethanolic extract (100 mg/ml) was mixed with 1ml Folin-Ciocalteu reagent and incubated for 3 min at room temperature. After incubation, 1ml of 35 % saturated Na₂CO₃ solution was added in the reaction mixture, volume adjusted to 10 ml with distilled water and incubated in the dark for 90 min, after which the absorbance was monitored at 725 nm with a spectrophotometer. Gallic acid was used as standard. Total phenol content of the sample was expressed as mg of gallic acid equivalents per gram of extract.

Determination of total flavonoid content:

Flavonoid concentration was determined by the method as described²³. Mushroom extract (100 mg/ml) was diluted with 4.3 ml of 80% aqueous methanol and 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1 M aqueous potassium acetate were added to it. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard.

Determination of total β-carotene and lycopene content:

β-carotene and lycopene was determined by the processes as suggested²⁴. In brief, 100 ml of mushroom extract (10 mg/ml) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 min and absorbance of the mixture was measured at 453, 505 and 663 nm. β-carotene and lycopene contents were calculated according to the following equations:

$$\text{Lycopene (mg/100mg)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}.$$

$$\beta\text{-carotene (mg/100mg)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Determination of ascorbic acid content:

Ascorbic acid content was determined by a method as described²⁵ with a little modification. Standard ascorbic acid (100 µg /ml) was taken in a conical flask and made up to 10 ml by 0.6% oxalic acid. It was titrated with a dye, 2, 6-dichlorophenol indophenol (21 mg sodium bicarbonate and 26 mg of dye in 100 ml water). The amount of dye consumed (V₁ ml) is equivalent to the amount of ascorbic acid. The sample (w µg/ml) was similarly titrated with the dye (V₂ ml). The amount of ascorbic acid was calculated using the formula,

Ascorbic acid ($\mu\text{g}/\text{mg}$) = $[(10 \mu\text{g}/V_1\text{ml}) \times V_2\text{ml}] \times w \mu\text{g} \times 1000$.

Total antioxidant capacity assay:

The 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 pH²⁶. The tubes containing extract (1 mg/ml) and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

Different concentrations of EfraPf (0.5 to 2 mg) was added to 0.004% methanolic solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark. Absorbance was measured at 517 nm against a blank. EC₅₀ value is the effective concentration of extract that scavenged DPPH radicals by 50% and it was obtained by interpolation from linear regression analysis.

Reducing power

Reducing power of EfraPf was determined following the method of Oyaizu, 1986²⁷. Variable concentrations of EfraPf were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 min. 2.5 ml of 10% trichloroacetic acid was added to the mixture and was centrifuged at 12000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml 0.1% ferric chloride and absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture was taken to mean an increase in reducing power of the sample.

Chelating effect on ferrous ions

The ability of the extract of EfraPf to chelate ferrous ions was estimated by the method of Dinis et al, 1995²⁸. Briefly, the extract was added to a solution of 2 mM FeCl₂ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), and the mixture was then shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated as $[(A_0 - A_1) / A_0] \times 100$, where A₀ was the absorbance of the control, and A₁ of the mixture containing the extract or the absorbance of a standard solution.

Results

Recovery percent and antioxidant components:

In the present study, table 1 shows the percent yield, total phenol, flavonoids, ascorbic acid, beta-carotene and lycopene content in the EfraPf. Data shows that ascorbic acid, total phenols and flavonoids were the major antioxidant components whereas, -carotene and lycopene were found in vestigial amounts.

Table 1: yield percentage and antioxidant components of ethanolic extract of *Pleurotus flabellatus* (EfraPf). Values are the mean \pm standard deviation of three separate experiments, each in triplicate. Total phenols are expressed in gallic acid equivalent (GAE) and flavonoids as quercetin equivalent (QE).

	Flavonoids	Total phenols	Ascorbic acid	- carotene	Lycopene
Yield %	($\mu\text{g}/\text{mg}$)				
1.8%	1.0625 \pm 0.375	6.875 \pm 0.45	8.75 \pm 0.416	0.00717 \pm 0.000006	0.00459 \pm 0.0005

Total antioxidant capacity:

Total antioxidant capacity of EfraPf was determined by the formation of green phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. Total antioxidant activity of the extract was estimated, using ascorbic acid as standard. Analysing the data, it was found that 1mg of extract is as functional as approximately $40 \pm 3.7 \mu\text{g}$ of ascorbic acid, expressed as 40 μg AAE.

DPPH assay:

DPPH is a stable free radical that has a characteristic absorption at 517 nm. The use of stable DPPH radical has the advantage of being unaffected by side reactions such as enzyme inhibition and metal chelation^{6, 29}. Such reactivity has been widely used to test the ability of a compound or extract as free radical scavengers. Upon treatment with an increasing concentration of the EfraPf, a marked decrease in absorption was observed, indicating a potent DPPH scavenging ability of the extract (Fig. 1). EC_{50} of DPPH radical scavenging activity was $1.8 \pm 0.02 \text{ mg/ml}$.

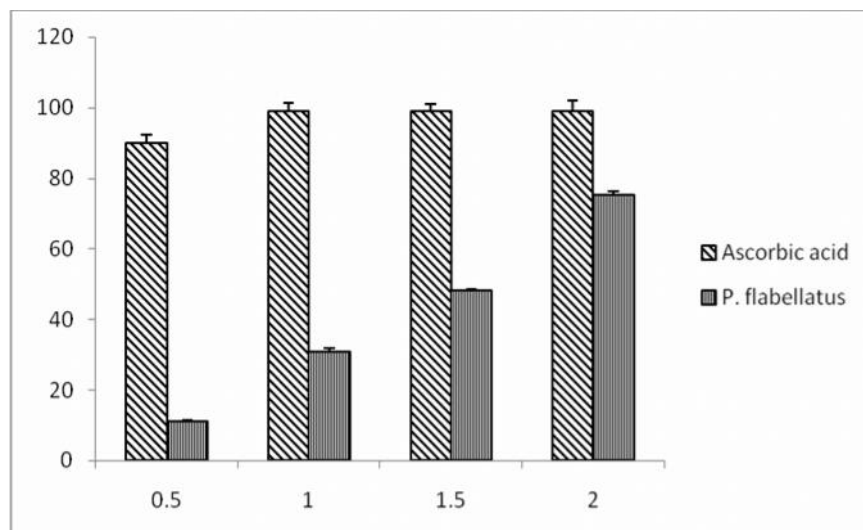


Fig. 1: DPPH radical scavenging activity of EfraPf compared with that of the standard, ascorbic acid.

Chelating effect on ferrous ions:

Like many transition metals, Ferrous ions in a biological system could catalyse Heber-Weiss and Fenton-type reactions leading to the formation of hydroxyl radicals. Antioxidants chelate these transition metal ions resulting in the suppression of hydroxyl radical generation and inhibition of peroxydation process of biomolecules. The range and the mean of Fe^{++} chelating capacity is directly related with antioxidant potentiality. At 100 – 500 $\mu\text{g/ml}$ the chelating effects of the EfraPf was between 14.6 and 72.3% (fig 2). At the same concentration range, the chelating effects of the known metal chelator EDTA, was between 69% - 90%. In the present study, EfraPf showed significant correlation between total phenolics and ferrous ion chelating activity ($R^2 = 0.826$) and flavonoids and ferrous ion chelating activity ($R^2 = 0.188$). It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidised forms of metal ions³⁰.

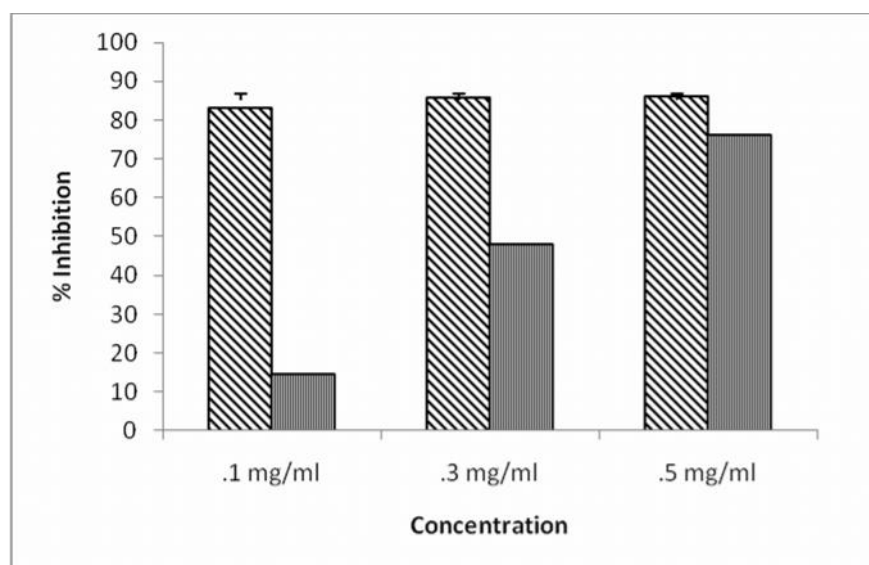


Fig 2: Chelating effects of EfraPf on ferrous ions compared with that of EDTA, used as standard. Values are the mean \pm standard deviation of three separate experiments, each in triplicate.

Reducing power:

Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity²⁷. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. In the reducing power assay the presence of antioxidants in the EfraPf would effect in the reduction of Fe^{3+} to Fe^{2+} by the donation of an electron. The increasing absorbance at 700 nm by measuring the formation of Perl's Prussian Blue indicates an increase in reductive ability. A steady increase in reducing power was observed (fig 3). Results showed that EC_{50} for the reducing power of EfraPf was of 0.84 ± 0.03 mg/ml.

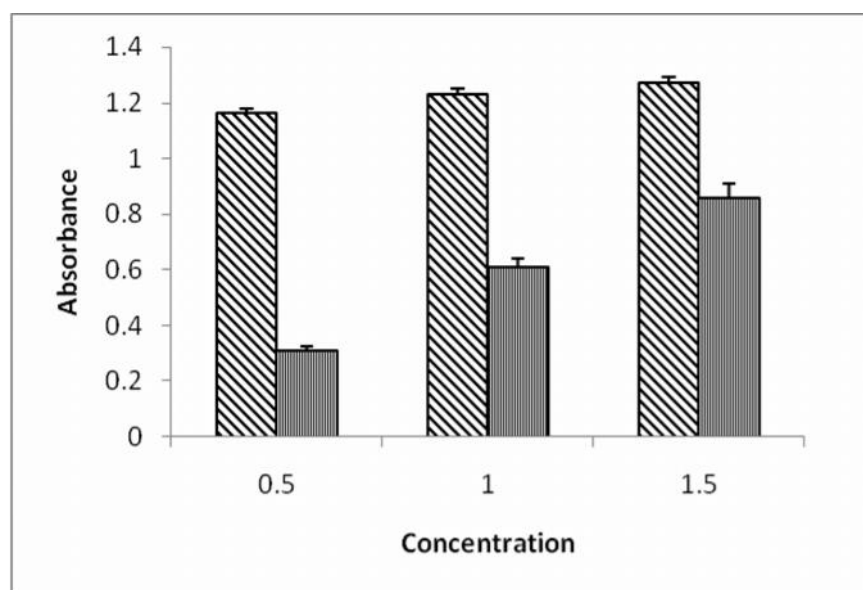


Fig 3: Reducing power of EfraPf with respect to that of ascorbic acid used as standard.

Table 2: EC₅₀ values of the extract for different antioxidant properties

	Chelating	DPPH	Reducing
EC ₅₀ (mg/ml)	.26 ± .007	1.8 ± .02	.84 ± .03

The EC₅₀ values of each antioxidant assay were correlated with each other to find out the relationships, if any, between each activity. Table 3 shows a clear correlation among each antioxidant activity. Chelating and reducing activities show a very strong positive correlation, signifying both of these activities can occur at the same level, at the same time. Correlations of total phenol, flavonoids and ascorbic acid content with EC₅₀ value of each antioxidant attribute were established and correlation coefficients (R^2) are shown in table 4. It seems that contents of these components were moderately to highly associated, R^2 ranging between 0.188 and 0.924, with antioxidant properties.

Table 3: Correlation between each of the antioxidant activities of EfraPf.

	Chelating	DPPH	Reducing
chelating	1		
dpph	-0.44925	1	
reducing	0.979864	-0.61859	1

Table 4: Correlation between each antioxidant property and major antioxidant components.

	Chelating	DPPH	Reducing
Flavonoids	0.1889	0.924	0.825
Total phenol	0.826	0.5	0.048
Ascorbic acid	0.773	0.792	0.901

Discussion:

Total antioxidant capacities of EfraPf was analysed by the phosphomolybdenum method. A high absorbance value of the sample indicates high antioxidant activity. The total antioxidant activity of EfraPf was found to be equivalent to the activity of $40 \pm 3.7 \mu\text{g}$ of ascorbic acid. The total antioxidant capacity of EfraPf may be attributed to their chemical composition and phenolic acid content. A recent study³¹ showed that some bioactive compounds from citrus fruits had strong total antioxidant activity, which was probably due to the presence of flavonoids, carotenoids and ascorbic acid.

DPPH is a stable free radical and possesses a characteristic absorbance at 517 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atom or electron to become a stable diamagnetic molecule. The use of stable DPPH radical has the advantage of being unaffected by side reactions, such as enzyme inhibition and metal chelation. The EC₅₀ value of ethanolic fraction of *Pleurotus flabellatus* (EfraPf) was lower than the ethanolic extract of *Calocybe gambosa*, *Armillaria mellea*, *Clitocybe odora* and *Coprinus cometus*¹. In our earlier investigations, the EC₅₀ value for the ethanolic extract of *Meripilus giganteus*⁶, *Ramaria aurea*⁷ showed very low EC₅₀ value. Therefore, the DPPH radical scavenging activity of the ethanolic extract from different basidiocarps were in descending order *Meripilus giganteus* ~ *Ramaria aurea* > *Pleurotus flabellatus* > *Coprinus cometus* > *Clitocybe odora* > *Armillaria mellea* > *Calocybe gambosa*. In a related study, the edible mushroom *Volvariella volvacea*, showed 57.8% DPPH scavenging at a concentration of 9 mg/ml³². Thus it can be said that the ethanolic extract of *Pleurotus flabellatus* has significant DPPH radical scavenging ability.

Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxy and alkoxy radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The main mechanism of the ferrous ion chelating activity is the ability to deactivate and/or chelate Fe^{2+} which can promote Fenton reaction and hydroperoxide decomposition. Iron toxicity is associated with an increased risk of free radical damage and cancer. Chelation therapy may possibly reduce iron related free radical damage and increase the overall survival in cardiovascular diseases³³. The ferrous ion chelating ability of EfraPf was effective and the EC_{50} value was found to be 0.36 mg/ml. Previous investigators have shown that the EC_{50} value of the ethanolic extract for *Russula delica*³⁴ and *Hypsizigus marmoreus*³⁵ were more than 3mg/ml which is much higher than that of EfraPf. In our earlier investigations, the EC_{50} value of the ethanolic extract of *Tricholoma giganteum* was very close to 1 mg/ml¹³. Hence, the studied mushroom extract shows higher interference with the formation of ferrous and ferrozine complex and can be considered as a good chelator of ferrous ions.

Reducing power of a compound serves as a significant indication of its potential antioxidant activity. The presence of reducers (i.e., antioxidants) causes the reduction of Fe^{3+} /Ferrocyanide complex to ferrous form. The yellow colour of the test solution changes to various shades of green and blue, which depends on the reducing power of each compound. EfraPf was found to be a potent reducing agent, with an EC_{50} value of 0.84 mg/ml. Compared with the reducing powers of previously studied edible mushrooms from previously reported studies, the ethanolic extract of *Pleurotus flabellatus* was an excellent reducer of ferric ions. The reducing power of ethanolic extracts of different edible mushrooms in descending order are *Hypsizigus marmoreus*³⁵ > *Calocybe gambosa*³⁷ > *Armillaria mellea*³⁷ > *Clitocybe odora*¹ > *Tricholoma giganteum*¹³ > *Coprinus comatus*¹ > *Pleurotus flabellatus*. Apparently the ethanolic extract of *P. flabellatus* is an excellent reducing agent.

Phenolic compounds are known to be powerful chain-breaking antioxidants and they possess scavenging ability due to their hydroxyl groups. The phenolic compounds contribute directly to the antioxidative action. In the present study, the total phenolic content of EfraPf ($6.875 \pm 0.45 \mu\text{g}/\text{mg}$) was found to be higher than that of the ethanolic extract of *P. ostreatus*, which was reported to be $5.49 \mu\text{g}/\text{mg}$ ³⁸, but was comparatively less than *P. squarrosulus*, which was $18.1 \mu\text{g}/\text{mg}$ ⁴⁰ and relatively similar to *P. citrinopileatus*, that being $8.62 \mu\text{g}/\text{mg}$ ³⁶.

Ascorbic acid is reported to interact directly with radicals such as O_2^- and $\text{OH}\cdot$ in plasma, thus preventing damage to red cell membranes, it also assists α -tocopherol in inhibition of lipid peroxidase by recycling the tocopherol radical³⁹. In the present study, the ascorbic acid content of *P. flabellatus* extract was high ($8.75 \mu\text{g}/\text{mg}$) when compared to the values reported from other *Pleurotus* species such as *P. ostreatus* (25 ng/1 mg) and *P. citrinopileatus* (31 ng/mg). Many other naturally occurring antioxidant components, including α -carotene, lycopene and flavonoids are known to possess strong antioxidative characteristics⁴¹. In this study α -carotene and lycopene were found in vestigial amounts, i.e. $7.17 \text{ ng}/\text{mg}$ and $4.59 \text{ ng}/\text{mg}$ respectively, which are lower than that of the methanolic extract of *P. squarrosulus*, which were 570 ng and 225 ng per mg respectively⁴⁰, but its α -carotene content was found to be higher than *P. ostreatus*³⁸. α -carotene was not detected in the ethanolic extract of *P. citrinopileatus*³⁶. The estimated flavonoid content of EfraPf is $1.0625 \mu\text{g}/\text{mg}$ which is lower than *P. squarrosulus*, where it was reported to be $3.07 \mu\text{g}/\text{mg}$ ⁴⁰ but higher than *P. citrinopileatus*, $71.2 \text{ ng}/\text{mg}$ ³⁶. Total phenols, ascorbic acid and flavonoids were the major naturally occurring antioxidant components estimated in this study. The higher amounts of these components in this extract might explain its more effectiveness in antioxidant properties.

Conclusion:

As a result of the study, the ethanolic extract of *Pleurotus flabellatus* was found to be an effective antioxidant in different *in vitro* assays including Ferrous iron chelating, ferric iron reducing, DPPH free radical scavenging and total antioxidant activity, and can be suggested as a natural additive in food and pharmaceutical industries.

Acknowledgement:

The author M. Rai gratefully acknowledges the financial support of University Grant Commission [F.PSW-080/11-12(ERO)], India.

References:

1. Khatua S., Paul S. and Acharya K., Mushroom as the potential source of new generation of antioxidant: a review, Res. J. Pharm. Tech., 2013, 6(5),496-505.
2. Rai M., Biswas G., Mandal S.C. and Acharya K., Free radicals and human diseases. In *Herbal Drugs: A modern approach to understand them better*, ed. S.C. Mandal, New Central Book Agency (P) Ltd., Kolkata, India, 2011,479-496.
3. Lin H.M., Yen F.L., Ng L.T. and Lin C.C., Protective effects of *Ligustrum lucidum* fruit extract on acute butylated hydroxytoluene-induced oxidative stress in rats, J. Ethnopharmacol., 2007, 11,129-136.
4. Nabavi S.F., Ebrahimzadeh M.A., Nabavi S.M. and Eslami B., Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss, Grasas Aceites., 2010, 61(3),244-250.
5. Mitra P., Khatua S. and Acharya K., Free Radical Scavenging and NOS Activation Properties of Water Soluble Crude Polysaccharide from *Pleurotus ostreatus*, Asian J. Pharm. Clin. Res., 2013, 6(3),67-70.
6. Acharya K. and Rai M., Proximate composition, free radical scavenging and NOS activation properties of a wild edible mushroom, Int. J. Pharm. Pharm. Sci., 2013, 5(1),67-72.
7. Rai M. and Acharya K., Proximate composition, free radical scavenging and NOS activation properties of *Ramaria aurea*, Res. J. Pharm. Tech., 2012, 5(11),1421-1427.
8. Rai M. and Acharya K., Evaluation of antioxidant and nitric oxide synthase activation properties of *Volvariella volvacea*, Int. J. Pharm. Pharm. Sci., 2012, 4(4),460-463.
9. Acharya K., Chatterjee S. and Ghosh S., Comparative evaluation on the free radical scavenging activity of eleven Indian cultivated strains of *Pleurotus ostreatus*, Pharmacologyonline, 2011, 1,440-450.
10. Acharya, K., Medicinal properties of mushroom, In *Advances in Medicinal Plant Research*, ed. S.N. Acharya and J.E. Thomas, Research Signpost, Kerala, India, 2007,215-236.
11. Biswas G., Chatterjee S. and Acharya K., Chemopreventive activity of the ethanolic extract of *Astraeus hygrometricus* (pers.) morg. on Ehrlich's Ascites Carcinoma cells, Digest J. Nanomat. Biostruc., 2012, 7,185-191.
12. Chatterjee S., Biswas G., Chandra S., Saha G.K. and Acharya K., Apoptogenic effects of *Tricholoma giganteum* on Ehrlich's Ascites Carcinoma cell, Bioprocess. Biosys. Eng. 2013, 36,101-107.
13. Chatterjee S., Biswas G., Basu S.K. and Acharya K., Antineoplastic effect of mushrooms: a review, Aust. J. Crop Sci., 2011, 5, 904-911.
14. Biswas G., Rana S., Sarkar S. and Acharya K., Cardioprotective activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg, Pharmacologyonline., 2011, 2,808-817.
15. Biswas G. and Acharya K., Hypoglycemic activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. in alloxan-induced diabetic mice, Int. J. Pharm. Pharm. Sci., 2013, 5 (Suppl 1),391-394.
16. Biswas G., Sarkar S. and Acharya K., Free Radical Scavenging and Anti-Inflammatory Activities of the Extracts of *Astraeus hygrometricus* (Pers.) Morg, Lat. Am. J. Pharm. 2010, 29,549-543.
17. Chatterjee S., Dey A., Dutta R., Dey S. and Acharya K., Hepatoprotective Effect of the Ethanolic Extract of *Calocybe indica* on Mice with CCl₄ Hepatic Intoxication, Int. J. PharmTech. Res., 2011, 3,2162-2168.
18. Acharya K., Chatterjee S., Biswas G., Chatterjee A. and Saha G.K., Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in mice, Int. J. Pharm. Pharm. Sci., 2012, 4,285-88.
19. Chatterjee S., Datta R., Dey A., Pradhan P. and Acharya K., In vivo Hepatoprotective Activity of Ethanolic Extract of *Russula albonigra* against Carbon Tetrachloride-Induced Hepatotoxicity in Mice, Res. J. Pharm. Tech., 2012, 5(8),1034-1038.
20. Giri S., Biswas G., Pradhan P., Mandal S.C. and Acharya K., Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India, Int. J. PharmTech. Res., 2012, 4(4),1554-1560.
21. Lai T.K., Biswas G., Chatterjee S., Dutta A., Pal C., Banerji J., Bhuvanesh N., Reibenspies J.H. and Acharya K., Leishmanicidal and anticandidal activity of constituents of Indian edible mushroom *Astraeus hygrometricus*, Chem. Biodivers., 2012, 9,1517-1524.
22. Singleton V.L. and Rossi J.A., Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents, Am. J. Enol. Vitic., 1965, 16,144-158.
23. Adebayo E.A., Oloke J.K., Ayandele A.A. and Adegunlola C.O., Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius* –LAU 09 (JF736658), J. Microbiol. Biotech. Res., 2012, 2(2),366-374.

24. Nagata M. and Yamashita I., Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit, *Nippon Shokuhin Kogyo Gakkaishi*, 1992, 39(10),925–928.
25. Rekha C., Poornima G., Manasa M., Abhipsa V., Pavithra D.J., Vijay K.H.T. and Prashith K.T.R., Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits, *Chem. Sci. Trans.*, 2012, 1(2),303-310.
26. Prieto P., Pineda M. and Aguilar M., Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.*, 1999, 269,337–341.
27. Oyaizu M., Studies on product of browning reaction prepared from glucose amine, *Jpn. J. Nut.*, 1986, 44,307-315.
28. Dinis T.C.P., Madeira V.M.C. and Almeida L.M., Action of phenolic derivatives (acetoaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers, *Arch. Biochem. Biophys.*, 1994, 315,161-169.
29. Waltasinghe M. and Shahidi F., Antioxidant and free radical scavenging properties of ethanolic extracts of defatted borage (*Berapa officinalis* L.) seeds, *Food Chem.*, 1999, 63,399-414.
30. Sowndhararajan K., Joseph J.M. and Manian S., Antioxidant and Free Radical Scavenging Activities of Indian Acacias: *Acacia Leucophloea* (Roxb.) Willd., *Acacia Ferruginea* Dc., *Acacia Dealbata* Link. and *Acacia Pennata* (L.) Willd, *Int. J. Food Prop.* 2013, 16(8),1717-1729.
31. Jayaprakasha G.K., Girenavar B. and Patil B.S., Radical scavenging activities of Rio Red grapefruits and sour orange fruit extracts in different in vitro model systems, *Bioresour. Technol.*, 2008, 99,4484–4494.
32. Cheung L.M., Cheung P.C.K. and Ooi V.E.C., Antioxidant activity and total phenolics of edible mushroom extracts, *Food Chem.*, 2003, 81,249-255.
33. Halliwell B. and Gutteridge J.M.C., Role of free radicals and catalytic metal ions in human disease: an overview, *Meth. Enzymol.*, 1990, 86,1-85.
34. Yaltirak T., Aslim B., Ozturk S. and Alli H., Antimicrobial and antioxidant activities of *Russula delica* Fr., *Food Chem. Toxicol.*, 2009, 47,2052-2056.
35. Lee Y.L., Yen M.T. and Mau J.L., Antioxidant properties of various extracts from *Hypsizius marmoreus*, *Food Chem.*, 2007, 104,1-9.
36. Lee Y.L., Huang G.W., Liang Z.C. and Mau J.L., Antioxidant properties of three extracts from *Pleurotus citrinopileatus*, *LWT - Food Sci. Technol.*, 2007, 40(5),823-833.
37. Vaz J, Barrosa L., Martinse A., Santos-Buelgaf C., Vasconcelosb M.H. and Ferreira I.C.F.R., Chemical composition of wild edible mushrooms and antioxidant properties of their water soluble polysaccharidic and ethanolic fractions, *Food Chem.*, 2011, 126(2),610-616.
38. Jayakumar T., Thomas P.A. and Geraldine P., In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*, *Innovative Food Sci. Emerg. Technol.*, 2009, 10(2),228-234.
39. Beyer W.N., Conner E. and Gerould S., Estimates of soil ingestion by wildlife. *J. Wildl. Manage.* 1994, 58,375-382.
40. Pal J., Ganguly S., Tahsin K.S. and Acharya K., In vitro free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont.) Singer., *Indian J. Exp. Biol.*, 2010, 48(12),1210-1218.
41. Dziedzic S.Z. and Hudson G.J.F., Polyhydrochalcones and flavanones as antioxidants for edible oils, *Food Chem.*, 1983, 12,205-212.
