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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF *PLEUROTUS FLORIDA* MUSHROOM

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ABSTRACT

Objective: An attempt was made to investigate the antioxidant activity of the methanolic extract of *Pleurotus florida* mushroom.

Methods: Antioxidant activity of *Pleurotus florida* mushroom was evaluated using DPPH (1, 1- Diphenyl -2- picrylhydrazyl) radical, reducing power, superoxide radical, hydroxyl radical, nitric oxide and metal chelating assay.

Results: The methanol extract showed the most potent radical-scavenging activity at a maximum concentration of 100 µg/ml and the scavenging effects was 78% on DPPH radicals. The IC_{50} value of the extract was found to be 50 µg/ml. The extract manifested significant reducing power (0.911) which exceeded even that of ascorbic acid (0.610) at a concentration of 500 µg/ml. At a maximum concentration of 1000 µg/ml, the extract showed 81.8% inhibition of nitric oxide and 65% of superoxide radicals; also the extract exhibited the scavenging effects of 64 and 59% inhibition on chelation of ferrous ions and hydroxyl radical respectively.

Conclusion: The present investigation suggest that the methanolic extract of *P. florida* mushroom has significant antioxidant activity, could be served as an easily accessible item of natural rich antioxidant food, as a promising food supplement or it may be utilized as a potential source of therapeutic agent.

Keywords: Antioxidant, DPPH, Methanol extract, *Pleurotus florida*, Radical scavenging activity.

INTRODUCTION

There are thousands of medicines and natural products have been closely linked through the use of traditional medicines and natural poisons. Natural products represent a wealthy source of biologically active compounds with recognized potential drug discovery and development [1]. In addition to the ancient utilization of plants the medicinal use of mushrooms has also an incredibly long custom. Among the large resources of fungi, higher Basidiomycetes, particularly mushrooms are unlimited sources of remedially useful biologically active agents [2]. Since ancient times mushrooms have been used as folk medicine throughout the world and they are used as nutritionally functional food and beneficial nontoxic medicines [3]. Mushrooms comprise an untapped source of powerful new pharmaceutical products. In particular, the most significant medicinal effect for modern medicine in mushrooms and their metabolites are that they attracted the attention of the public and they represent an unlimited source of polysaccharides with antitumor and immunostimulating properties [4].

Pleurotus species are commonly called as Oyster mushrooms. It is a lignocellulolytic fungus that grows naturally in the temperate and tropical forests on dead and decaying matter and it is in second grade among the important cultivated mushrooms in the world. There are about 40 species are coming under *Pleurotus* mushroom, in that 25 species are commercially cultivated [5].

Oxidation is essential to many living organisms for production of energy for biological processes. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxidation of cell and leads to cell death and tissue damage. Free radicals are responsible for aging and causing various human diseases such as atherosclerosis, diabetes, cancer, hypertension, alzheimer's disease, parkinsonism and cirrhosis [6]. Antioxidants dealing with an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves [7]. The antioxidants in the human diet are of great interest as possible protective agents to help human body to reduce oxidative damage. To prevent lipid oxidation food industries have long using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest in antioxidant substances from natural resources [8].

Several species of mushroom contains a wide variety of free radicals or reactive oxygen species scavengers which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development [9]. Barros *et al.* (2008) reported that mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system [10]. But to date, there is still relatively scarce information on antioxidant activities of the mushroom *P. florida*, which is widely cultivated in India. Hence, the present study aims to evaluate the antioxidant activities of methanolic extract of *P. florida* by free radical scavenging ability, reducing power and chelating effects on ferrous ion.

MATERIALS AND METHODS

Chemicals

The chemical agents such as 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Phenazine Methosulphate (PMS), Nicotinamide Adenine Dinucletide (NADH), Nitro Blue Tetrazolium (NBT) and Thiobarbituric acid (TBA), Methanol, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteu Phenolic reagent, Ferrozine, Sodium carbonate, Potassium ferricyanide, L- Ascorbic acid, Ethylenediamine tetraacetic acid (EDTA) disodium salt, Hydrogen peroxide (H_2O_2), Trichloroacetic acid (TCA) and Sodium hydroxide (NaoH) were purchased from Sigma Chemicals Co. (St. Louis, USA) or Merck (Darmstall, Germany) or Himedia, Mumbai. All the chemicals used were of analytical grade.

Methanol extraction

Since horse gram supplement enhanced the growth of *P. florida* mushrooms [11], the mushroom cultivated in horse gram nutrient was used in the present study. The *P. florida* mushrooms was cultivated, air dried in shade and powdered. About 10 g of the powdered mushroom was extracted with 200 ml of 95% methanol using a Soxhlet apparatus and filtered through muslin cloth. The filtered extract was evaporated under reduced pressure and vacuum drier to get the viscous residue and used for the estimation of antioxidant activities.

DPPH radical scavenging assay

The antioxidant activity of the methanolic extract of *P. florida* mushroom was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux [12]. About 1 ml of 100 µM DPPH solution in methanol, equal volume of the extract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30 min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using a spectrophotometer at 517 nm (Cyberlab UV 100, USA). The different concentrations of ascorbic acid were used as reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

Percentage of DPPH assay = (Control OD – Sample OD / Control OD) x 100

Superoxide radical scavenging assay

The superoxide radical scavenging activity of the methanol extract was studied by using the method of Liu *et al.* [13]. Superoxide radicals are generated in phenazine methosulphate (PMS) - Nicotinamide adenine dinucletide (NADH) systems by oxidation of NADH and assayed by the reduction of Nitro Blue Tetrazolium (NBT). About 200 µl of methanol extract of different concentrations were taken in a series of test tubes. Superoxide radicals were generated by 1 ml of Tris-HCl buffer (16 mM, pH-8), 1 ml of NBT (50 µM), 1 ml of NADH (78 µM) solution and 1 ml of PMS (10 µM). The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm. A control tube containing Tris-HCl buffer was also processed in the same way without methanolic extract of *P. florida* mushroom. Different concentration of ascorbic acid was used as reference compound. The percentage inhibition of superoxide anion generation was calculated as follows:

Percentage Inhibition = $[(A_0 - A_1) / A_0] \times 100$,

Where A_0 is the absorbance of the control; A_1 is the absorbance of the sample extract/ standard.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan *et al*. [14]. When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions. About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the *P. florida* methanolic extract (200 - 1000 µg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1-naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding *P. florida* extract.

Hydroxyl radical scavenging assay

Hydroxyl radicals (OH) are generated from Fe^{2+} - ascorbate - EDTA - H_2O_2 system (Fenton's reaction) which attack the deoxyribose and set off a series of reactions that eventually result in the formation of malondialdehyde (MDA), measured as a pink MDA-TBA chromogen at 535nm. The hydroxyl radical scavenging activity of *P. florida* methanolic extract was measured according to the method of Halliwell *et al.* [15]. Stock solutions of EDTA (1 mM) were prepared in DMSO and $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The method was carried out by adding 100 µl of EDTA, 10 µl of $FeCl_3$, 100 µl of H_2O_2, 360 µl of deoxyribose, 1000 µl of the extract (100 to 500 µg/ml) dissolved in distilled water, 330 µl of phosphate buffer (50 mM, pH 7.4) and 100 µl of ascorbic acid. This mixture was then incubated at 37°C for 1 h. About 1 ml of incubated mixture was mixed with 1 ml of 10% TCA and 1 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% butylated hydroxyl anisole) and the development of pink chromogen was measured spectrophotometrically at 532 nm. The hydroxyl radical scavenging activity of the mushroom extract was reported as percentage inhibition of deoxyribose degradation and was calculated by using the formula:

$$OH^{\bullet}\text{- scavenged (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 was the control absorbance and A_1 was the mixture containing the extract absorbance or the standard absorbance.

Metal chelating ability of ferrous ions

The chelating of ferrous ions by methanol extract of *P. florida* was estimated by the method of Hinneburg *et al.* [16]. The different concentration of extract was added to a solution of 2 mM/L $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left at 37°C for 10 min and the absorbance was measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard and the results were expressed as µg EDTA equivalent/g extract.

Reducing power assay

The reducing power was determined according to the method of Berker *et al.* [17]. The *P. florida* methanolic extract (100-500 µg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After the addition of 2.5 ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (µg extract/ml) is the effective concentration at which the 0.5 absorbance for reducing power and ascorbic acid was used as a standard.

RESULTS

***In vitro* free radical scavenging assays**

The methanolic extract of *P. florida* mushroom was investigated for the effects on the *in vitro* generation of free radicals and antioxidant profile. The results of the study showed that the maximum extent inhibition of free radical generation and maximum amount of antioxidant capacity, identification of nature of the active principle and methanol extract of *P. florida* mushroom was analyzed using antioxidant profile against a battery of oxidant moieties that included radicals like DPPH, SO, NO, OH, the metal chelating and reducing power assay.

DPPH radical scavenging assay

DPPH free radical compound has been widely used to test the free radical scavenging ability of various food samples; the antioxidant present neutralizes the DPPH• by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH• could be determined by colour changes from purple to yellow by read at 517 nm. The methanolic extract of *P. florida* demonstrated H-donor activity in our study. The DPPH radical scavenging activity of extracted material was detected and compared with standard antioxidant - vitamin C. The extract of *P. florida* tested against DPPH stable radicals spectrophotometrically which reveals that the radical scavenging activity of *P. florida* methanol extract possessed excellent antioxidant capacity by increased with the increasing concentration of the extract [Fig. 1]. At a concentration of 100µg/ml of methanol extract the percentage of inhibition was found to be 78%. However, the scavenging activity of ascorbic acid at the same concentration was 85.02%. The IC_{50} values of methanol extract of *P. florida* was found at the concentration of 50 µg/ml [Table 1].

Table 1: IC_{50} values of the methanolic extract from *P. florida* for antioxidant properties

S. No.	Antioxidant profile	IC_{50} values (µg/ml)	
		Control (Ascorbic acid)	Methanolic extract of *P. florida* mushroom
1	DPPH radical	24	50
2	Superoxide radical	451	542
3	Nitric oxide radical	240	510
4	Hydroxyl radical	360	410
5	Metal chelating	243	355
6	Reducing power assay	355	110

Superoxide radical scavenging assay

The result of superoxide radical (SO) scavenging activity obtained for the extract of *P. florida* showed dose dependent free radical scavenging activity and the percentage inhibition was shown in [Fig. 2]. In the present study, the *P. florida* mushroom extract was found to be a notable scavenger of superoxide radicals generated in the riboflavin-NBT light system.

The scavenging activity of methanolic extract of *P. florida* (IC_{50} 542 µg/ml) was compared with the vitamin C (451 µg/ml) as standard. The methanol extract in a concentration of 200 µg/ml showed 29.2% whereas, in 1000 µg/ml concentration the percentage of inhibition (O_2^- is scavenged and thus blue formazan formation is inhibited) was recorded as 65%. This clearly indicates the inhibition activity was dose dependent.

Nitric oxide radical scavenging assay

The methanolic extract of *P. florida* mushroom effectively reduced the generation of nitric oxide from sodium nitroprusside. IC_{50} value of *P. florida* methanol extract showed nitric oxide scavenging activity at the concentration of 510µg/ml while the standard vitamin C was showed 240 µg/ml [Table 1]. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore was measured at 546 nm in the presence of the extract. *P. florida* extract proved to decrease in amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. The methanol extract recorded maximum percentage of NO activity of 81.8% at the concentration 1000 µg/ml [Fig. 3].

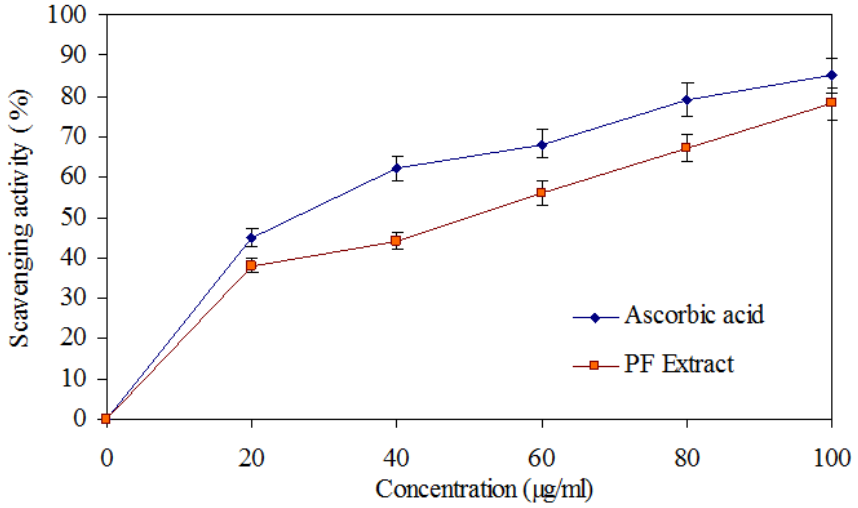

Fig. 1: The scavenging activity on DPPH radical of methanolic extract of *P. florida* mushroom

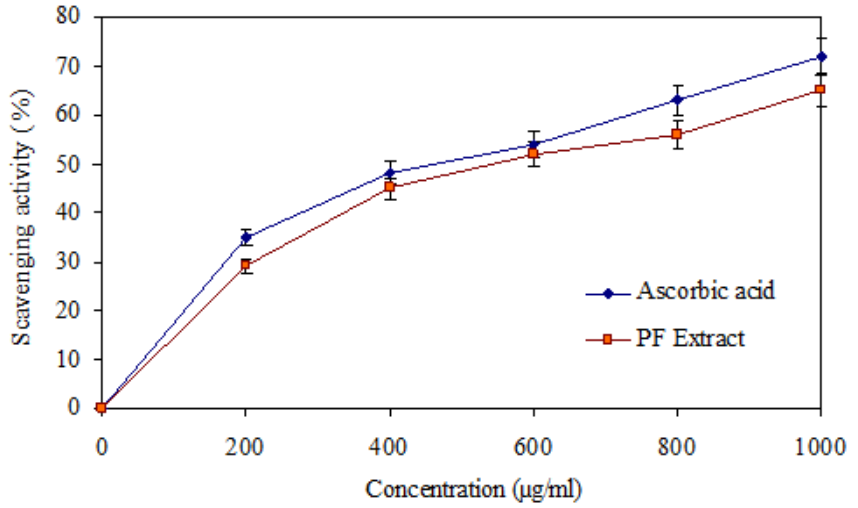

Fig. 2: The scavenging activity on superoxide radical of methanolic extract of *P. florida* mushroom

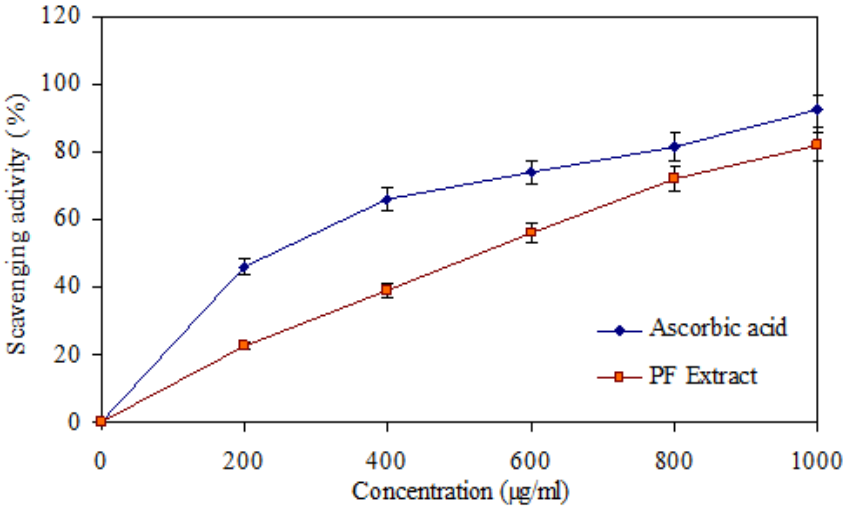

Fig. 3: Nitric oxide scavenging assay of methanolic extract of *P. florida*

Hydroxyl radical scavenging assay

Hydroxyl radicals (OH) generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen upon heating with TBA at low pH. Ferric – EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4.While the addition of methanol extract to the reaction mixture found that they removed hydroxyl radical from the sugar and prevented their degradation. The methanol extract of *P. florida* mushroom showed potent hydroxyl radical scavenging activity. The potential methanol extract of the *P. florida* mushroom, inhibit hydroxyl radical-mediated deoxyribose damage which was assessed by means of the iron (II) - dependent DNA damage assay. In the present study, the hydroxyl radical-scavenging effect of the methanol extract in a concentration of 500 µg/ml was found to be 59.2% and ascorbic acid was used as a standard since it is reported to be significantly effective in inhibition of hydroxyl radicals, which shows 63% scavenging effect at the same concentration [Fig. 4]. The IC_{50} value of *P. florida* and ascorbic acid was found at the concentration of 410µg/ml and 360µg/ml respectively [Table 1], further the shown hydroxyl radical scavenging activity as dose dependent.

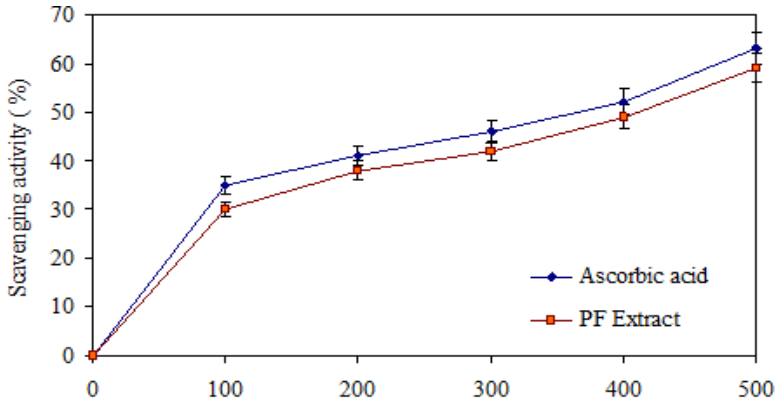

Fig. 4: Scavenging activity on hydroxyl radical of methanolic extract of *P. florida* mushroom

Metal chelating ability of ferrous ions

Transition metals have been proposed as the catalysts for the initial formation of radicals. Chelating agents may stabilize transition metals in living systems and inhibit generation of radicals, consequently reducing free radical-induced damage. To estimate the antioxidant potential of the *P. florida* mushroom extract, its chelating activity was evaluated against Fe^{2+}. Ferrozine quantitatively forms complexes with Fe^{2+}. The chelating effects of the mushroom extract and EDTA on ferrous ions evaluated as increased with increasing concentrations [Fig. 5]. The methanol extract chelated 64% at the concentration of 500 µg/ml and the IC_{50} value of *P. florida* was found to be at the concentration of 355 µg/ml [Table 1]. However, synthetic metal chelator (EDTA) showed an excellent ability of 81% at the same tested concentration.

Reducing power assay

To find the active species which is capable of donating hydrogen and subsequently its leads to the reducing power activity was determined. The high reducing power is indicative of the hydrogen donating ability of the active species present in the extract. Antioxidant potential of HGS *P. florida* methanol extract was estimated by using potassium ferric cyanide reduction method. In the present study, the reducing power of the methanolic extract of *P. florida* was found to be excellent and steadily increase in direct proportion to the increasing concentrations of the extract [Fig. 6]. The reducing power of a 500 µg/ml concentration of the mushroom extract was found to be 0.911, which was relatively more pronounced than that of standard ascorbic acid (0.61). IC_{50} value in reducing power of *P. florida* was found to be 110µg/ml while comparing with the standard antioxidant ascorbic acid at the concentration of 355µg/ml [Table 1].

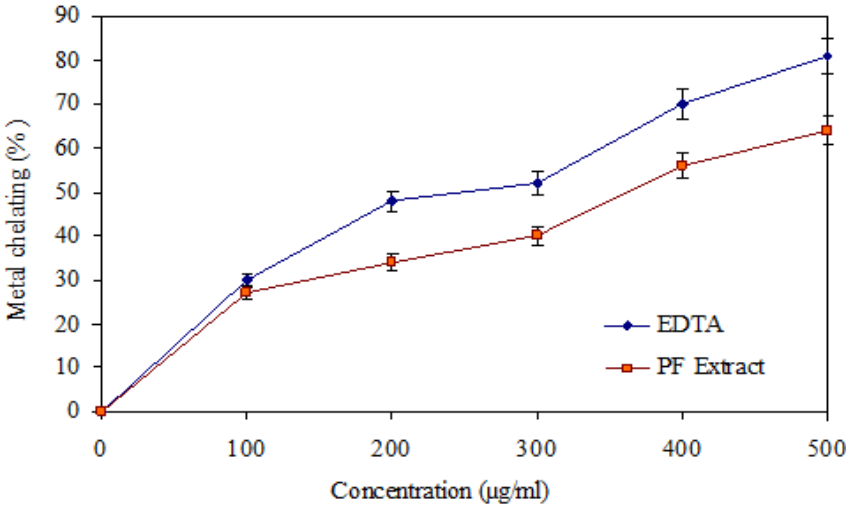

Fig. 5: Metal chelating activity of methanolic extract of *P. florida*

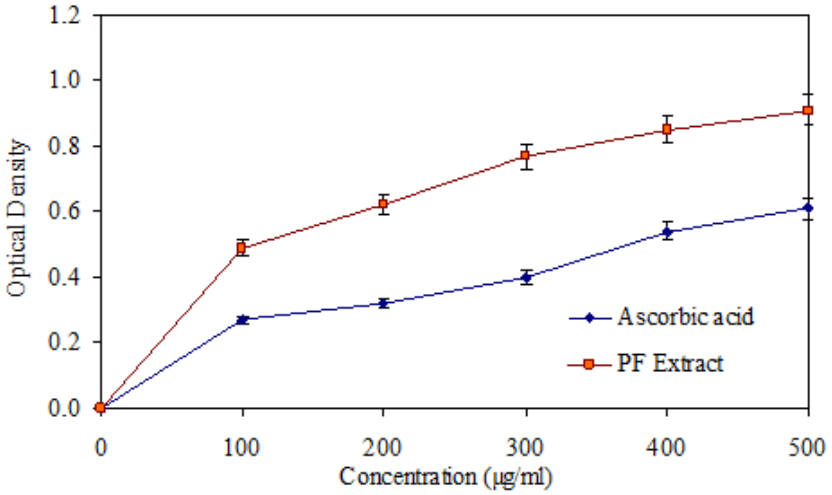

Fig. 6: The reducing power assay of methanolic extract of *P. florida*

DISCUSSION

In the free radical scavenging assays, DPPH is stable and possesses a distinctive absorbance at 517 nm, which significantly decreases on exposure to radical scavengers by donating a hydrogen atom to become a stable diamagnetic molecule. DPPH radical has certain advantage of being unaffected by side reactions *i.e.,* enzyme inhibition and metal chelation [6]. The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine [18]. The reducing capacity of extract may be serving as a significant indication of its probable antioxidant capacity and the reducing properties of antioxidants are generally associated with the presence of reductones, e.g., ascorbic acid [13].

Superoxide radical is a highly toxic species, which is generating by numerous biological and photochemical reactions [19]. Superoxide radical can further interact with other molecules to generate secondary ROS (e.g., hydroxyl radical, hydrogen peroxide and singlet oxygen), either directly or prevalently through enzyme or metal catalyzed processes [20]. The hydroxyl radical is the most reactive of the ROS which induces severe damage in adjacent biomolecules [21]. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins [22]. Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxyl and alcoxyl radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [23]. Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal [24].

Different range of concentrations was used, *P. florida* mushroom showed a dose-dependent percentage of inhibition on the scavenging assays. Therefore, the methanolic extract showed significant antioxidant potential that may reveal its therapeutic potentials for several diseases. Results of the present study revealed that methanol extract of *P. florida* mushroom showed relatively high reducing power and DPPH radical scavenging assay (IC_{50} value110µg/ml and 50 µg/ml). At a maximum concentration of 1000 µg/ml, the extract showed 81.8% inhibition of nitric oxide and 65% of superoxide radicals; also the methanolic extract exhibited the scavenging effects of 64% and 59% inhibition on chelation of ferrous ions and hydroxyl radical respectively. The radical scavenging activity of *P. ostreatus* mushroom is reported to be higher (6 mg/ml) than those of other mushrooms like *Agaricus bisporus, Volvariella volvaceae, Calocybe indica* and *Hybsizus ulmarius* [25]. When compared to *P. ostreatus* mushroom the methanolic extract of *P. florida* has higher chelating activity against ferrous ion [26]. The IC_{50} value of ethanolic extract of *A. bisporus* was 0.37 and 0.38 mg/ml, methanolic extract of *Boletus edulis* was 0.34

and 0.73 mg/ml in reducing power and DPPH radical scavenging assay respectively [27, 28]. Finimundy *et al.* (2012) reported that IC_{50} value of DPPH scavenging ability of aqueous extract of *P. sajar-caju* showed 9.01 % and the EC_{50} values of *P. abalones* in DPPH radicals scavenging ability and reducing power were 8.68 and 4.68 mg/ml respectively [29, 30]. The IC_{50} value of hot water extract of *P. squarrosulus* was found to be 340 µg/ml [6]. Therefore, the scavenging activity of *P. florida* was much effective than those mentioned above. Apparently, the reducing power of the methanolic extract of *P. florida* is excellent, when compared to that of other commercial and medicinal mushrooms. A large number of antioxidant components such as phenolic compounds, flavonoids, carotenoids and vitamins C and E have been isolated from the fruit bodies of *Pleurotus* mushrooms [31]. The quantity of these antioxidant phytochemicals components vary from strain to strain and also depend on cultivation using different nutrient supplement.

CONCLUSION

The results obtained from this study strongly suggest that the methanolic extract of *P. florida*, has significant antioxidant activity, could serve as an easily accessible item of natural rich antioxidant food which may enhance the immune system against oxidative damage, or it may be utilized as a potential source of therapeutic agent.

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REFERENCES

1. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003; 66: 1022-37.
2. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulatory polysaccharides. Appl Microbiol Biotechnol 2002; 60: 258-74.
3. Wasser SP, Elisashvili VI, Tan KK. Effects of carbon and nitrogen sources in the medium on *Tremella mesenterica* Retz.: Fr. (Heterobasidiomycetes) growth and polysaccharide production. Int J Med Mushrooms 2003; 5: 49-56.
4. Jose N, Janardhanan KK. Antioxidant and antitumor activity of *Pleurotus florida.* Curr Sci 2000; 79 (Suppl 7): 941-43.
5. Singh M, Vijay B, Kamal S, Wakchaure GC. Mushrooms: Cultivation, Marketing and Consumption. Directorate of Mushroom Research, Indian Council of Agricultural Research (ICAR), Solan (India); 2011.
6. Pal J, Ganguly S, Tahsin KS, Acharya K. In vitro free radical scavenging activity of wild edible mushroom Pleurotus squarrosulus (Mont.) Singer. Indian J Experimen Biol 2010; 47: 1210-18.
7. Sies H. Oxidative stress: oxidants and antioxidants. Experimen Physiol 1997; 82 (Suppl 2): 291-95.
8. Naveena BM, Sen AR, Vaithiyanathan S, Babji Y, Kondaiah N. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Sc 2008; 80 (Suppl 4): 1304-08.
9. Guerra-Dore CMP, Azevedo TCG, de Souza MCR, Rego LA, de Dantas JCM, Silva FRF, Rocha HAO, Baseia IG, Leite EL. Anti-inflammatory, antioxidant and cytotoxic actions of β-glucan-rich extract from *Geastrum saccatum* mushroom. Int Immunopharmacol 2007; 7 (Suppl 9): 1160-69.
10. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 2008; 46 (Suppl 8): 2742-47.
11. Menaga D, Mahalingam PU, Rajakumar S, Ayyasamy PM. Evaluation of phytochemical characteristics and antimicrobial activity of *Pleurotus florida* mushroom. Asian J Pharm Clin Res 2012; 5 (Suppl 4): 102-6.
12. Molyneux P. The use of the stable free radical diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004; 26 (Suppl 2): 211-19.
13. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci 1997; 60: 763-71.
14. Govindharajan R, Rastogi S, Vijayakumar M, Rawat AKS, Shirwaikar A, Mehrotra S, Pushpangadan P. Studies on antioxidant activities of *Desmodium gangeticum*. Biol Pharm Bull 2003; 26: 1424-27.
15. Halliwell B, Gutteridge JMC. Cross CE. Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med 1992; 119: 598–620.
16. Hinneburg I, Dorman HJD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem 2006; 97: 122-29.
17. Berker K, Guclu K, Tor I, Demirata B, Apak R. Total Antioxidant Capacity Assay Using Optimized Ferricyanide/Prussian Blue Method. Food Anal Methods 2010; 3: 154-68.
18. Sreeyan N, Rao M. Free radical scavenging activity of curcuminoids. Drug Res 1996; 46: 169-171.
19. Govindarajan R, Vijaya kumar M, Rawat AKS, Shanta M. Free radical scavenging potential of *Picorrhiza kurroa Royle Ex Benth* . Ind J Exp Biol 2003a; 41: 875-879.
20. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44–84.
21. Gutteridge MC. Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid reactive material from deoxy sugars, nucleosides and benzoate. Biochem J 1984; 224: 761-67.
22. Spencer JPE, JennerA, Aruoma OI. Intense oxidative DNA damage promoted by L-DOPA and its metabolites, implications for neurodegenerative disease. FEBS Letters 1994; 353: 246-250.
23. Halliwell B, Gutteridge JMC. Free radicals in Biology and Medicine (Oxford University Press, Oxford, UK, 1989, 1.
24. Wang BS, Chen JH, Liang YC, Duh PD. Effects of Welsh onion on oxidation of low density lipoprotein and nitric oxide production in macrophage cell line RAW 264.7, Food Chem 2005; 91: 147.
25. Ramkumar L, Ramanathan T, Thirunavukkarasu P, Arivuselvan N. Antioxidant and radical scavenging activity of nine edible mushrooms extract. Int J Pharmacolog 2010; 6 (Suppl 6): 950-3.
26. Mohamed Imran M, Mohamed Mahroop Raja M, Abdul Basith J, Asarudeen A. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous.* Int Food Research J 2011; 18: 574-577.
27. Liu J, Jia L, Kan J, Jin CH. *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food Chemical Toxicol 2013; 51: 310-6.
28. Vamanu E, Nita S (2013). Antioxidant capacity and the correlation with major phenolic compounds, anthocyanin, and tocopherol content in various extracts from the wild edible *Boletus edulis* mushroom. Hindawi Publishing Corporation. BioMed Research International 2013; 1-11.
29. Finimundy TC, Gambato G, Fontanab R, Camassolab M, et al. Aqueous extracts of Lentinula edodes and Pleurotus sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity. Nutrition Research 2013; 33 (Suppl 1): 76-84.
30. Wang Q, Li H, Chen TT, Han JR. Yield, polysaccharides content and antioxidant properties of *Pleurotus abalones* and *Pleurotus geesteranus* produced on asparagus straw as substrate. Scientia Horticulturae 2012; 134: 222-26.
31. Jayakumar T, Thomas PA, Sheu JR, Geraldine P. *In-vitro* and *in-vivo* antioxidant effects of the oyster mushroom *Pleurotus ostreatus*. Food Res Int 2011; 44: 851-61.