

**ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF
PLEUROTUS PULMONARIUS (FRIES) QUEL-CHAMP.**

Sachin L. Badole, Prasad A. Thakurdesai, Subhash L. Bodhankar*

Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth University,
Erandwane, Paud Road, Pune-411038, India.

Summary

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. Several commercial mushrooms are reported to be effective against various diseases by virtue of their antioxidant potential. In this investigation, we examined the antioxidant activities of aqueous extract of oyster mushroom, *Pleurotus pulmonarius* (Fr.) through various in vitro models. *P. pulmonarius* showed strong antioxidant activity in DPPH and reducing power assay. It also showed antioxidant potential against hydroxyl radical, nitric oxide, superoxide anion scavenging and hydrogen peroxide scavenging. In addition, the *Pleurotus pulmonarius* found to contain a significant phenolic content. These results suggested that the *P. pulmonarius* can be used as natural antioxidants.

Keywords: *Pleurotus pulmonarius*; antioxidant activity, DPPH, reducing power, total phenols, hydroxyl, nitric oxide, superoxide anion, hydrogen peroxide radical.

***Address for correspondence:**

Dr. Subhash L. Bodhankar,
Professor and Head,
Department of Pharmacology,
Poona College of Pharmacy,
Bharati Vidyapeeth University,
Erandwane 411 038, Pune, India.
E-mail: sbodh@yahoo.com,
Tel. No. : +91-20-24537237 (Ext. 29). Fax No.: +91-20-25439386

Introduction

Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging (1-3) and so they are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, completers of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (4). The antioxidant capacity refers to a full spectrum of antioxidant activity against various reactive oxygen/nitrogen radicals. Antioxidants are often used in oils and fatty foods to retard their auto-oxidation.

Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years (5) and many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds including food items.

Mushrooms have been valued throughout the world as both food and medicine for thousands of years (6, 7). Mushrooms are a group of fleshy macroscopic, fungi, which until recently, as other fungi, were induced in the plant kingdom because of cell wall and spores. Edible mushrooms are a good source of protein, certain vitamins, minerals and fiber. China and Japan have long recognized the nutritional and medicinal properties of various genera and species of mushrooms. When tested *in vitro* and in animal based models, mushrooms have been shown to contain valuable sources of bioactive agents that result in beneficial health effects such as antitumor/anticancer, antiviral and hypolipidemic activity (8, 9).

There are many varieties of mushrooms species of which *Pleurotus* (commonly known as “oyster mushroom” (10) are characterized by a white spore print, attached to gills, often with an eccentric stip, or no stip at all. Several other commercial mushrooms are reported to be effective against various diseases by virtue of their antioxidant potential (11-15). Badole et al reported hypoglycemic (16)(Badole, et al. 2006a) activity of *Pleurotus pulmonarius* extract and its interaction with rosiglitazone (17), glyburide(18) and acarbose (19). However, no data are available in the literature on the antioxidant active activity of *Pleurotus pulmonarius*.

Therefore, we undertook the present investigation to examine the antioxidant activities of aqueous extract of *Pleurotus pulmonarius* (Fr.) through various *in vitro* models.

Material and Methods

Chemicals

Butylated hydroxytoluene (BHT), L-Ascorbic acid, gallic acid, curcumin, quercetin, α -tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, ethylenediaminetetra acetic acid (EDTA), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2-deoxy ribose, ammonium thiocyanate and ferric chloride were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant material and extract

The mushroom of *Pleurotus pulmonarius* (Fries) Quel-Champ. (Family: Lentinaceae) was provided to us as a gift sample from Bajaj Orchard, Pvt. Ltd., Mumbai, India. It was identified and authenticated by the Dr. A. M. Mujumdar, Department of Botany, at Agharkar Research Institute, Pune. Weighed quantity powder of air-dried *Pleurotus pulmonarius* was added to distilled water (1:15), boiled for 20 min on water bath, cooled to room temperature, filtered. The filtrate was dried on tray dryer at 70 °C. (Yield - 24% w/w). The dried extract thus obtained was used for the assessment of antioxidant activity through various *in vitro* assays.

Determination of free radical scavenging activity

The free radical scavenging activity of the *Pleurotus pulmonarius* extract and butylated hydroxy toluene (BHT) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (20). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (10-120 μ g/ml).

Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50} . The IC_{50} value was defined as the concentration (in $\mu\text{g/ml}$) of extracts that inhibits the formation of DPPH radicals by 50%.

Determination of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (21). Various concentrations of the extracts (10-120 $\mu\text{g/ml}$) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 7000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared FeCl_3 solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power is given in ascorbic acid equivalent (ASE ml^{-1}) that shows the amount of ascorbic acid expressed in mM those reducing power is the same than that of 1 ml sample.

Determination of total phenolic content

Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard compound. 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm.

The concentration of total phenols was expressed as mg/g of dry extract (22). The concentration of total phenolic compounds in the extract was determined as μg of gallic acid equivalent using an equation obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.0008 \times \text{gallic acid } (\mu\text{g})$$

Determination of hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell (23). Stock solutions of EDTA (1 mM), FeCl_3 (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of FeCl_3 , 0.1 ml of H_2O_2 , 0.36 ml of deoxyribose, 1.0 ml of the extract (10 – 120 $\mu\text{g}/\text{ml}$) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated as

$$\text{OH}^- \text{ Scavenged } (\%) = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Determination of superoxide anion radical scavenging activity

Measurement of super oxide anion scavenging activity of the *Pleurotus pulmonarius* extract based on the method described by Liu et al (24) with slight modification. Super oxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In this experiment, the super oxide radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1.0 ml of NBT (50 μM) solution, 1.0 ml NADH (78 μM) solution and sample solution of the extracts (100-120 $\mu\text{g}/\text{ml}$) in water. The reaction started by adding 1.0 ml of phenazine methosulphate (PMS) solution (10 μM) to the mixture.

The reaction mixture was incubated at 250 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicates increased super oxide anion scavenging activity. The % inhibition of super oxide anion generation was calculated using the following formula:

$$\text{SOD Scavenged (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Determination of nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric Oxide (25-27) which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (26, 27). Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10 – 120 µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25 °C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent.

$$\text{NO Scavenged (\%)} = \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100$$

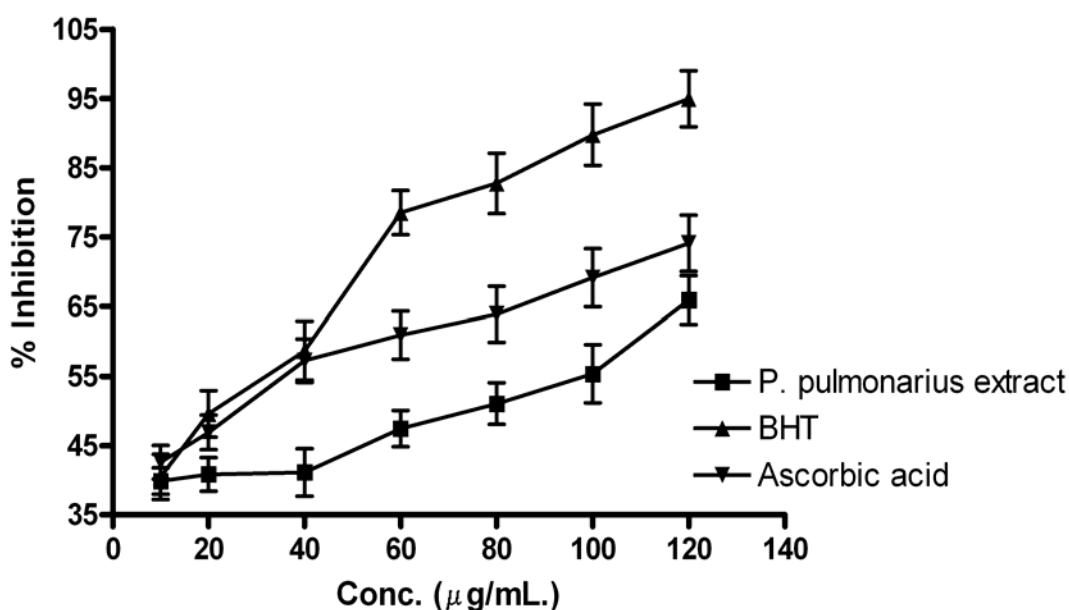
where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Results and Discussion

Inhibition of free radical scavenging

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Fig. 1 illustrates a significant ($P < 0.05$) decrease in the concentration of DPPH radical due to the scavenging ability of soluble solids in the *Pleurotus pulmonarius* and the standard BHT, as a reference compound, presented the highest activity at all concentrations. A maximum effect of *Pleurotus pulmonarius* and BHT was found to be 65.94 and 94.98% inhibition, respectively and the IC_{50} values were found to be 53.79 (± 0.10) and 18.46 (± 0.07) $\mu\text{g/ml}$ for *Pleurotus pulmonarius* and BHT respectively. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (28).

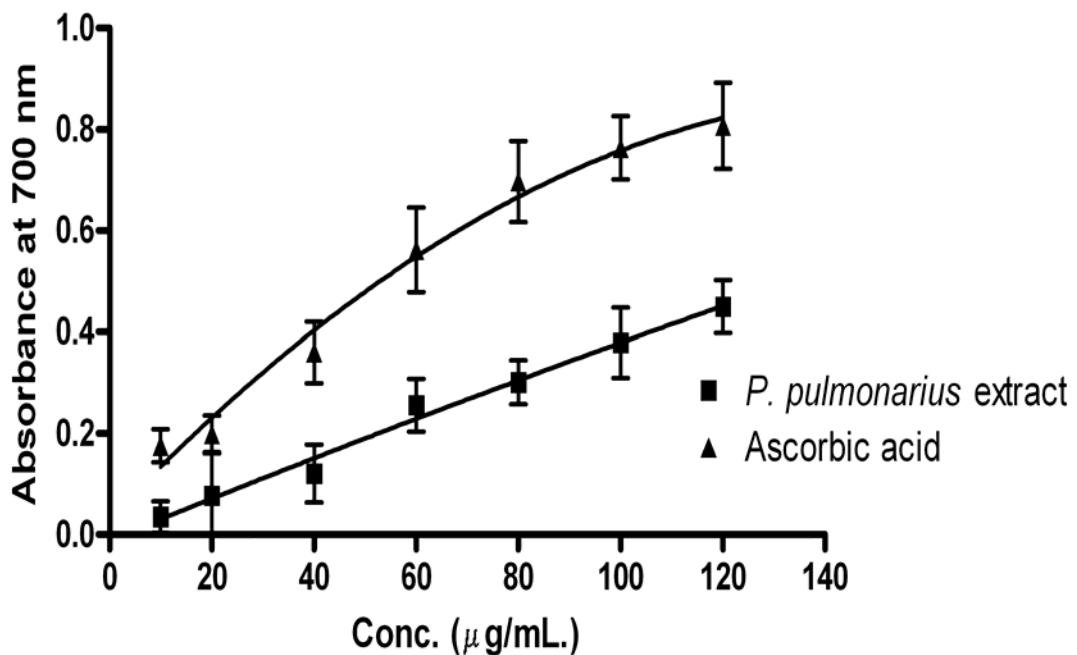
Fig. 1: DPPH and hydroxyl radical scavenging activity of *Pleurotus Pulmonarius*



Reducing ability

Reducing power of the selected diluted extract found to be significant ($P < 0.01$) and as good as L-Ascorbic acid (Fig.2). The antioxidant activity has been reported to be concomitant with development of reducing power (15). The reducing power of *Pleurotus pulmonarius* increased with increasing amount of sample. *Pleurotus pulmonarius* showed significant activities in all the tested concentrations when compared to control ($P < 0.01$). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant (15). For the measurements of the reductive ability, we investigated the Fe^{3+} - Fe^{2+} transformation in the presence of the *Pleurotus pulmonarius* (21).

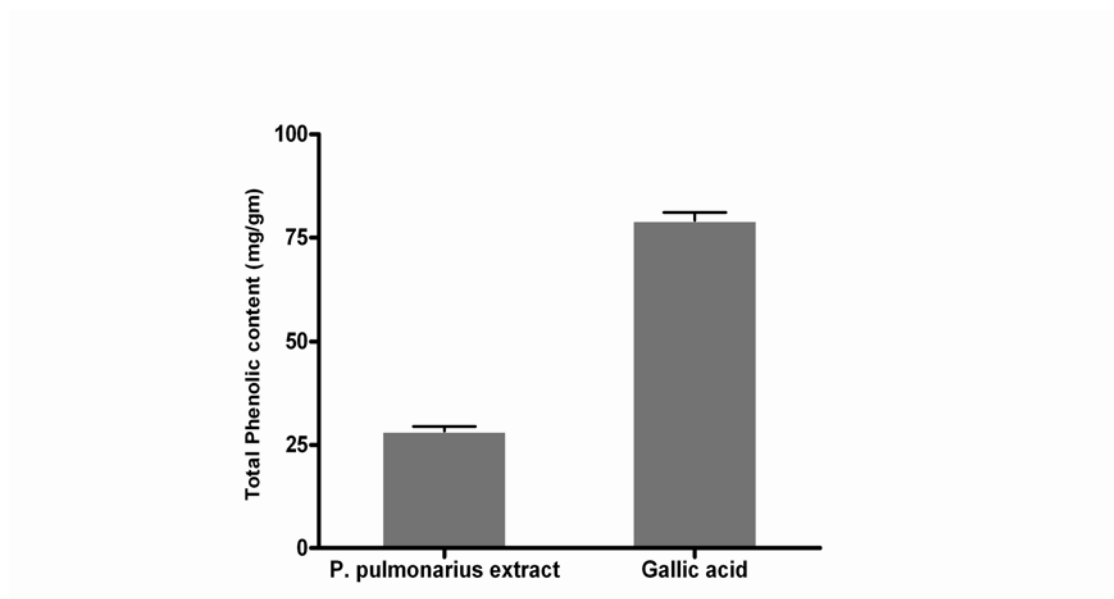
Fig. 2: Reducing power assay of *Pleurotus pulmonarius*



Amount of total phenolic compounds

The total amount of phenolic content present in *Pleurotus pulmonarius* was shown in Fig. 3. In the *Pleurotus pulmonarius* (1g), 28.31 mg gallic acid equivalent of phenols was detected. Phenolic compounds are known as powerful chain breaking antioxidants (29). Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (30). The phenolic compounds may contribute directly to antioxidative action (31). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1g daily from a diet rich in fruits and vegetables (32). The Folin-Ciocalteu assay has been utilized to measure polyphenols for a number of different plants, including mushrooms. A correlation has been shown between higher antioxidant activity and larger amount of total phenolics in extracts from various commercial mushrooms (33).

Fig. 3: Presence of total phenolic content in *Pleurotus pulmonarius*



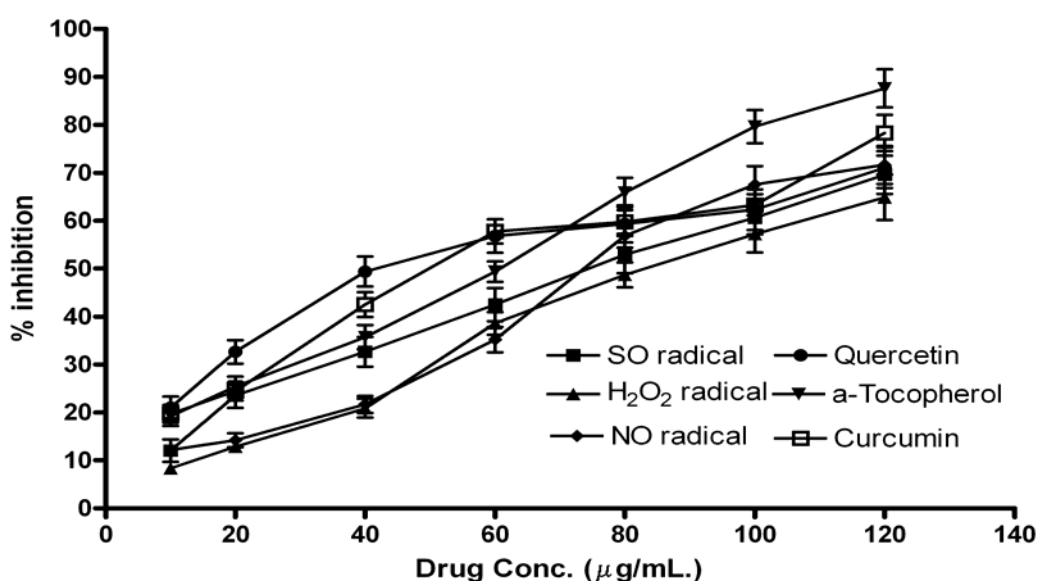
Phenolic compounds are one of the most widely distributed plant secondary products. The ability of these compounds to act as antioxidants has been well established. Polyphenols are multifunctional by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (34).

In order for a polyphenols to be considered an antioxidant it must first be present in low concentrations relative to the substrate to be oxidized and delay, retard or prevent the autooxidation or free radical mediated oxidation. Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been to be act as excellent antioxidants (14, 35).

Inhibition of hydroxyl radical

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins (36). The effect of *Pleurotus pulmonarius* on the inhibition of free radical-mediated deoxyribose damage was assessed by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH), which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Fig. 4 shows the effect of the extracts on the Iron (II)- dependent deoxyribose damage. The *Pleurotus pulmonarius* was the capable of reducing DNA damage at all concentrations. The *Pleurotus pulmonarius* was the capable of reducing DNA damage at all concentrations ($\text{IC}_{50} = 68.84 (\pm 0.02) \mu\text{g/ml}$). Ascorbic acid, used as a standard was highly effective in inhibiting the oxidative DNA damage.

Fig. 4: Nitric oxide (NO), Super oxide (SO) and Hydrogen peroxide (H_2O_2) radical scavenging activity of *Pleurotus pulmonarius*



Inhibition of nitric oxide radical

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by *Pleurotus pulmonarius*. Fig. 4 showed the percentage inhibition of nitric oxide generation by *Pleurotus pulmonarius*. Curcumin was used as a reference compound. The concentration of *Pleurotus pulmonarius* needed for 50% inhibition was found to be 72.69 (± 0.03) $\mu\text{g/ml}$ whereas IC_{50} for curcumin was 49.92 (± 0.03) $\mu\text{g/ml}$.

Inhibition of superoxide anion radical

In the early nineties, Prior and Cao (37) developed an assay called the oxygen radical absorbance capacity (ORAC) to quantify the antioxidant capacity of a number of products including fruits and vegetables. In this study, we have used PMS/NADH-NBT system, in which superoxide anions are derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Fig. 4 shows the superoxide radical scavenging activity of 10 to 120 $\mu\text{g/ml}$ of *Pleurotus pulmonarius* in comparison with same dose range of quercetin. *Pleurotus pulmonarius* had strong superoxide radical scavenging activity and exhibited nearly equal superoxide radical scavenging activity like quercetin. The results were found statistically significant ($P < 0.05$).

Inhibition of H_2O_2 radical

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (38). Thus, removing H_2O_2 as well as O_2^- is very important for protection of food systems. The scavenging ability of *Pleurotus pulmonarius* extract on hydrogen peroxide is shown in Fig. 4 and compared with that of α -tocopherol as standard. *Pleurotus pulmonarius* extract and α -tocopherol exhibited significant scavenging activity on H_2O_2 radical ($\text{IC}_{50} = 82.76 (\pm 0.017)$ and $48.73 (\pm 0.05)$ $\mu\text{g/ml}$ respectively). The aqueous extract of *Pleurotus pulmonarius* showed strong antioxidant activity by inhibiting DPPH and reducing power activities when compared with different standards such as BHT, and L-ascorbic acid respectively. It also showed significant activity against hydroxyl radical, nitric oxide, superoxide anion scavenging and hydrogen peroxide

scavenging as compared to the standard compounds L-ascorbic acid, curcumin, quercetin, and α -tocopherol respectively.

In addition, the *Pleurotus pulmonarius* found to contain a noticeable amount of total phenols and can play a major role in exhibiting antioxidant potential. The results of this study indicated that oyster mushroom (*Pleurotus pulmonarius*) can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. Further works on the isolation and identification of the antioxidant components in *Pleurotus pulmonarius* is warranted.

Acknowledgements

The authors acknowledge Dr. S. S. Kadam, Vice-Chancellor and Dr. K.R. Mahadik, Principal, Poona College of Pharmacy, Bharati Vidyapeeth University, Pune, India for providing necessary facilities to carry out the study. We are also thankful to Bajaj Orchard, a division of Trinity Bio-Tech, Mumbai, India, for a gift sample of *P. Pulmonarius*.

References

1. Lee KG, Mitchell AE, Shibamoto T: Determination of antioxidant properties of aroma extracts from various beans. J Agric Food Chem. 2000; 48: 4817-20.
2. Middleton E, Jr., Kandaswami C, Theoharides TC: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000; 52: 673-751.
3. Pietta P, P. S, Mauri P: Antioxidant activity of selected medicinal plants. J. Agric. Food Chem. 1998; 46: 4487-4490.
4. Andlauer W, Furst P: Antioxidative power of phytochemicals with special reference to cereals. Cereal Foods World. 1998; 43: 356-359.

5. Jayaprakasha GK, Selvi T, Sakariah KK: Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extract. Food Res. Int. 2003; 36: 117-122.
6. Tribe I, Tosco U: The world of mushrooms. Orbis Publishing, London, 1973.
7. Wright T: Medicinal mushrooms Nutraceuticals World. Rodman Publishing, Ramsey, NJ, 2004, pp. 26- 29.
8. Beelman RB, Royse D, Chikthimmah N: Bioactive components in button mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetidae) of nutritional, or biological importance. Int. J. Med. Mushrooms. 2003; 5: 321-327.
9. Chang ST, Buswell JA: Medicinal Mushrooms-A Prominent Source of Nutraceuticals of the 21st Century. Curr. Top. in Nutraceutical Res. 2003; 1: 257-280.
10. Miles PG, Chang S-T: Mushroom Biology: Concise basics and current development. World Scientific Publishing Company, New York, 1997.
11. Fu H, Shieh DE: Antioxidant and Free Radical Scavenging Activities of Edible Mushrooms. J. Food Lipids. 2002; 9: 35-46.
12. Kim KC, Kim IG: Ganoderma lucidum extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. Int J Mol Med. 1999; 4: 273-277.
13. Mau JL, Lin HC, Chen CC: Antioxidant properties of several medicinal mushrooms. J Agric Food Chem. 2002; 50: 6072-7.
14. Mau JL, Lin HC, Song SF: Antioxidant Properties of Several Specialty Mushrooms. Food Res. Int. 2002; 35: 519-526.

15. Yang JH, Lin H, C., Mau JL: Antioxidant properties of several commercial mushrooms. Food Chem. 2002; 77: 229-235.
16. Badole SL, Shah SN, Patel NN, Thakurdesai PA, Bodhankar SL: Hypoglycemic activity of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel.-Champ in alloxan induced diabetic mice. Pharma. Biol. 2006; 44: 421-425.
17. Badole SL, Bodhankar SL, Thakurdesai PA: Study of interaction of aqueous extract of *Pleurotus pulmonarius* (fr.) Quel-Champ with rosiglitazone in alloxan induced diabetic mice. Pharmacologyonline 2006; 3: 64-72.
18. Badole SL, Patel N, Thakurdesai PA, Bodhankar SL: Study of interaction of aqueous extract of *Pleurotus Pulmonarius* (Fr.) Quel-champ. with glyburide in alloxan induced diabetic mice. Evidence Based Complementary and Alternative Medicine In Press, 2006.
19. Badole SL, Bodhankar SL: Interaction of aqueous extract of *Pleurotus pulmonarius* (Fr.) Quel.-Champ with acarbose in alloxan induced diabetic mice. Journal of Applied Biomedicine In Press, 2007.
20. Blois MS: Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-1200.
21. Oyaizu M: Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 1986; 44: 307-315.
22. Kim D, S J, Lee C: Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003; 81: 321-326.
23. Halliwell B: Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med. 1991; 91: 14S-22S.

24. Liu F, Ooi VE, Chang ST: Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci.* 1997; 60: 763-71.
25. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [¹⁵N]nitrate in biological fluids. *Anal Biochem.* 1982; 126: 131-8.
26. Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardes-Albert M: Antioxidant action of *Ginkgo biloba* extract EGb 761. *Methods Enzymol.* 1994; 234: 462-75.
27. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L: The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun.* 1994; 201: 748-55.
28. Soares JR, Dinis TC, Cunha AP, Almeida LM: Antioxidant activities of some extracts of *Thymus zygis*. *Free Radic. Res.* 1997; 26: 469-78.
29. Shahidi F, Wanasundara PK: Phenolic antioxidants. *Crit Rev Food Sci Nutr.* 1992; 32: 67-103.
30. Hatano T, Ogawa N, Kira R, Yasuhara T, Okuda T: Tannins of cornaceous plants. I. Cornusins A, B and C, dimeric monomeric and trimeric hydrolyzable tannins from *Cornus officinalis*, and orientation of valoneoyl group in related tannins. *Chem Pharm Bull (Tokyo).* 1989; 37: 2083-90.
31. Duh PD, Tu YY, Yen GC: Antioxidant activity of water extract of Harnng Jyur (*Chrysanthemum morifolium* Ramat). *Lebensm-Wiss. Technol.* 1999; 32: 269-277.

32. Tanaka M, Kuei CW, Nagashima Y: Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi* 1998; 47: 1409-1414.
33. Cheung PC, Lee MY: Fractionation and characterization of mushroom dietary fiber (nonstarch polysaccharides) as potential nutraceuticals from sclerotia of *Pleurotus tuber-regium* (Fries) singer. *J Agric Food Chem.* 2000; 48: 3148-51.
34. Rice-Evans CA, Miller NJ, Paganga G: Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.* 1996; 20: 933-56.
35. Ishikawa Y, Morimoto K, Hamasaki T: Flavoglaucin, a Metabolite of *Eurotium Chevalieri*, Its Antioxidation and Synergism with Tocopherol. *J. Am. Oil Chem. Soc.* 1984; 61: 1864-1868.
36. Spencer JP, Jenner A, Aruoma OI, Evans PJ, Kaur H, Dexter DT, Jenner P, Lees AJ, Marsden DC, Halliwell B: Intense oxidative DNA damage promoted by L-dopa and its metabolites. Implications for neurodegenerative disease. *FEBS Lett.* 1994; 353: 246-50.
37. Prior RL, Cao G, Prior RL, Cao G: Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *J AOAC Int.* 2000; 83: 950-6.
38. Halliwell B, Gutteridge JM, Aruoma OI: The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem.* 1987; 165: 215-9.