Study on *in vitro* antioxidant potential of some cultivated *Pleurotus* species
(Oyster mushroom)

Gajendra S Jeena, H Punetha, Om Prakash, Mahesh Chandra and K P S Kushwaha

1Department of Biochemistry, 2Department of Chemistry, College of Basic Sciences & Humanities, 3M RTC, GBPUA &T, Pantnagar-263145, Uttarakhnad, India

Received 2 July 2012; Accepted 4 November 2013

The antioxidant potential and quantitative phenolic from the methanolic extract of *Pleurotus sajor-caju* (Fr.) Singer, *P. ostreatus* (Jacq ex Fr.) P. Kumm. and *P. sapidus* (Schulzer & Kalchbr.) Sacc. at mature fruiting stage and mycelium stage were investigated. Fruiting bodies of *Pleurotus* species exhibited the most potent free radical scavenging activity (*P. sajor-caju* 88.9 % at 10 mg/mL) and reducing power (1.98 at 10 mg/mL) while mycelia exhibiting higher chelating activity at 10 mg/mL (*P. sapidus* 99 %). Gallic acid, BHT and Catechin used as a standard were highly effective in inhibiting hydroxyl and superoxide radicals, showing IC$_{50}$ values below 1 mg/mL. *Pleurotus sajor-caju* exhibited maximum antioxidant potential as compared to other two mushrooms. Analysis of total phenols and flavonoids in *Pleurotus* species has been carried out *in vitro*. *Pleurotus sajor-caju* fruiting contains highest phenol content (1.53 mg/g) and flavonoids (1.88 mg/g). These results suggest that these mushrooms may be used as a potential source of natural antioxidants for food supplements as well as in the development of nutraceuticals.

**Keywords:** *Pleurotus* species, Oyster, Mushrooms, Antioxidant activity, Phenols, Flavonoids,

**IPC code:** Int. cl. (2013.01)−A61K 36/00

**Introduction**

Oyster mushroom is an edible white-rot fungus and is classified into the genus *Pleurotus* (Jacq. ex Fr.) Kumm. comprises about 40 species. Mushrooms have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fibre and poor in fat. Edible mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E). Edible mushrooms could be a source of many different nutraceuticals such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids. Thus, they might be used directly in diet to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. Oxidation is essential to many living organisms for the production of energy to fuel in biological processes. Free radicals are produced in normal and or pathological cell metabolism. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Antioxidant properties of phenolic compounds may help the endogenous defence system, assuming a major importance as possible protector agents reducing oxidative damage. The edible mushrooms analysed could be directly used in the human diet to combat oxidative stress, while inedible species could represent a source of extractable phenolic compounds to be used as additives in the food industry or as components in pharmaceutical and cosmetic formulations, due to their well-known antioxidant properties. Till now, research has tended to focus on the dietary value and cultivation of edible mushrooms; but, there is limited data in the literature concerning the antioxidant properties of the edible wild mushrooms and comparison between fruiting stage and mycelia stage. Hence, the present study focussed to determine antioxidant potential and *in vitro* phenolic at fruiting and mycelia stage of some cultivated *Pleurotus* species.

**Materials and Methods**

**Preparation of methanolic extract**

Three kinds of oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer, *P. ostreatus* (Jacq ex Fr.)
P. Kumm. and P. sapidus (Schulzer & Kalchbr.) Sacc (Plate 1) were harvested during early mature fruiting stage at Mushroom Research Training Centre, Pantnagar. To obtain the fungal mycelia of these oyster mushrooms, inoculums were grown in potato dextrose broth at 120 rpm, 25°C for 10-12 days. The fruit bodies were cleaned to remove any residue and mycelia were extracted through filtration. The freeze dried mushrooms were ground using a blender and then stored at -80°C along with mycelia for further analysis. A 20 g dried mushroom and mycelia sample was extracted using 100 mL of methanol overnight at room temperature, and the extract was filtered using a Whatman No.2 filter paper. The residue was then extracted with two additional portion of methanol under same condition. The methanolic extracts of mushrooms were combined and evaporated using a rotary evaporator at 40°C for dryness. For analysing samples, the dried extract was resolubilized in methanol and then five different concentrations of mushroom methanolic extracts (2, 4, 6, 8, 10 mg/mL) were prepared.

Scavenging effect on 1, 1-Diphenyl-2-picrylhydrazyl radical
The scavenging activity was determined by DPPH method developed earlier. 1 mL of methanolic extract was mixed with 5 mL of 0.004% methanolic solution of DPPH. Each mixture was placed in dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer.

\[
\text{% scavenging activity} = (1 - \frac{A_{\text{sample}}}{A_{\text{control}}}) \times 100
\]

Reducing power
Reducing power was determined by the method developed earlier. Each concentration of methanolic extracts (1 mL) was mixed with 2.5 mL phosphate buffer (200mM, pH6.6) and 2.5 mL of 1% potassium ferricyanide followed by incubate at 50°C for 20min. After incubation, a 2.5 mL of 10% trichloroacetic acid was added to the mixture and then centrifuged at 650 rpm for 10min. The supernatant (5 mL) was mixed with 5 mL distilled water and 1 mL of 1% ferric chloride, and then its absorbance was measured at 700 nm.

Chelating effects on ferrous ions
Chelating ability was determined according to the standard method. Each extract (1 mL) was mixed with 0.1 mL of 2 mM FeCl₂. The reaction was initiated by the addition of 5 mM ferrozine and make up volume to 5 mL by adding methanol. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm % chelating activity = \((1 - \frac{A_{\text{sample}}}{A_{\text{control}}}) \times 100\)

Total phenolic contents (TPC)
Total phenolic content of the methanolic extract of mushroom were measured according to the standard method developed earlier. Each extract (1 mL) was mixed with 1 mL of saturated sodium carbonate solution and 0.4 mL Folin-Ciocalteau reagent. Made the volume up to 10 mL by adding distilled water. Incubate at 27°C for 1h and absorbance was recorded at 765 nm. TPC were expressed as Gallic acid equivalents.

Ortho-Dihydroxy phenols
Ortho-Dihydroxy phenols in the mushroom extract were determined by standard method. Each extract (1 mL) was mixed with 1mL of 0.5N HCL and Arnow’s reagent. To the reaction mixture add 2 mL of 1N aqueous sodium hydroxide and then absorbance was measured at 515 nm.

Total flavonoid contents (TFC)
Flavonoid content in the methanolic extract of oyster mushroom was determined by the method
developed earlier and results were expressed as mg (+) catechins equivalents per gm of mushrooms. Standard solution or mushroom extract (1 mL) was mixed with 1.25 mL of distilled water and 75 µL of 5% NaNO₂ solution. After incubation of 5 min, 150 µL of 10 % AlCl₃·H₂O was added. After 6 min, 500 µL of 1M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed and intensity of pink colour was measured at 510 nm.

Statistical analysis

All the analyses were performed in triplicates and these results were reported as means ± standard deviation (SD). The significance of differences among treatment means were determined by one way analysis of variance (ANOVA) with a significant level of (p < 0.05).

Results and Discussion

DPPH radical scavenging activity

The DPPH radical scavenging activity of the methanolic Oyster mushroom extracts increased with increase in concentrations. At 10 mg/mL the radical scavenging activities of P. sajor-caju, P. ostreatus and P. sapidus fruiting bodies (fb) were 88.9, 87.4 and 85 % which is much higher as compared to their mycelia (53.9, 44.1 and 75%). Fruiting bodies of P. sajor-caju and P. ostreatus showing free radical scavenging activity equivalent to that of standard antioxidants, BHT, gallic acid and catechin at 10 mg/mL. DPPH radical scavenging activities of P. ostreatus were 81.8% at 6.4 mg/mL and 68.4% at 5 mg/mL. P. eryngii (DC. ex Fr.) Quel., P. dijamor (Fr.) Boedjin and P. flabellatus (Berk. & Br.) Sacc. have higher radical free scavenging activity than P. sajor-caju and P. florida at 100 % mycelia extract. Our results showed that P. sajor-caju (fb) has the lowest IC₅₀ value which is about 2 mg/mL. Among the mycelia, P. sapidus was found to be more efficient than P. sajor-caju and P. ostreatus with IC₅₀ value about 3 mg/mL. Free radical scavenging activity has been known as an established phenomenon in inhibiting lipid oxidation, which otherwise can be deleterious to the cellular components and cellular function. With the presence of radical scavenging activity, consumption of wild mushrooms might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses.

Reducing power

Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reducers (antioxidants) causes the reduction of the Fe³⁺/ ferricyanide complex to the ferrous form. In present study, P. sajor-caju fruiting had an excellent reducing power (0.759 at 2 mg/mL and 1.980 at 10 mg/mL), showing that its reducing ability was more effective than the fruiting of P. sapidus and P. ostreatus (1.97 and 1.78 at 10 mg/mL) (Fig. 2). Among the mycelia of these Pleurotus species, highest reducing power was shown by P. sajor-caju (1.672) followed by P. ostreatus (1.371) and P. sapidus (1.010) at 10 mg/mL (Fig 2). Ethanolic extract of the mushroom Pleurotus citrinopileatus Singer has been reported to exhibit the

Fig.1—Scavenging effects of DPPH radical of the methanolic extracts of some Pleurotus species at different concentrations. Each value is expressed as mean ± SD (n=3).

Fig. 2—Reducing power of methanolic extracts of some Pleurotus species at different concentrations. Each value is expressed as mean ± SD (n=3).
reducing power of 1.05 at 10 mg/mL\(^{18}\). The antioxidant activity has been reported to be concomitant with the development of reducing capacity\(^{19}\). Therefore, reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity\(^{20}\). However, absorbance of BHT at 20 mg/mL was much higher (2.660) indicating synthetic antioxidant has better reducing ability compared to antioxidant from wild mushrooms. Similarly, the reducing power of the wild mushrooms was much lower than other edible mushrooms reported elsewhere\(^{21}\). However, the reducing power of the methanolic extract from \textit{P. porrigens} (Pers. ex Fr.) Kumm. steadily increases to 0.2 at 20 mg/mL\(^{22}\) which is found to be lower as compared to the edible mushrooms used in present study.

**Chelating effect on ferrous ion**

The range and the mean of Fe\(^{2+}\) chelating capacities varied significantly among different types of mushrooms. In this assay, extract of the mushroom species interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Chelating effect of methanolic extracts from different oyster mushrooms on ferrous ions increased as the concentration increases from 2 mg/mL to 10 mg/mL (Fig. 3). At 10 mg/mL, fruiting bodies of \textit{P. sapidus} had an outstanding chelating ability (94 %) which is more than the synthetic metal chelators EDTA (92.3 %) and citric acid (87.6 %) while \textit{P. ostreatus} and \textit{P. sajor-caju} has 88.2 % and 69.7 % chelating ability. \textit{Pleurotus} species at their mycelia stage have greater chelating effect on ferrous ion as compared to their fruiting bodies. \textit{P. sapidus} was found to be the lowest IC\(_{50}\) value below 1.5 mg/mL which shows that \textit{P. sapidus} has highest ferrous ion chelating activity. The order of chelating effect on ferrous ion is \textit{P. sapidus} (99 %) > \textit{P. ostreatus} (89.4 %) > \textit{P. sajor-caju} (73 %). Reverse case has been observed to that of DPPH radical scavenging activity and reducing power. Fig. 3 reveals that the methanolic extract of edible mushrooms in this study showed a marked capacity for iron binding, suggesting that their action as per oxidation protector may be related to its iron binding capacity. Fruit bodies of \textit{P. citrinopileatus} have been reported to chelate ferrous ion by 82.1% at 5 mg/mL, showing that it is more effective than its mycelia\(^{17}\). Chelating abilities of \textit{P. eryngii}, \textit{P. ferulae} and \textit{P. ostreatus} at 5 mg/mL were 41.4-64 %\(^{14}\). For commercial mushrooms including winter, oyster mushrooms and shiitake, their methanolic extracts chelated 45.6-81.6 % of ferrous ion at 1.6 mg/mL\(^{13}\). A heteroglycan isolated from the mycelia of \textit{P. ostreatus} also have a marked capacity for iron binding ability of 54.82 % at a concentration of 1 mg/mL\(^{23}\).

**Total phenolic contents (TPC)**

The measurement of the amount of total phenolic compounds as well as the identification of the main phenolics in mushrooms, have great importance in nutritional and functional characterization\(^{24}\). The TPC of oyster mushrooms fruiting body were in the order of \textit{P. sajor-caju} (1.53 mg/g) > \textit{P. ostreatus} (1.32 mg/g) > \textit{P. sapidus} (1.10 mg/g) while the TPC of the mycelia were found to be \textit{P. sajor-caju} (0.69 mg/g), \textit{P. ostreatus} (0.68 mg/g) and \textit{P. sapidus} (0.50 mg/g) (Table 1). These TPC results could show the significant correlation to the antioxidant activities of the three oyster mushrooms in terms of radical

![Fig. 3—Chelating Effects on Ferrous Ion of Methanolic Extracts of Some Pleurotus Species at Different Concentrations. Each Value is Expressed as Mean ± SD (n=3).](image-url)
scavenging, reducing power, and chelating effects. Phenolics are the major naturally occurring antioxidant components found in methanolic extract of several mushroom species\textsuperscript{25}. The most abundant polyphenols reported in edible mushroom extracts are \textit{p}-benzoic acid, \textit{p}-phenyl acetic acid, \textit{o}-coumaric acid, ferulic acid and chrysin\textsuperscript{26}. Phenolic groups such as flavonoids, lignin, and phenolic acids were found in \textit{Agaricus bisporus} (J.E. Lange) Emil J. Imbach. (Button mushroom), \textit{P. ostreatus} (Oyster mushroom) and \textit{Lentinus edodes} (Berk.) Pegler (Shiitake mushroom) which contribute to the high phenolics content\textsuperscript{27}. Numerous studies have showed the consumption of foods high in phenolics can reduce the risk of heart disease by slowing the progression of atherosclerosis due to their antioxidative properties\textsuperscript{28-29}. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid per oxidation\textsuperscript{30}.

### Total flavonoid contents (TFC)

Flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones and flavonoids which are responsible for the orange, red and blue color in fruits and vegetables. Generally, deep-colored fruits, vegetables or foods are recognized as more healthy to human body\textsuperscript{31}. There has been a growing interest in pigment components of natural food, which may promote human health or lower the risk for diseases. As shown in Table 1, total phenolic contents in all the three mushrooms were higher than corresponding mycelia. Similar case was observed in total flavonoid contents. \textit{P. sajor-caju} fruiting has higher flavonoid content (1.88 mg/g) as compared to \textit{P. ostreatus} (1.82 mg/g) and \textit{P. sapidus} (1.39 mg/g). The concentration of flavonoids varies depending on the mushroom species and the total flavonoid content does not correlate with the phenolic concentration. Thus, \textit{Lactarius deliciosus} (L. ex Fr.) S.F.Gray has the higher amount and \textit{A. bisporous} and \textit{P. ostreatus} present the lower flavonoid content. Total flavonoid content found in \textit{P. ostreatus} is below 1 mg/g\textsuperscript{32}. Total flavonoid content was estimated in oyster mushroom (\textit{P. ostreatus}) dark grey, yellow and pink strains\textsuperscript{33}. Reportedly dark grey strain has the highest flavonols (2.16 mg/g). Our results showed the significant increase in the flavonoid content at fruiting and mycelia stage.

### Conclusion

This study showed that the methanolic extracts from different \textit{Pleurotus} species had considerable antioxidant activities. Especially, \textit{P. sajor-caju} fruiting exhibited the strongest antioxidant activities including scavenging ability and reducing power as compared to \textit{P. ostreatus} and \textit{P. sapidus}. Furthermore, antioxidant properties of three different oyster mushrooms were intimately linked with phenolic compounds content. \textit{P. sajor-caju} fruiting body has highest phenol and flavonoid content. Therefore, these results indicate that matured fruiting of oyster mushroom could be very beneficial for defending radical mediated toxicity and used as a potential source of natural antioxidants for food supplements as well as in the development of nutraceuticals.

### Acknowledgements

Authors are thankful to DES Pantnagar and Joint Director MRTC for providing necessary facilities.

### References

10. Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents.
American Journal of Enology and Viticulture, 1965, 16, 144-158.


Lin JY and Tang CY, Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation, Food Chem, 2007, 101, 140-147.
