Antioxidant and antitumour activity of *Pleurotus florida*

Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicines. They have been used in folk medicine throughout the world since ancient times. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments. The most significant medicinal effect of mushrooms and their metabolites that attracted the attention of the public is their antitumour property. Mushroom metabolites are usually used as adaptogens and immunostimulants and they are now considered to be one of the most useful antitumour agents for clinical uses. Hence, search for new antitumour substances from mushrooms has been a matter of great importance.

*Pleurotus* species are commonly called Oyster mushrooms. There are about 40 species of this mushroom. They enjoy worldwide distribution, both in temperate and tropical parts of the world. Oyster mushrooms now rank second among the important cultivated mushrooms in the world.

Reactive oxygen species such as superoxide anion radical (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$) are considered to be important factors in the etiology of several pathological conditions such as cardiovascular diseases, diabetes, inflammation, cancer, etc. They are implicated in carcinogenesis-induced mutation and tumour promotion. Antioxidants act as a major defence against radical-mediated toxicity by protecting the damages caused by free radicals. Inhibition of free radical generation can serve as a facile system for identifying cancer preventive agents.

Here we report the antioxidant and antitumour activity of a commercially cultivated edible Oyster mushroom, *Pleurotus florida* (Figure 1).

![Figure 1. Pleurotus florida growing on paddy straw.](image)

Figure 1. *Pleurotus florida* growing on paddy straw.

Ethyl acetate and methanol extracts were concentrated and evaporated to dryness under vacuum. The aqueous extract was lyophilized. For the *in vitro* antioxidant assay, the ethyl acetate extract was presolubilized in 10% DMSO or in PBS, methanol and aqueous extracts were dissolved in 0.9% saline.

Male Balb/c mice of 4–5 weeks age (20 to 25 g) were used for antitumour studies. The animals were maintained under environmentally controlled conditions with free access to standard food (Lipton, India) and water.

Nitroblue tetrazolium (NBT) was purchased from Sisco Research Lab, Mumbai; 2-deoxy-β-ribose from Sigma, St. Louis, USA and thiobarbituric acid from BDH Laboratories, England. The other chemicals and reagents used were of analytical grade. Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) cell lines were obtained from Cancer Institute, Adyar, Chennai and maintained on mice in our laboratory.

Superoxide radical (O$_2^-$) was generated from the photoreduction of riboflavin and was detected by NBT reduction method of Mc Cord and Fridovich. The reaction mixture contained EDTA (6 mM) with 3 mg NaCN, riboflavin (2 μM); NBT (50 μM); KH$_2$PO$_4$–Na$_2$HPO$_4$ buffer (67 mM, pH 7.8) and various concentrations of the *P. florida* extract in a final volume of 3 ml. The tubes were illuminated under incandescent lamp for 15 min. The optical density (OD) at 530 nm was measured before and after illumination. The inhibition of superoxide radical was determined by comparing the absorbance values of the control with those of the treatments. Catechin was used as the standard.

Lipid peroxidation induced by Fe$^{2+}$–ascorbate system in rat liver homogenate by the method of Bishaya and Balasubramaniyan was estimated as thiobarbituric acid reacting substance (TBARS) by the method of Ohkawa et al. 1987. The reaction mixture contained rat liver homogenate 0.1 ml (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO$_4$(Ni)l$_2$; SO$_2$; 7H$_2$O (0.16 mM); ascorbate (0.06 mM); and various concentrations of *P. florida* extract in a final volume of 0.5 ml. The reaction mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was removed and treated with 0.2 ml sodium dodecyl sulphate (SDS) (8.1%); 1.5 ml thiobarbituric acid (0.8%); and 1.5 ml acetic acid (20%; pH 3.5). The total volume was made up to 4 ml with distilled water and then kept in a water bath at 95 to 100°C for 1 h. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15 : 1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol–pyridine layer was removed and its absorbance at 532 nm was measured to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the OD of treatments with that of the control. Quercetin was used as the standard.

Hydroxyl radical was generated from Fe$^{2+}$–ascorbate–EDTA–H$_2$O$_2$ system (Fenton’s reaction) which degraded deoxyribose resulting in TBARS. The reaction mixture contained deoxyribose (2.8 mM); FeCl$_3$ (0.1 mM); KH$_2$PO$_4$–KOH buffer (20 mM, pH 7.4); EDTA (0.1 mM); H$_2$O$_2$ (1.0 mM); ascorbic acid (0.1 mM) and various concentrations of *P. florida* extract in a final volume of 1 ml. The reaction mixture was incubated at 37°C for 1 h. The TBARS formed was
estimated by the method of Ohkawa et al. The hydroxyl radical scavenging activity was determined by comparing the absorbance of control with that of treatments. Catechin was used as a standard.

Cytotoxicity of ethyl acetate, methanol and aqueous extracts was tested using EAC and DLA cell lines by trypan blue exclusion method. Antitumour activity of *P. floridus* was assayed using ascites tumour and solid tumour models. In both the experiments, male Balb/c mice of 4 to 5 weeks age and 20 to 25 g weight were employed. For antitumour assay against ascites tumour, animals were divided into five groups with six animals in each group. The animals were injected intraperitoneally (ip) with EAC cells (10⁷/0.1 ml). Methanol extract of *P. floridus* at a dose of 50, 100 and 250 mg/kg body weight was given ip 24 h after the tumour implantation to the three groups. The administration of the drug was continued for alternate days and the total dose given was five. One group administered with EAC cells alone served as positive control. Cisplatin was used as the standard. The mortality rates were noted in each group and the per cent increase in life span (ILS) of the drug-treated group was calculated using the formula % ILS = [(T – C)/C] x 100 (T is the mean survival time of treated mice and C that of the control). For determining the solid tumour reducing activity, the animals were divided into five groups with six animals in each group. Animals were injected subcutaneously with EAC cells (10⁷/0.1 ml) on the right hind limb. Methanol extract of *P. floridus* was given ip at a dose of 250, 500 and 1000 mg/kg body weight after 24 h of tumour inoculation and the drug administration continued for 10 days. Cisplatin was used as the standard and the group administered with EAC cells alone served as positive control. The development of tumour on animals in each group was measured using a vernier callipers and the tumour volume was calculated using the formula V = (4/3)πr₁²r₂, where r₁ and r₂ are the radii of the tumours.

Experimental data were expressed as mean ± S.E. Student’s *t* test was applied for expressing significance *P* < 0.05.

Ethyl acetate and methanol extracts of *P. floridus* showed significant lipid peroxidation inhibition activity. IC₅₀ (inhibition concentration 50%) values for ethyl acetate and methanol extracts were 496 and 320 µg/ml, respectively (Table 1). Methanol extract possessed significantly higher activity than catechin. However, the aqueous extract did not show lipid peroxidation inhibition activity. The ethyl acetate, methanol and aqueous extracts of *P. floridus* also showed significant scavenging activity of hydroxyl radical generated from Fe²⁺-ascorbate–EDTA–H₂O₂ system (Table 1). The IC₅₀ values for ethyl acetate, methanol and aqueous extracts were 530, 263.33 and 260 µg/ml, respectively. These extracts possessed significantly higher activity than catechin. The results indicated that ethyl acetate and methanol extracts of *P. floridus* possessed significant antioxidant activity. However, none of the extracts of *P. floridus* tested was found to have superoxide radical scavenging activity.

Short-term bioassay for cytotoxicity showed that ethyl acetate, methanol and aqueous extracts of *P. floridus* did not exhibit toxicity against DLA and EAC cell lines up to a concentration of 1 mg/ml.

Methanol extract of *P. floridus* was not found to have any tumour reducing activity against ascites tumour induced by EAC cells up to a concentration of 250 mg/kg body weight. However, the extract showed significant tumour growth inhibition against the solid tumour induced by EAC cell lines at concentrations 250, 500 and 1000 mg/kg body weight (Table 2). Tumour reducing effect of the extract at a concentration of 1000 mg/kg body weight was almost equal to Cisplatin at a dose of 4 mg/kg.

Free radicals of oxygen, hydrogen peroxide and organic peroxides have been identified as agents that contribute to tumour promotion probably by forming oxidized DNA bases which can act as cancer initiators as well as promoters. Free radical intermediates are of significant importance in mechanisms associated with the action of many compounds that lead to tissue damages. Lipid peroxidation-mediated membrane damage is one of the deleterious effects of free radicals. The results of the investigations reveal that ethyl acetate and methanol extracts of *P. floridus* have potent hydroxyl radical scavenging and lipid peroxidation inhibition activities. The methanol extract possesses higher antioxidant activity than the other extracts. The significant antioxidant activities of *P. floridus* extracts thus suggest the therapeutic value of this mushroom. However, no superoxide scavenging activity of the extracts

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**Table 1. In vitro antioxidant activity of *Pleurotus floridus* extracts (IC₅₀ µg/ml)**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Ethyl acetate</th>
<th>Methanol</th>
<th>Water</th>
<th>Catechin</th>
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</thead>
<tbody>
<tr>
<td>Hydroxyl radical scavenging activity</td>
<td>530 ± 29.4</td>
<td>263.33 ± 24.9</td>
<td>260 ± 23.09</td>
<td>850 ± 20</td>
</tr>
<tr>
<td>Inhibition of lipid-peroxidation</td>
<td>496 ± 4.7</td>
<td>320 ± 10</td>
<td>Nil</td>
<td>418 ± 28.6</td>
</tr>
</tbody>
</table>

Values represented as mean ± SD. *n* = 3 values are significant. *P* < 0.001 v/s standard.

**Table 2. Effect of methanol extract of *Pleurotus floridus* on solid tumour**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumour volume (cm³)</th>
<th>% Decrease in tumour volume</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.395 ± 0.096</td>
<td></td>
</tr>
<tr>
<td>Standard (Cisplatin 4 mg/kg)</td>
<td>0.046 ± 0.001</td>
<td>96.60</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>0.698 ± 0.202</td>
<td>50.47</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>0.347 ± 0.177</td>
<td>75.16</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>0.1576 ± 0.049</td>
<td>88.69</td>
</tr>
</tbody>
</table>

Values represented as mean ± SD. *n* = 6 values are significant. *P* < 0.001 v/s standard.
even at a concentration of 1 mg/ml was found by the assay method employed in this study. The reason for the absence of superoxide scavenging activity is unknown. However, herbs that scavenge superoxide contain a component of flavanoids which are widely distributed in plants. "The general capability of flavanoids to scavenge superoxide radical has been reported."

P. florida is an excellent edible mushroom, hence the possibility of cytotoxicity of the extract of the mushroom cannot be envisaged. Our experimental results support the hypothesis according to the criteria established by the National Cancer Institute, USA. Results indicate that the methanol extract of P. florida has remarkable capacity to inhibit the growth of solid tumour induced by EAC cell line in a dose-dependent manner in experimental animals. This suggests the antitumour property of this mushroom. The hot water extracts of the fruit bodies of several mushrooms were reported to show marked host-mediated antitumour activity against sarcoma 180 in Swiss albino mice. However the antitumour activity of the methanol extract of P. florida at a high dose is almost comparable to the recommended dose of an established anticancer drug, Cisplatin. Several epidemiological findings demonstrated that increased intake of dietary antioxidants might contribute to chemoprevention of some human cancers.

P. florida is cultivated on a commercial scale in many parts of the world, including India. This is a delicious edible mushroom. The results of the present investigations indicate that P. florida is a nutritionally functional food with valuable therapeutic use. The best known therapeutic agent stated to be of potential use for correcting hypercholesterolemia is lovastatin and its analogues. Pleurotus species are reported to be the best known source of this drug. The Oyster mushrooms thus have important therapeutic use for the prevention and control of cancer and cardiovascular diseases.


ACKNOWLEDGEMENTS. We thank Dr Sukumara Varma, Plant Pathology Department, Kerala Agricultural University, Trichur for his help during the course of this investigation. The financial assistance from Science and Technology Environment Committee, Government of Kerala for the research project is gratefully acknowledged.

Received 22 May 2000; revised accepted 25 July 2000

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Annual breeding cycle and spawning behaviour of *Hyla annectans* Jerdon 1870 in Nagaland, India

A study of the breeding cycle and spawning behaviour is essential to plan conservation measures for the species included in the IUCN red list category. *Hyla annectans* had been categorized as a lower-risk near-threatened species. There are several contributions on these aspects of anuran amphibians from India. *H. annectans* is the only species reported from India of the family Hylidae and genus *Hylaurenti*7. This species was recorded from Kashi hills and Upper Burma. In Nagaland (25°15′–27°04′N latitude and 93°20′–95°15′E longitude) its distribution is along the Borail range at various altitudes ranging from 1400 to 2440 m ASL. The general climatic condition of the habitat is as follows: temperature 4°C in January to 27°C in July, humidity 44% in January to 92% in July, precipitation 3 to 15 mm in January to 289 to 489 mm in July. Vegetation is temperate evergreen forest.

The frog is leafy-green dorsally and yellowish-white ventrally (Figure 1). Male (SVL 40 mm) and female (SVL 48 mm) frogs show the same colouration. A light