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# *In vitro* Antioxidant and Antilipidperoxidative potential of *Pleurotus florida*

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## Abstract

The ethanolic extract of *Pleurotus florida* was studied for its free radical scavenging property on different in vitro models like 1,1 –diphenyl-picryl hydrazyl (DPPH)Assay, Ferric Reducing Antioxidant Power (FRAP) assay and *in vitro* antilipidperoxidative assay using goat liver homogenate and RBC Ghost model .The *in vitro* Lipid peroxidation (LPO) was inhibited to a good extent by the *Pleurotus florida* ethanolic extract and the extent of inhibition being higher in the RBC membrane model than the liver homogenate model. The mushroom extract showed good dose-dependent free radical scavenging property in both the models.

**Keywords** : free radical scavenging, *Pleurotus florida,* Antioxidant.

## Introduction

The study of lipid per oxidation (LPO) is attracting much attention in recent years due to its role in disease processes. Membrane lipids are particularly susceptible to LP due to the presence of polyunsaturated fatty acids. Since membranes form the basis of many cellular organelles like mitochondria, plasma membrane , endoplasmic reticulum, lysosomes, peroxisomes ,etc. the damage caused by LP is highly detrimental to functioning of the cell and its survival. . It has been implicated in the pathogenesis of a number of diseases and clinical conditions. These include atherosclerosis, cancer, adult respiratory distress syndrome, Alzheimer's disease, Parkinson's disease, ischaemia-reperfusion injury of various organs, chemical and radiation-induced injury, diabetes, etc. Experimental and clinical evidence suggests that aldehyde products of LP can also act as bioactive molecules in physiological and pathological conditions [1].

The most deleterious effect caused by ROS (Reactive oxygen species) is the peroxidation of membrane lipids. Exogenous chemicals and radiation produce peroxidation of lipids leading to structural and functional damage to cellular membranes [2]. Polyunsaturated fatty acids present in cellular membranes are especially prone to damage by ROS and the resulting LP can have serious consequences. LP plays a major role in mediating oxidative-damage in biological systems. There are also several toxic by-products of per oxidation which can damage other biomolecules away from the site of generation[3&4]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation.

Among the fungi, mushrooms have been used for untold centuries as food and medicine. Edible and medicinal mushrooms not only convert the huge lignocellulosic biomass waste into human food, but most remarkably, can produce notable myco-pharma ceuticals; myco-nutriceuticals myco-cosmeceuticals. Mushroom accu mulates a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Also, a mushroom phenolic compound has been found to be an excellent anti-oxidant and synergist that is not mutagenic [5]

*Pleurotus florida* species are commonly called Oyster mushrooms and is an excellent edible mushroom. 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 [6]. The best known therapeutic agent stated to be of potential use for correcting hypercholesterolemia is levostatin and its analogues. *Pleurotus* species are reported to be the best known source of this drug [7].

## Materials and Methods:

*Collection and Preparation of Sample*: Mushroom samples were collected from Blue hill Mushroom Producers and the sample is been preserved in the Department of Biochemistry Dr N.G.P Arts and Science College, Coimbatore,and shade dried and made into a coarse powder . The coarse powder was extracted using ethanol for 72 hours in a soxhlet apparatus. The ethanol was evaporated and the extract was concentrated and was used for the assay.

### Preparation of goat liver homogenate :

Fresh goat liver was obtained from local slaughter house, washed free of blood and removed fat deposits, if any, a 5% homogenate was prepared in ice cold TBS (Tris Buffered Saline) and used for assay. The assay procedure given by Okhawa et al., (1979) [8] has been followed.

### Preparation of Erythrocyte Ghosts :

About 50ml of fresh venous whole blood of goat was collected into a cleaned sterile bottle and defibrinated immediately using acid-washed stones. The defibrinated blood was then transferred into sterile centrifuge tubes and spun at 3000rpm for 10min to pellet out the cells and the supernatant was discarded. The pellet of RBCs was washed in isotonic TBS, thrice successively. The washed pellet was then treated with hypotonic TBS and incubated at 37ÚC for 1 hour for lysis to occur. The lysate was centrifuged at 5000rpm for 15-20 min at 4ÚC. The pale pellet containing the erythrocyte ghost membranes' was then suspended in 1.5ml of TBS. The assay procedure given by Dodge et al., (1963) [9] has been followed to study the anti-lipidperoxidative effect of the mushroom.

### Radical Scavenging Activity :

The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-coloured methanol solution of 1,1- Diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Brand-Williams 1999 ) [10] . 2 to 1 ml of various concentrations of the ethanolic mushroom extract was added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated as follows:

I (%) = (Ablank – Asample / Ablank) x 100

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition (%) against extract concentration. Tests were carried out in triplicate.

### Ferric Reducing Ability of Plant (FRAP) as Measuring of Antioxidant Power :

FRAP assay was carried out by the method of (Pulido 2000 ) [11]with minor modification. The method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe3+-TPTZ) to the ferrous form ($Fe^{2+}$-TPTZ). The ethonal extract of mushroom in varying concentration(20-100ug) were added to 10 mM ferric-TPTZ reagent and the increase in absorbance at 593 nm was measured at 8 min.

## Results

***Determination of in vitro LPO*** : The result obtained are shown in Table 1 .

The extracts tested were effective in reducing the production of TBARS in a dose –dependent manner. The *in vitro* LPO was inhibited to a good extent by the *Pleurotus florida* extract and the extent of inhibition being higher in the RBC membrane model than the liver homogenate model.

***Determination of in vitro Antioxidant assay (DPPH and FRAP) :*** Since the role of free radicals has been implicated in a large number of diseases, the antioxidant activity of mushroom is of significant importance in exploiting their therapeutic potential. The ethanolic extract was subjected to screening for their possible antioxidant activity. Two complementary test system, namely DPPH Free Radical Scavenging and FRAP assay were used for the analysis. Result indicated in the Table 2 and 3.

## Discussion

The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and found variable therapeutic activity such as anticarcinogenic, anti-inflammatory, immunosuppressor and antibiotic[12].

The Lipid peroxidation (LPO) has been broadly defined as the oxidative deterioration of polysaturated lipids. Peroxyl and hydroxyl radicals are important agents that mediate lipid peroxidation, thereby damaging cell membranes [13]. A number of toxic compounds are generated during this process of LPO. TBARS are produced as by-products of LPO that occurs in the hydrophobic core of biomembranes [14].A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom[15].Two different model systems namely goat liver homogenate and RBC ghost (plasma membrane) were used to compare the membrane models which differ in their lipid composition. Mammalian cells have evolved migrate interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism [16]. The effect of mushroom extracts on LP show significant inhibition of TBARS formation. The present finding strongly suggests that the use of the mushroom extracts prevent LP leading to membrane damage consequent to radiation and to certain chemicals which generate potent ROS.

DPPH , a stable free radical with a characteristic adsorption at 517 nm, was used to study the radical scavenging effects of the extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of the radical scavenging. All the concentration which were studied showed free radical scavenging activity. The 50% of inhibition value for *Pleurotus florida* (IC50= 62.05ìg ethanolic extract was necessary to obtain 50% of DPPH degradation).Ethanolic extract seems to be fairly significant when compared to commonly used synthetic antioxidant alpha-tocopherol (á- tocopherol IC 50 = 18.2 ìg ). The inhibition value increases with increase in concentration. Current research is now directed towards finding naturally occurring antioxidants which could reduce or minimize the deleterious effect caused by ROS .The result of the investigation reveal that ethanolic extract of *Pleurotus florida* have potent lipid peroxidation inhibition and showed the highest antioxidant capacity when determined by DPPH and FRAP assays.

**Table 1**

**Extent of Inhibition of *in vitro* Lipid Peroxidation in RBC Ghost and Goat Liver Homogenate by Ethanolic Extract of *Pleurotus florida***

| S.No | Concentration of Extract in( μg) | Percentage Inhibition | |
|---|---|---|---|
| | | RBC Ghost | Liver Homogenate |
| 1. | 20 | 13.60 ±0.11 | 07.30± 0.21 |
| 2. | 40 | 27.24 ± 0.01 | 12.45 ±0.11 |
| 3. | 60 | 40.80 ± 0.21 | 26.20 ± 0.03 |
| 4. | 80 | 54.41 ± 0.10 | 40.92 ± 0.01 |
| 5. | 100 | 68.28±0 .04 | 54.85 ± 0.79 |

Values represented as mean ± SD: n= 3.

**Table 2 : *In vitro* Antioxidant Capacity of Ethanolic Extract Of *Pleurotus florida* (DPPH Assay)**

| S.No | Concentration of Extract in( μg) | Percentage Inhibition |
|---|---|---|
| 1. | 20 | 16.20 ±0.11 |
| 2. | 40 | 32.04 ± 0.01 |
| 3. | 60 | 48.40 ± 0.21 |
| 4. | 80 | 64.02 ± 0.11 |
| 5. | 100 | 81.41 ± 0.03 |
| α- tocopherol IC 50 = 18.2 μg | | |

Values represented as mean ± SD: n= 3.

**Table 3 :**
***In vitro* Antioxidant Capacity of Ethanolic Extract of *Pleurotus florida***
**(FRAP Assay)**

| S.No | Concentration of Extract in( µg) | Percentage inhibition |
|---|---|---|
| 1. | 20 | 0.073 ± 0.01 |
| 2. | 40 | 0.147 ± 0.10 |
| 3. | 60 | 0.220 ± 0.02 |
| 4. | 80 | 0.288 ± 0.01 |
| 5. | 100 | 0.368 ± 0.04 |

Values represented as mean ± SD: n= 3.

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