



Antimicrobial, Antioxidant and Anticancer Activity of the Ethanol Extract of *Pleurotus ostreatus*

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Abstract

The current study evaluates antimicrobial, antioxidant and anticancer activity of the ethanol extract of *Pleurotus ostreatus*. Chemical analysis showed the presence of alcohol, amides, alkanes, ether, phenol and sulphides. Most of the chemical constituents were found to be esters and carboxylic acid derivatives. The extract showed highest resistant against *Bacillus cereus*. *P. ostreatus* ethanol extract showed comparable antioxidant activity with ascorbic acid in both DPPH and FRAP method and also showed an IC_{50} of 39.29 $\mu\text{g/ml}$ against HL60 cells.

Keywords: MTT, DPPH, HL-60 cells, FRAP, GC-MS

1. Introduction

Pleurotus ostreatus usually denoted as oyster mushroom is broadly cultivated for edible purpose and widespread among farmers for its vast adaptableness. Although, about 40 species of oyster mushroom have been diagnosed and documented scientifically, most effective 12 species are being cultivated in different locations of India. Production of oyster mushroom in India is anticipated to be 15-20,000 metric tons every twelve months¹. Various species of *Pleurotus* have unique temperature requirement for growing and development which makes them ideally fit for cultivation all through the year in diverse areas of India. Several lignocellulosic wastes inclusive of corncobs, sawdust, and so on had been used in cultivating

Pleurotus species. The distinct substrates utilized in cultivating mushrooms do have effect at the functional, organoleptic and chemical residences of mushrooms². A preliminary investigation on the yield and proximate composition of *Pleurotus ostreatus* planted in a few humid wooded substrates has been achieved. In normal human diet, it is harmless to eat mushrooms which also plays a key role as it consists of resources of vitamins, minerals, carbohydrates, proteins, unsaturated fatty acids specifically oleic acid and linoleic acids and sodium. In addition, being a rich resource of vitamins with high food charge, mushrooms comprise of many therapeutically important energetic composites³. Free radical formation is linked to ordinary metabolism in our body and also inflicts numerous illnesses⁴. Most of the food trades practice artificial

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antioxidants together with Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) as the preservatives to thwart the oxidation of lipid which results in off-odor in lipid rich food⁵. These synthetic antioxidants utilized in food have some carcinogenic impact. However, the limited usage of those antioxidants has led to an increase in antioxidant material from natural assets. Generally, the antioxidant materials obtained from the herbal resources are considered as harmless as they're available lavishly in vegetables and lots of different plant sources⁶. Several natural resources have been studied and are found to possess antioxidant materials but the dietary mushroom is found to have twice that of any other natural resources which are fit to be eaten⁷. However, mushrooms are believed to be a big source of amino acids and for this reason they are known as rich in nutritional assets. Mushrooms also are stated to have a lot of radical scavenging molecules which include polysaccharides and polyphenols⁸. Along, with phenolics, large quantity of vitamin A, nutrition C and β -carotene in mushroom are recognized to be the prime client for its antioxidant assets. The foremost idea of this scheme is to analyze the qualitative analysis of phytochemical screening, antimicrobial, antioxidant and anticancer activity of the ethanol extract of *Pleurotus ostreatus*.

2. Materials and Method

2.1 Preparation and Extraction of Sample

The mushroom sample was washed with distilled water and dried. The dried sample was pulverized to a powder form. The pulverized mushroom sample was extracted with ethanol in liquid bath at 50°C for 330 minutes. The extract was filtered and used for further analysis.

2.2 Phytochemical Screening

2.2.1 Test for Proteins

The hyphae extract of 1ml was added with 2% NaOH and 0.3% of CuSO_4 . The existence of pink color exposed the occurrence of proteins.

2.2.2 Test for Tannins

The hyphae extract of 1ml and 5% ferric chloride were mixed, and the green shade occurrence specifies the occurrence of tannins.

2.2.3 Test for Saponins

The hyphae extract was agitated vigorously with 5 ml of purified water, the persistence of foam even after 15 minutes specifies the occurrence of saponins.

2.2.4 Test for Flavonoids

1 ml of the hyphae extract was mixed well with 1 ml of 2N NaOH and the yellow color formation designates the existence of flavonoids.

2.2.5 Test for Alkaloids

The hyphae extract of 1 ml and 2 ml of conc. HCl were mixed and then few droplets of Mayer's reagent were added. The white color precipitate specifies the existence of alkaloids.

2.2.6 Test for Terpenoids

1 ml of hyphae isolate, and 2 ml of chloroform were mixed and then few drops of concentrated H_2SO_4 was added. The occurrence of terpenoids was established by the appearance of red brown color at the interface.

2.2.7 Test for Phenols

1 ml of hyphae extract, and 2 ml of condensed water were mixed and then few droplets of 10% ferric chloride was added. The occurrence of phenols was indicated by formation of green color.

2.2.8 Test for Steroids

2 ml of chloroform and few drops of condensed H_2SO_4 was added to 5ml aqueous extract. The top layer showed red while the H_2SO_4 layer appeared yellow with greenish fluorescence, which signifies presence of steroids.

2.2.9 Test for Lipids

1 ml of hyphae isolate was added with 0.1N alcoholic potassium hydroxide along with a few drops of phenolphthalein which was heated in the water bath

for 1 hour. The presence of soapy appearance indicates the presence of lipids.

3. Structural/Compound Estimation of *Pleurotus ostreatus*

3.1 GC-MS

The analysis was performed in GC-MS (Perkin Elmer model: Clarus 680) with mass spectrometer (Clarus 600 (EI) and were evaluated utilizing (TurboMassver5.4.2) software. Intertwined silica was packed with Elite-5 MS. Flow rate of helium was 1 ml/min. The temperature of the injector was 260°C. The sample of 1 µL was injected into the apparatus, the temperature sequence was 60°C (2 mins); followed by 300°C at the proportion of 10°C min⁻¹; and 300°C for 6 mins. Temperature of transfer line was 240°C: and ionization mode electron impact at 70 eV, the term time of output was 0.2 sec and the scan interval were 0.1 sec. The fragments were between from 40 to 600 Da. The results were analyzed with the database accumulated in the GC-MS NIST library.

3.2 FTIR Analysis

FTIR helps in distinguishing proof of compound bond in an atom by delivering an infrared assimilation range. The spectra produce a profile of the sample, an atomic unique mark that can be utilized to screen and provide output tests for a wide range of segments⁹. For the recognition of functional group and characterizing covalent bonding data, FTIR is the successful logical procedure.

3.3 Antioxidant Profile of *Pleurotus ostreatus*

The antioxidant activity was studied by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method and Ferric Reducing Ability of Plasma (FRAP) method.

3.4 DPPH Assay

The antioxidant capacity of *Pleurotus ostreatus* was studied by 2, 2 Diphenyl-1-picrylhydrazyl (DPPH) test. Equal volumes samples at different concentrations

were mixed with 0.1mM of DPPH. Then the mixture was stored in a dark place for 30 minutes¹⁰. The color change from violet to yellow indicated the presence of antioxidants. The experiments were carried out in triplicates. Ascorbic acid was utilized as the standard to compare with samples and IC50 (inhibition concentration) was calculated for both sample and standard. The percentage of inhibition was evaluated applying the formula;

$$\% \text{ of inhibition} = [A_0 - A_1 / A_0] \times 100$$

Where A₀ is absorbance of control (i.e., DPPH solution without sample) and A₁ is absorbance of sample or standard (i.e., DPPH solution with sample/standard).

3.5 FRAP Assay

Samples (50, 100, 250, 500 g/ml) was added with 1 ml of H₂O and mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.1 ml, 1%). The mixture was maintained at 50°C for 2 minutes. 2.5 ml of trichloro acidic acid (10%) was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was added with FeCl₃ (0.5 ml, 0.1%) and the absorbance was estimated at 700 nm. Ascorbic acid was used as standard reference.

3.6 Antibacterial Assay

3.6.1 Agar Well Diffusion Method

The antibacterial test was carried out by Agar well diffusion method. The antibacterial assay of *Pleurotus ostreatus* was carried out against four bacterial cultures viz., *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli*. The study was performed in accordance with Riaz *et al.*, 2012¹¹ and zone of inhibition was calculated.

3.7 Anticancer Activity

3.7.1 MTT Assay

Anticancer tests were carried out in accordance with ISO 10993:5. HL60 (osteosarcoma) was used as primary cytotoxic assay at a dose of 100 µM for 48 hours (MTT assay). A 0.1 mL aliquot of the cell suspension (5 × 10⁶ cells/100 µl) and 0.1 mL of the test solution (10-50 µl)

were added to the wells, and the plates were kept in an incubator (5% CO₂) at 37 °C for 18 h. After 18 hours, 1 mg/ml of MTT was added and the plates were kept in the CO₂ incubation for 4 h, followed by the addition of propanol (100 µl). The plates were secured with aluminum foil to shield them from light and fomented in a rotating shaker for 10–20 min, and the absorbance was measured in a ELISA reader at 517 nm. Cytotoxicity and cell viability was calculated using the formula;

$$\text{Cytotoxicity} = [(Control-Treated)/Control] * 100$$

$$\text{Cell viability} = (Treated/Control) * 100$$

4. Result and Discussion

The phytochemical analysis of the ethanol isolate of *Pleurotus ostreatus* showed positive result to proteins, tannins, saponins, flavonoids, alkaloids, terpenoids, phenols and lipids (Table 1).

Table 1. Phytochemical screening of *Pleurotus ostreatus*

Secondary metabolites	Inference
Tannins	Present
Saponins	Present
Flavonoids	Present
Alkaloids	Present
Terpenoids	Present
Phenols	Present
Proteins	Present
Lipids	Present

The fragments were analyzed between 40 to 600 Da. The data showed the presence of phenol,2-methoxy-3-(2-propenyl)-, eugenol, n-hexadecanoic acid,

Table 2. GC-MS analysis of ethanol extract of *Pleurotus ostreatus*

S. No	Retention Time	Compound Name	Molecular Formula	Molecular Weight	Peak Area
1	11.502	Phenol,2-Methoxy-3-(2-Propenyl)-	C ₁₀ H ₁₂ O ₂	164	9.693
2	11.702	Eugenol	C ₁₀ H ₁₂ O ₂	164	7.654
3	18.054	N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	6.400
4	19.535	9,12-Octadecadienoic Acid (Z,Z)_	C ₁₈ H ₃₂ O ₂	280	48.746
5	27.608	Dehydroergosterol 3,5- Dinitrobenzoate	C ₃₅ H ₄₄ O ₆ N ₂	588	23.190

9,12-octadecadienoic acid (z,z)- and dehydroergosterol 3,5-dinitrobenzoate with retention time of 11.502, 11.702, 18.054, 19.535, 27.608 respectively. Most of the chemical constituents were found to be esters and carboxylic acid (Table 2). The chromatogram of the GC-MS examination is represented in Figure 1.

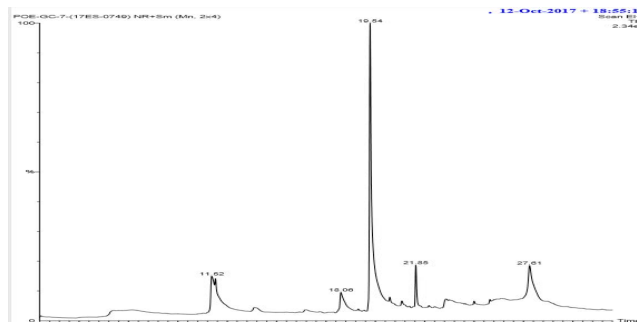


Figure 1. Chromatogram (GC-MS) of ethanol extract of *Pleurotus ostreatus*.

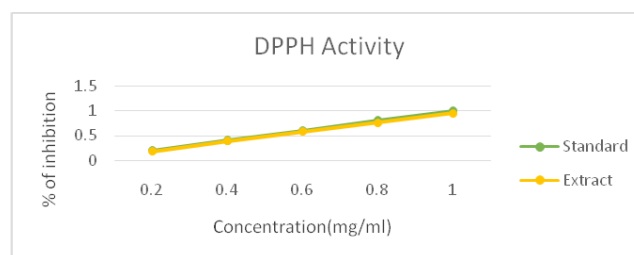


Figure 2. DPPH activity of *Pleurotus ostreatus*.

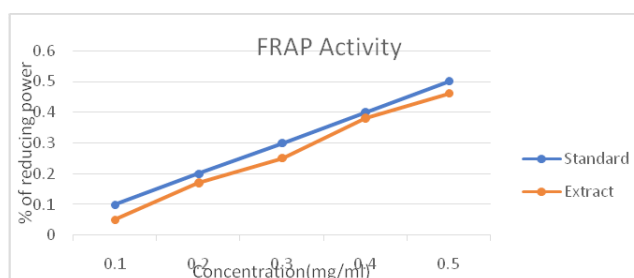


Figure 3. FRAP activity of *Pleurotus ostreatus*.

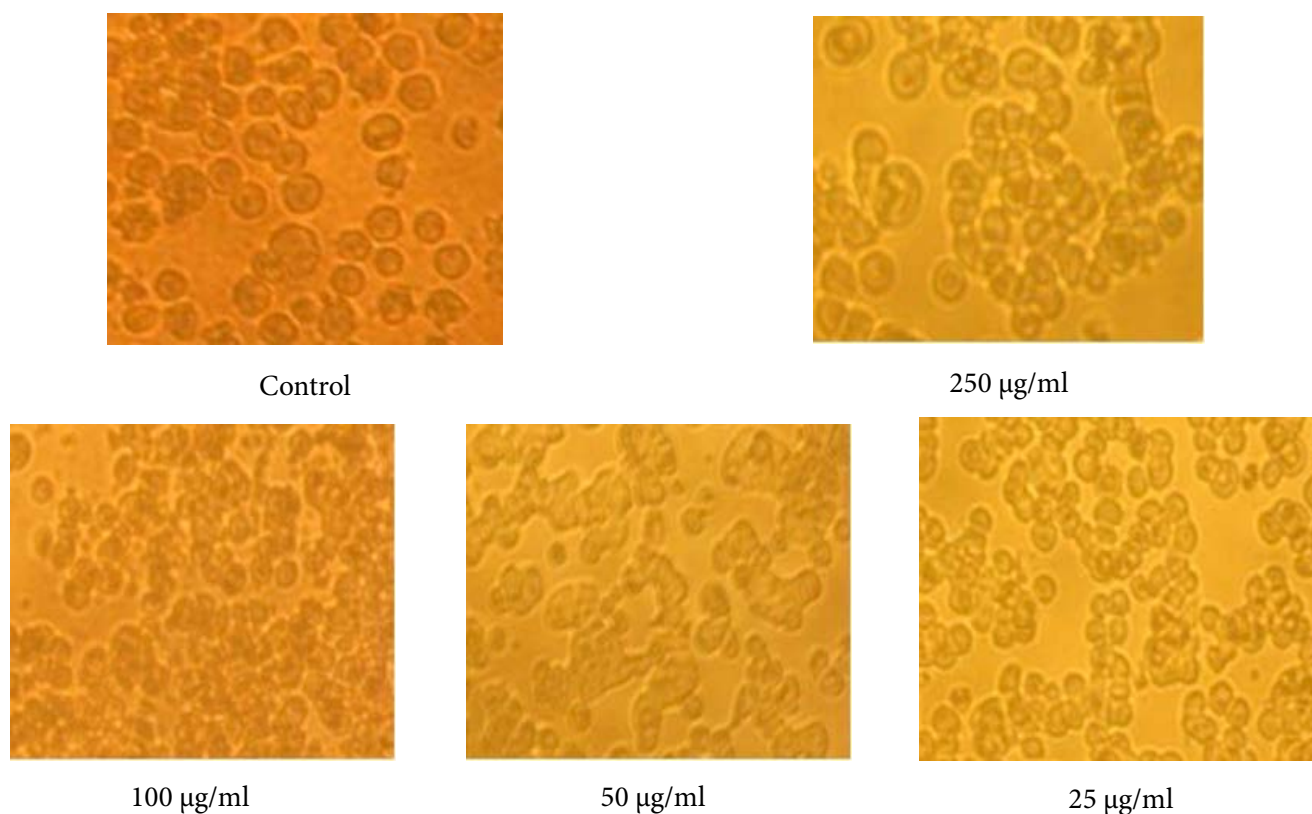


Figure 4. Cytotoxicity effect of *Pleurotus ostreatus* against HL60 cells.

FTIR spectra shows shift in peaks from 3570-3200 (due to OH stretch and polymeric OH stretch), 2935-2915 (due to methylene C-H asymmetry stretching), 2880-2860 (due to methyl C-H asymmetry/symmetry stretch), 1650-1590 (corresponding primary amine NH bend) 1650-1550 (NH bond second amine), 1650-1550 (imino compound NH-stretch), 1350-1260 (corresponding phenol or tertiary group), 1150-1050 (alkyl-substituted ether C-O stretch), 1190-1130 (due to CN stretch secondary amine), 1150-1050 (alkyl substituted ether C-O stretch), 500-470 (due to S-S stretch polysulphides and aryl disulfides S-S stretch). In brief FTIR analysis showed presence of alcohol, amides, alkanes, ether, phenol and sulphides.

Ethanol extract of *P. ostreatus* showed considerable antioxidant activity in DPPH and FRAP assay (Figures 2, 3). The ethanol extract of *Pleurotus ostreatus* showed inhibition in the order of *Bacillus cereus* > *Escherichia coli* > *Bacillus subtilis* > *Saccharomyces cerevisiae*.

The MTT results indicated that the extract has moderate impacts over the HL60 malignant growth

cells. The outcome indicated a diminished cell feasibility and cell development hindrance in a dose dependent manner (Figure 4, Table 3). IC_{50} of the ethanol extract was found to be 39.29 $\mu\text{g/ml}$.

Table 3. Effect of ethanol extract of *Pleurotus ostreatus* against HL60 cells

S.NO	CONCENTRATION ($\mu\text{g/ml}$)	% OF CELL DEATH	% OF LIVE CELLS
1	250	65.10	20.81
2	100	64.36	32.37
3	50	63.62	36.09
4	25	42.38	51.97
5	12.5	34.13	70.48
6	6.25	39.14	79.57
7	3.125	28.95	83.57
8	1.562	18.50	88.76
9	0.781	8.05	95.94

5. Conclusion

The current study concluded that the ethanol isolate of *Pleurotus ostreatus* consist of flavonoids, saponins, phenols, terpenoids, tannins, lipids, amino acids and alkaloids as confirmed by qualitative phytochemical analysis. The extract shows highest resistant against *Bacillus cereus* hence, it may be useful in curing diarrheal related diseases. FTIR analysis showed the presence of alcohol, amides, alkanes, ether, phenol and sulphides. Most of the chemical constituents were found to be esters and carboxylic acid derivatives. *P. ostreatus* ethanol extract showed comparable antioxidant activity with ascorbic acid in both DPPH and FRAP method and also showed an IC₅₀ of 39.29 µg/ml against HL60 cells.

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