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Phytochemical, Antioxidant and Antitumor activity of edible mushroom *Pleurotus ostreatus*

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Abstract

Pleurotus ostreatus is a common edible mushroom**.** It is a saprotroph, white-rot, wood decaying fungus and its mycelia were found to kill and digest nematodes. They are used traditionally as medicine for different diseases. Earlier studies showed that antitumor activity was found in the water-soluble polysaccharide (POPS-1) of *Pleurotus ostreatus.* The present study examines the phytochemistry, antioxidant and antitumor activity of two organic extracts of *Pleurotus ostreatus* obtained by using the solvents, ethyl acetate and methanol. Ethyl acetate extract (EAE) exhibited more antitumor activity than methanol extract (ME) and showed growth inhibition. Comparatively, the Ethyl acetate extract elicited higher total phenolic content and *in vitro* antioxidant capacity. Phytochemical analyses of the extracts revealed low to moderate levels of terpenoids, tannins and carbohydrates, while flavonoids, alkaloids, coumarins, phlobatanin, while the glycosides were not detected. The antioxidant activity was examined and estimated using Diphenylpicrylhydrazyl (DPPH) method and the antitumor activity was examined using melanoma cancer cells A375 and it was estimated by MTT assay. The results indicate that *P. ostreatus* possesses higher antioxidant potential in methanol extract and higher antitumor activity in Ethyl acetate extract.

Keywords: Phytochemistry, Antioxidant, Antitumor, *P.ostreatus*, DPPH, MTT

1. Introduction

Phytochemistry is the study of phytochemicals in which the plants contain secondary metabolic components. These constituents helpplants as a protective cover from foreign attacks and also the consumer can also get such protective functions. Extraction of these constituents can be done by organic solvents and also by chromatography techniques (Doughari, James Hamuel, 2013)[7]. In this study, the mushroom, *Pleurotus ostreatus*, was considered as a plant and the phytochemical tests were done by the extraction using organic solvents such as methanol and ethyl acetate with the dried mushroom. Before extraction, the carbohydrate, protein and lipid content were estimated from the fresh mushroom.

This species is widely used as an edible mushroom and it also has various medicinal values. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. The free radicals can cause cancer, heart diseases, decline in brain function and immune system etc., Antioxidants terminate the chain reaction causing damage to the cells (Bjelakovic G *et al.*, 2013)[2]. An Antitumor drug functions against the growth of cancerous cells. A tumor may be defined as an uncontrolled growth of cells, which may spread to other tissues and organs, causing cancer. They are

usually malignant in nature. In the case of melanoma (skin cancer) cell, the signs are change in size, shape, color or elevation of a mole. The appearances of new moles during pain, itching, redness around the sites are the other signs (Melanoma Skin Cancer, 2012)[1]. Antitumor drugs breakdown the growth of tumor cells which can be done by chemotherapy.

The word pleurotus refers to 'sideways' which shows the sideways growth of the stem with respect to the cap. The English common name, oyster refers to the shape of the cap. *P. ostreatus* is also called as the Tree Oyster Mushroom or the Grey Oyster Mushroom (*Stamets et al., 2000)*[22]. The shape differentiates it from other species in the genus. This is a common edible mushroom and it is also used industrially for mycoremediation purposes. They are widespread in many tropical and subtropical forests. It is a saprotroph, white-rot; wood decaying fungus and its mycelia can kill and digest nematodes. Mushroom belongs to the family of macro fungi, because of their naked fruiting body structure. They are hypogeous and epigeous where they can be picked by hand (Phillips & Roger, 2006)[16].

Oyster mushrooms are consumed for their medicinal and nutritional values. They are used traditionally as medicine for a wide variety of diseases. In this regard various worked and studied this fungus on many characteristic features *viz.*, characterization of fruit body morphology on various environmental conditions in *Pleurotus ostreatus* (Jang, *et al.*, 2003)[11]. The studies on phytochemical screening, antioxidant and antibacterial activity were broadly defined by Parihar *et al.* (2015)[15]. The *Pleurotus ostreatus* species were regarded as therapeutic food for their antioxidant, antimicrobial, antitumor and antiviral properties. These properties were obtained by the presence of various phytoconstituents, which includes terpenoids, tannins and carbohydrates in its various extracts. The antitumor activity was broadly explained by HaibinTong *et al.*,(2006)[24]. Their study showed that the water-soluble polysaccharide (POPS-1) was obtained from the fruiting bodies of *Pleurotus ostreatus* by hot water extraction, ethanol precipitation, and fractionated by DEAE-cellulose ion exchange chromatography. The Japanese mushrooms tested against solid type of sarcoma 180 by intraperitoneal or oral administration (Maruyama *et al.*, 1989)[13]. In this study, the phytochemicals, antioxidant of the ethyl acetate and methanol extracts of *Pleurotus ostreatus* were assayed and antitumor activity using *Homo sapiens* skin cancer cell line A375was assayed through *in vitro* condition.

2. Materials and Methods

Collection of samples

Edible *Pleurotus ostreatus* mushroom processed by S&R AGRO BIOTECH, Guduvancheri, Chennai was bought from the supermarket. The sample was dried in shade while a small part of the fresh sample was used for proximate analysis.

Estimation of Carbohydrates by Anthrone Method

The carbohydrate content was estimated with anthrone reagent as suggested by Yemm, E. W., & Willis, A. J. (1954)[27].

Estimation of Protein by Folin-Ciocalteau's Reagent

Protein content in the sample was estimated by the Lowry method (1951)[12] using Folin-Ciocalteau's Reagent.

Extraction of Lipid

The total lipid content in the sample was extracted by the method followed from Ryckebosch *et al.* (2012)[17].

Preparation of *Pleurotus ostreatus* extracts

The extracts were prepared by using Hexane, ethyl acetate and methanol solvent as suggested by Tambekar *et al.* (2006)[23]. The dried samples were weighed and ground into powder prior to extraction. Then it was subjected with solvents, maintained at room temperature for 24 hours, sequentially. The residual solvents were removed by evaporation at 40-80ºC for 30 minutes. The resulting organic extracts were stored in sterile capped bottle under room temperature for subsequent assays.

Phytochemical Analysis of *Pleurotus ostreatus* extracts

(Qualitative Analyses)

The qualitative analysis was done with standard procedure described in Phytochemical methods (1973)[3], Medicinal plants and traditional medicine in Africa (1993)[4], Pharmacognosy (1989)[5] and are shown in Table 1.

Quantitative Analysis

Total phenolic content determination

The total phenolic compounds present in the extracts were determined with Folin-Ciocalteau phenol reagent suggested by Slinkard and Singleton (1997)[21].

Total tannin content determination

Estimation of tannin in the extract was measured by Folin–Denis method suggested by Schanderi SH (1970)[19].

Antioxidant activity by DPPH assay

Antioxidant activity of extracts were determined using 2,2 – diphenylpicrylhydrazyl(DPPH) radical neutralization assay as described by Brand-Williams et al.(1995)[6].

$$\text{DPPH scavenging activity (I\%)} = [((A_{control} - A_{sample})/A_{control}) \times 100]$$

Cytotoxicity analysis

MTT assay (for cell viability)

The cell viability was tested by the standard procedure called as MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)assay described by Mossman(1983)[14]. This is based on the ability of live, but not dead cells, to reduce a yellow tetrazolium dye to a purple formazan product.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) x100;
Cytotoxicity % = 100 – Viability%

3. Results and Discussion

Estimation

The carbohydrate content in the *Pleurotus ostreatus* was found to be 7.594 ± 0.59 µg/ml (Figure 1.). The amount of total carbohydrate was lower as compared to previous date from literature of wild edible mushroom (Nigerian species) *Pleurotus ostreatus* 32.50 ± 0.12 %[8].The protein content in *Pleurotus ostreatus* was found to be 30.313 ± 2.313µg/ml (Figure 2.). This was in accordance with Egwin *et al.*, (2011)[8] who observed 27.13±0.38 % of protein in Nigerian species *Pleurotus ostreatus* content in his study. The amount of lipid extracted in *Pleurotus ostreatus* per 0.5g sample is 0.011g which was lower when compared with the previous literature of wild edible Nigerian species *Pleurotus ostreatus* (4.89 ± 0.13 %)[8].

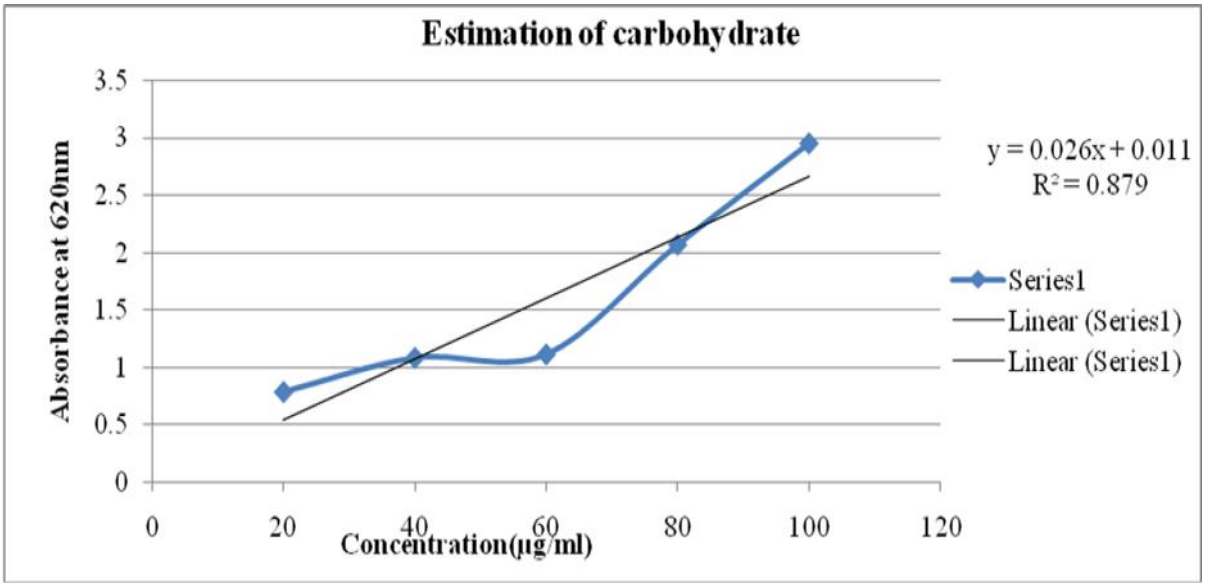

Figure 1.Estimation of Carbohydrate

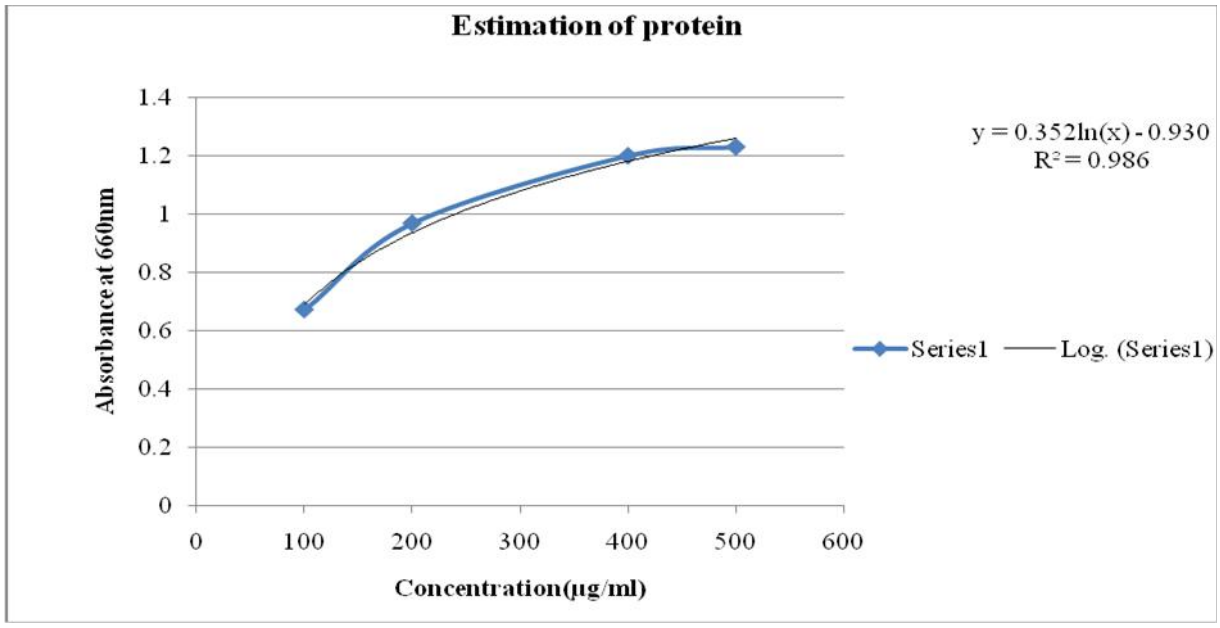

Figure 2. Estimation of Protein

Phytochemical Analysis of *Pleurotus ostreatus* extracts (Qualitative Analyses)

Phytochemical analyses of *Pleurotus ostreatus* disclosed presence of tannins, saponins, quinones, phenols in both extracts but with higher Terpenoids, steroids, phytosteroids, carbohydrates content in methanol extract fraction. Flavonoids, alkaloids, glycosides, coumorins, phlobatannins were not detected in both extracts.

P. ostreatus, an oyster mushroom is primarily consumed for its nutritive value and used industrially as a bioremediator (Solomko and Eliseeva, 1988; Fountoulakis *et al.*, 2002; Tsioulpas *et al.*, 2002)[10][9][25].

Among the solvents used for extraction, the extract obtained using methanol showed more number of phytoconstituents as compared to others. The following table shows the qualitative analysis of *Pleurotus ostreatus.*

Table 1. Qualitative analysis of *Pleurotus ostreatus*

Phytoconstituents	Ethyl acetate Extract	Methanol Extract
Carbohydrates	-	+
Tannins	+	+
Saponins	+	+
Flavonoids	-	-
Alkaloids	-	-
Quinones	+	+
Glycosides	-	-
Cardiac glycosides	+	-
Terpenoids	-	+
Phenols	+	+
Coumarins	-	-
Steroids	-	+
Phytosteroids	-	+
Phlobatannins	-	-

Quantitative Analysis of *Pleurotus ostreatus*

In this case, the total phenol content of 184.84 and 215.6 µg/ml GAE were elicited by ethyl acetate and methanol extracts respectively (figure 3.), which is lower when compared with petroleum ether(PE) and acetone extract(AE) (325.7 and 352.8 mg/L GAE) described in the study of (Iwalokun *et al.*, 2007)[10]. The total tannin content of 475.35 and 273.45 µg/ml using tannic acid standard were elicited by ethyl acetate and methanol extracts, respectively (figure 4.) at which the other species have tannin content in Agaricus species described in the study (Saiqa *et al.*, 2008)[18].

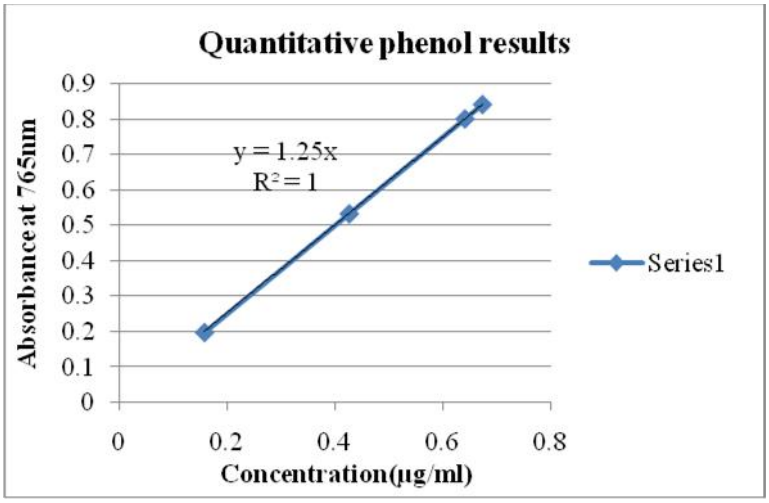

Figure3. Estimation of phenol

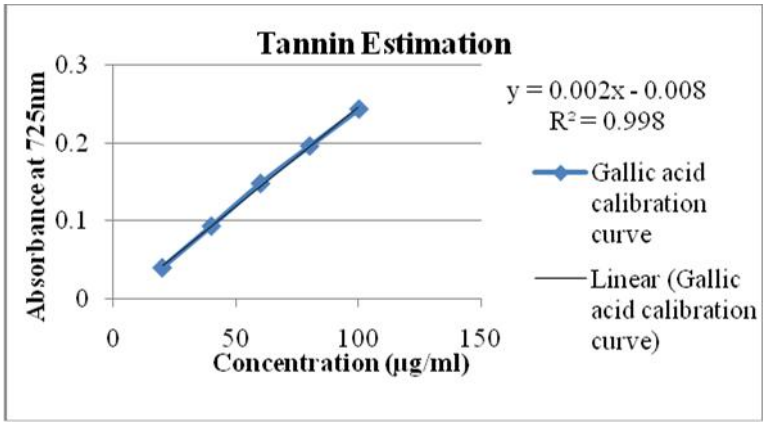

Figure4. Estimation of tannin

Antioxidant Activity of the extracts by Diphenylpicrylhydrazyl method

The DPPH method based on the reduction of methanolic DPPH solution, in the presence of hydrogen donating antioxidant, leading to the formation of non-radical form (DPPH-H). DPPH radical is a stable, free radical and when it reacts with an antioxidant compound which can donate hydrogen or electron, it is reduced to yellow colored DPPH. The reduction capability of the DPPH radical was determined by the decrease in its absorbance at 570nm.

Figure 5 shows the percentage inhibition of DPPH radicals by the two extracts. Results clearly showed that among the two extracts, methanol extract has the highest percentage scavenging activity for the concentration of 200 and 300µg/ml followed by ethyl acetate extract. The scavenging activity is characterized by the loss of the violet color.

The IC_{50} value of the sample, which is the concentration of the sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. The IC_{50} values have been represented in the figure. The lower the IC_{50} value, the higher the antioxidant power. The lower IC_{50} shown by methanol extract was 290µg/ml approx. Thus, methanol extracts have the highest antioxidant activity.

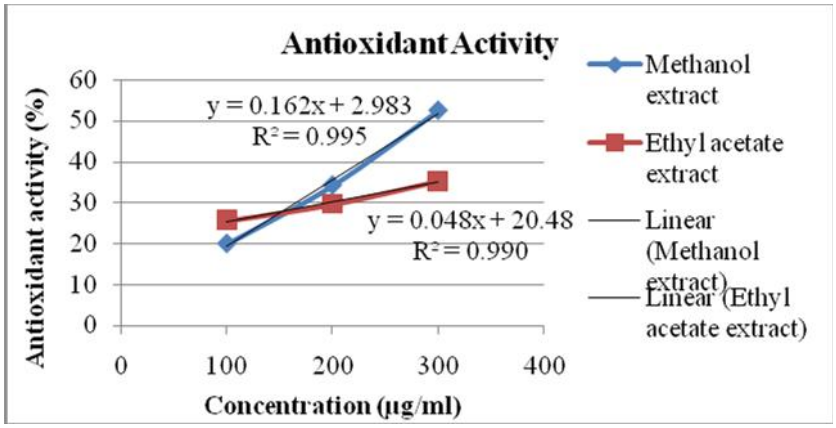

Figure 5. Antioxidant activity of ME and EAE

Cytotoxicity Analysis (MTT Assay)

The MTT assay is based on the ability of live, but not dead cells to reduce the yellow tetrazolium dye to a purple formazan product. The melanoma cancer cell lines A-375, maintained in DMEM medium, supplemented with 10% Fetal bovine serum and an antibiotic, were subjected to treatment, with both the extracts. The absorbance read at 570nm in a microtiter plate reader was found to be directly proportional to the percentage of cytotoxicity; the higher the absorbance, the higher the percentage of cytotoxicity.

The IC_{50} values of methanol and ethyl acetate extracts are represented in figure 6. The lower the IC_{50} value, the higher the cytotoxicity of the extract. The lower IC_{50} value shown by ethyl acetate extract was 150µg/ml approx. Thus, the ethyl acetate extract has the highest antitumor activity.

Figure 6 suggest that the anti-tumor activity (cytotoxicity) of ethyl acetate and methanol extracts of *Pleurotus ostreatus* against melanoma cancer cell lines A-37.

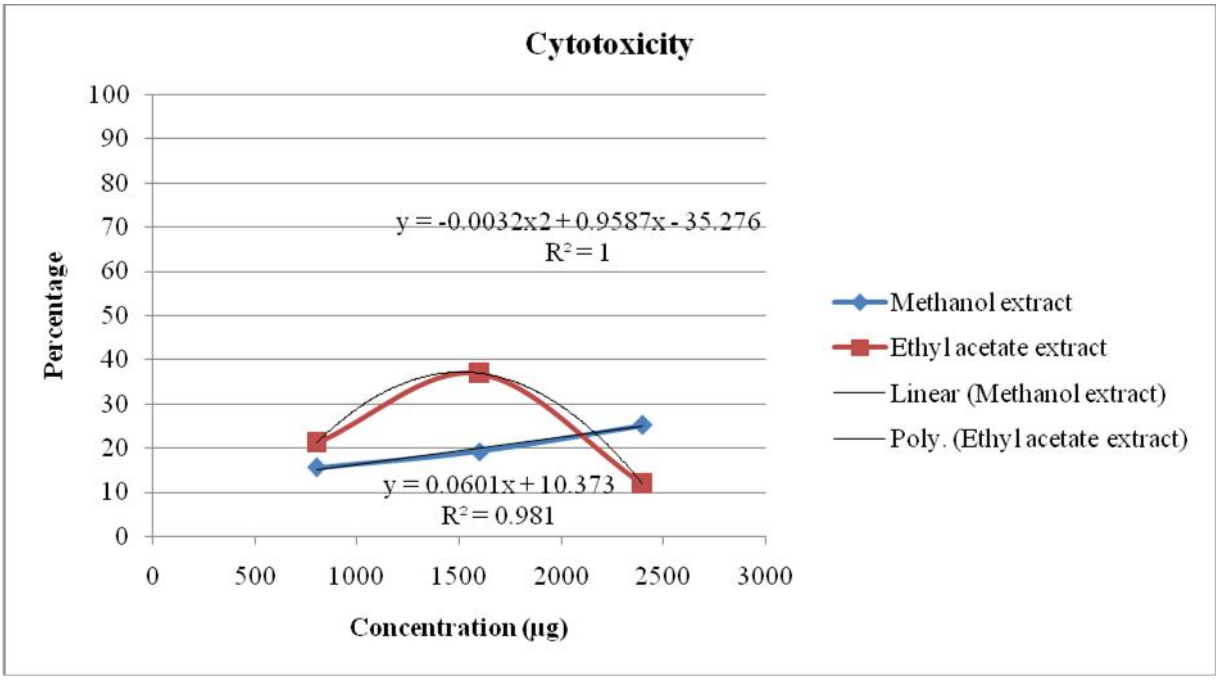

Figure 6. Cytotoxicity of ME and EAE

Conclusion

Cytotoxicity analysis revealed that the ethyl acetate extract showed the highest cytotoxic activity against the treated cell line. With the onset of new antitumor drugs and their adverse side effects, new non-invasive approaches for cancer treatment are being developed. Fungi have an innate ability to synthesize numerous phytochemicals that have potential anti-inflammatory (Schneider *et al.*, 2011)[20], antibacterial (Parihar et.al., 2015)[15], antitumor (Wang *et al.*, 2000)[26] and other medicinal properties. This study suggests that ethyl acetate extract of *Pleurotus ostreatus* has the potential antitumor activity against skin cancer cells. The compounds responsible for the cytotoxic activity can be isolated and used as potential antitumor agents.

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