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Evaluation of Antioxidant and Cytotoxic Activities of Mycelia Biomass and Culture Broth of three Edible Mushrooms, *Morchella conica, Hericium erinaceus* and *Pleurotus florida* Grown in Submerged Culture

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Abstract

Mycelia biomass of three edible mushrooms namely, Morchella conica, Pleurotus florida and Hericium erinaceus were grown as submerged culture in potato dextrose broth supplemented with yeast extract, potassium dihydrogen orthophosphate and magnesium sulphate. The mycelia biomass was extracted with 70% ethanol and culture broth with ethyl acetate. The solvents were evaporated to dryness at low temperature using a rotary vacuum evaporator and the residues thus obtained were used for the studies.. The extracts of mycelia biomass and culture broth were evaluated for antioxidant, cytotoxic and antiproliferative activities. Antioxidant activity of extracts was assayed by DPPH (2,2-dyphenyl-1- picrylhydozyl) radical scavenging assay, FRAP (Ferric ion reducing antioxidant power), superoxide radical scavenging and lipid peroxidation inhibiting assays. Cytotoxicity of mycelia and culture broth extracts were assayed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2-5 diphenyltetrazolium bromide) assay using HepG2 liver cancer cell line and antiproliferative activity by using DLA (Dalton's Lymphoma Ascites) cell line. Among three mushrooms examined, ethanol extract of mycelia of M.conica and ethyl acetate extract of culture broth of H. erinaceus possessed highest antioxidant activity.The extracts of M. conica and H. erinaceus had a significant cytotoxic effect against DLA cells than P. florida.. Ethyl acetate extract of culture broth of H. erinaceus showed the highest cytotoxic activity against HepG2 liver cancer cell lines. The experimental results suggest the possible use of the mycelia and culture broth extracts of these mushrooms as supplements in cancer treatment. The findings also reveal the mycelia and culture broth of medicinal mushrooms have similar therapeutic potential as the fruiting bodies.

Keywords: *Morchella conica, Pleurotus florida, Hericium erinaceus*, mycelia biomass, culture broth.

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INTRODUCTION

Cancer represents a leading cause of death among individuals of all ages. According to the recent reports, it is one of the primary causes of human mortality and is expected to reach thirteen million deaths by 2030 [11] Cancer treatment often leads to a number of complications and deleterious effects on normal cells, tissues, and organs. Some of the common side effects include anemia, appetite loss, bruising and bleeding (thrombocytopenia), constipation, diarrhea, edema, fatigue, flu-like symptoms, hair loss (alopecia), infection, cognitive problems, vomiting, neurodegeneration (Peripheral Neuropathy), inflammation and pain [19,20] A large number of anticancer drugs derived from

synthetic as well as natural sources are currently available. However, 60% of currently used drugs for chemotherapy are originated from natural products. Bioactive compounds from natural products including plants and microbes have been regarded as important sources for the development of novel anticancer drugs. Search for anticancer medicines from natural sources continue to remain an important area of research in modern medicine [14].

Among the microbial sources, mushrooms have gained great attention for the development of anticancer medicines in recent years. They are known for their delicacy and nutritional value from ancient times. In terms of nutrition, the mushrooms are highly rich in fiber, unsaturated fatty acids, vitamins, essential oils, lectins, lactones, alkaloids, terpenoids, antibiotics, metal-chelating compounds, proteins, enzymes, etc. Researchers found that mushrooms as a reliable source of many bioactive compounds that are useful for various therapeutic application [6,18] In this context, attempts are being made in recent years to discover potential bioactives from these mushrooms for therapeutic uses.

Morchella conica, is a member of the Morchella genus. This mushroom is an abundant source of nutrients such as proteins, minerals, alpha-carotene, and ergosterol. *Morchella conica* also contains a number of bioactive substances, including glutathione [17]. This edible mushroom possesses significant medicinal properties and biological activity.*Morchella conica* contain mannitol, arabinitol, linoleic acid, palmitic acid. A polysaccharide isolated from *Morchella conica* was designated as Neutral *Morchella Conica* Polysaccharides-2 (NMCP-2) [22]. Triterpenoids isolated from *M. conica* were found useful to treat cancer due to their immunomodulatory and antiproliferative effects [14].

*Hericium erinaceus*is a member of the Hericiaceae family. The mushroom is frequently referred as Lion's mane. Hericium erinaceus, is a nutritious mushroom with a variety of therapeutic benefits. *Hericium erinaceus* contains alkaloids, fatty acids, phenolics, steroids, and pyranones [24]. One of the main bioactive diterpenoids from the cultivated mycelia of *H. erinaceus* that exhibits strong antitumorigenic properties is erinacine A [21] By using cancer cell lines, the two extracts (HTJ5 and HTJ5A) produced from the broth *of H. erinaceus* were assessed for their anticancer effects against a variety of malignancies, including gastrointestinal cancers [16].

Pleurotus species are referred as oyster mushrooms. Oyster mushrooms are the second largest cultivated mushrooms in the world. They are excellently edible and extensively used for culinary purpose.Commonly cultivated in India,*P.florida* has excellent nutritional qualities, which includeprotein (25–50%), fat (2–5%), carbohydrates(17–47%), mycocellulose (7–38%), and minerals (potassium, phosphorus, calcium, and sodium) of roughly 8–12% [12] *Pleurotus florida*also contains biologically active compounds like polysaccharides (PS-I, PS-II), lectin, pleuran, β glucan, iron, and molybdenum [15]. Pleuran, is utilised as supplementary treatment in radiotherapy and chemotherapy. The compound also has a favourable impact on persons who experience prolonged stress [13] In addition to this *Pleurotus* species contain several other bioactive compounds such as ergosterol (provitamin D2), phenolic acids, amino acid, ergothioneine, and lovastatin [7].

Considering these attributes of medicinal mushrooms, we examined the antioxidant, cytotoxic and antiproliferative properties of mycelia biomass and culture broth of three edible mushrooms *Morchella conica, Hericium erinaceus* and *Pleurotus florida.*

MATERIALS AND METHODS

Cultures of *Morchella conica* and *Hericium erinaceus* were obtained from ICAR Directorate Mushroom Research, Solan whereas that of *Pleurotus florida* was obtained from Kerala Agricultural University, Mannuthy, Thrissur and maintained in Amala Cancer Research Centre.HepG2 liver cancer cell line was purchased from National Centre for Cell Science (NCCS), Pune, India, and maintained in vitro in DMEM purchased from Himedia, supplemented with 10% FBS,100 µg/ml gentamycin and 100 µg/ml streptomycin in a CO_2 incubator with 5% CO_2 supply at 37°C. DLA cells were collected from

tumor-bearing Swiss albino mice maintained at Amala Cancer Research Centre. All chemicals used in the study were of analytical grade.

Submerged Culture of Mycelia

Cultures of *M. conica, H. erinaceus, and P. florida* were maintained on Potato-dextrose –agar medium (PDA) supplemented with 0.5% yeast extract. Potato-dextrose broth (200g of peeled potato, 20g dextrose, 1g yeast extract, 5g KH_2PO_4, 2.5g of $MgSO_4$. $7H_2O$ dissolved in 1L distilled water) was used for growing the mycelia in submerged culture. One hundred ml of medium was poured into several Erlenmeyer flasks and sterilized in an autoclave at 15lb pressure for half an hour. The medium was inoculated with the cultures of the mushrooms and incubated at 24 - 27°C for 12-14 days on a rotary shaker at 170 rpm. After the submerged growth period the cultures were filtered through Whatman filter paper and the mycelia biomass and culture filtrate were separated. The mycelia biomass was dried at 40°C and the culture broth was stored at 4°C in a refrigerator.

Preparation of Extracts

Dried mycelia biomass of the mushrooms were extracted with 70% ethanol using Soxhlet apparatus and the culture filtrates were extracted with ethyl acetate by shaking in a separating funnel. The solvent extracts were evaporated to dryness at low temperature at 40°C using a rotary vacuum evaporator. The residues thus obtained were used for further assays.

Preliminary Chemical Analysis of Extracts for Phytochemical Profile

The preliminary analysis for phytochemical profile of the mycelia and culture broth extracts was carried out. The extracts were assayed for flavonoids, steroids, terpenoids, and polyphenols constituents.The following qualitative analysis was carried out to determine the presence and absence of phytochemicals [2]. The extracts were dissolved in distilled water/methanol for the purpose of assays.

Test for Steroids

An equal amount of extract and concentrated H_2SO_4 were mixed well and the formation of wine red colour in the extract indicated the presence of steroids.

Test for Terpenoids

2ml chloroform was added to 2ml extract and then 2ml $H_2SO_4$was slowly added in a test tube. Production of the golden yellow layer at the interface indicated the presence of terpenoids.

Test for Total Flavonoids

A few drops of diluted NaOH solution was added into a test tube containing 1ml of extract which was dissolved in 5ml 95% ethanol. Production of intense yellow colour indicated the presence of flavonoids.

Test for Polyphenols

500 µl extract was added with 500 µl ferric chloride and incubated for 5min at 22°C. Then 2 ml of 20% Na2CO3 was added and incubated for 10-15 mins. The blue colour indicated the presence of polyphenols.

***In vitro* Antioxidant Assays**

DPPH Radical Scavenging Assay

DPPH assay was done based on the method of Aquino et al. [8, 9]. A stable free radical DPPH [2,2-Diphenyl-1-Picryl hydrazyl] soluble in methanol was used to perform the free radical scavenging assay. DPPH appeared in deep purple color in its radical state, on reduction, the color of DPPH get converted into yellow or becomes colorless by the antioxidant compound. DPPH was prepared in methanol and the OD was adjusted to 0.7 prior to the experiment. An aliquot of different concentrations (10µg, 20µg, 30µg, 40µg, 50µg, 60µg, 70µg, 80µg, 100µg, 120µg, 140µg, 160µg, 180µg) of 70% ethanolic extract

of mycelia) and ethyl acetate extract of culture filtrate (100µg,150µg, 200µg) was added to 1ml DPPH solution. After 20 minutes of incubation, the absorbance was taken at 515nm. By comparing the absorbance value of the control with that of the treated, the free radical scavenging ability was determined. 1ml DPPH in methanol was used as a control

Percentage inhibition was calculated by using the formula:

$$\% \text{ inhibition } = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}}$$

Ferric Reducing Antioxidant Power (FRAP) assay

The reducing power of the extract can be determined by using the FRAP method described by Benzie and Strain [10]. The assay is based on the reduction of Fe3+- TPTZ (2,4,6-tripyridyl-s-triazine) to Fe2+ TPTZ complex which on reaction produces a purple–blue colour at a low pH. Different concentrations of 70% ethanolic extract of mycelia and ethyl acetate extract of culture filtrate (20µg,40µg, 60µg,80µg, 100µg) were mixed with 900µl FRAP reagent (300mM acetate buffer -pH 3.6, 10mM $FeCl_3$, 40mM HCl in a ratio of 10:1:1) and made up to 1ml final volume. After 15 minutes of incubation, the OD was taken at 595nm. An increase in absorbance value indicated higher antioxidant potential.

ABTS Radical Scavenging Assay

The stable free radicals obtained from 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) were allowed to react with different concentrations (20 µg, 40 µg, 60 µg, 80 µg, 100 µg) of ethyl acetate extract of culture filtrate and 70% ethanolic extract of mycelia. To the ABTS solution(7mM), potassium persulphate (2.45mM) was added and made to react in the dark for 24 hours at room temperature. ABTS and persulphate react with one other which led to partial oxidation of ABTS and the formation of $ABTS^+$ radical. The $ABTS^+$ radical solution was diluted with ethanol to get an absorbance of 0.75 at 735nm.ABTS+ radical solution was added to each concentration of extracts and made up to 1ml final volume. After 10 min of initial mixing of both the extracts and radical solution, absorbance was measured and the reduction in absorbance in comparison to ethanol was noted [4].

Percentage inhibition was calculated by using the formula:

$$\% \text{ inhibition } = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}}$$

Lipid Peroxidation Assay

The inhibition of lipid peroxidation was tested by Thiobarbituric acid-reactive species (TBARS) assay [5]. This test is based on the end product's reaction. Malondialdehyde (MDA) is combined with TBA to generate an adduct, a pink chromogen resulting from of lipid peroxidation. Different concentrations of ethyl acetate extract of culture filtrate and 70% ethanolic extract of mycelia (25µg, 50µg,100µg) were added to 250 µl of 10% egg yolk homogenate and made up to 500 µl by adding distilled water. The whole experiment was set up in dark condition. To the reaction mixture 25 µl of 0.07 M $FeSO_4$ was added. After 30 min mixture 25 µl of 20% trichloroacetic acid, 750 µl of 20% glacial acetic acid (pH 3.5) and 750 µl of 0.8% of TBA (prepared in 1.1% of sodium dodecyl sulphate) were added to the reaction mixture then vortexed, and kept in the water bath for 60 min. After cooling down the absorbance was measured at 532nm using spectrophotometer. Percentage of lipid peroxidation inhibition (LPI%) was calculated using the equation:

$LPI\% = [(AC - AS)/AC] \times 100$
(AC = control absorbance, AS = sample absorbance)

In vitro cytotoxicity screening

Trypan Blue Exclusion Assay

Short-term cytotoxicity activity of different concentrations of the extracts was assayed using DLA cells by trypan blue assay [3]. DLA (Dalton lymphoma ascites) cells were collected from the peritoneal

cavity of tumour-bearing mice and centrifuged at2000 rpmfor 5 min. The supernatant was discarded and the pellet was resuspended in 1ml PBS solution. The above procedure was repeated twice. After centrifugation, the cells were suspended in 20 ml of PBS solution. The DLA cells were treated with different concentrations of ethyl acetate extract of culture filtrate and 70% ethanolic of extract mycelia (10µg, 25µg, 50µg, 100µg). 100µl of PBS solution was added to the suspension and incubated for 3 hours at 37˚C. After the incubation, 100 µl of trypan blue was added into the cell suspension making the final volume of 1ml and incubated for 1 min in room temperature. Then a drop of the mixture was applied onto the hematocytometer and placed on the stage of the binocular microscope for counting the viable (unstained) and nonviable (stained) cells separately.

$$\% \text{ cytotoxicity } = \frac{\text{No. of dead cells}}{\text{No. of live cells } + \text{ No. of dead cells}} \times 100$$

MTT Assay

Cell viability was estimated by the MTT assay, adopting the method of [1] The cytotoxicity assay was carried out using HepG2 liver cancer cell line and 3T3 mouse embryonic fibroblastic cell lines. Cells were seeded in 96 well plates at a seeding density of 5000cells/ well and incubated for 24 hours at 37℃ and 5 % CO_2. After the incubation, the cells were treated with different concentrations (50µg,100µg, 200µg) of ethyl acetate extract of culture filtrate and 70% ethanolic extract of mycelia of *H. erinaceus* and *M. conica* respectively, and incubated for 24 hours. At the end of the treatment period, discard the media and gently washed the cells with PBS. For analyzing the cell viability, they were treated with 100 µl of MTT (1mg/ml in serum free media) for 3 hours. After 3 hours of the incubation, 100 µl of DMSO was added into each well to dissolve the formazan crystals. The absorbance was measured at 570nm using Elisa reader. IC50 value was determined by plotting the percentage of cell death against the drug concentrations. Doxorubicin was used as standard.

The percentage growth inhibition of cells exposed to treatments was calculated as follows.
% Cell death = $([OD\ of\ control - OD\ of\ sample]/OD\ of\ control) \times 100$

Cell Morphology Analysis by Phase Contrast Microscopy

By using a Phase contrast microscope, the morphological changes of HepG2 cells were observed after treating the cells with different concentrations of ethyl acetate extract of culture filtrate and 70% ethanol extracts of mycelia of *H. erinaceus* and *M. conica*.

Statistical Analysis

All the tests were conducted in triplicates, and the data were statistically analyzed with Graph Pad prism software and expressed as mean ± SD. The analysis was done using one-way analysis of variance (ANOVA) followed by Dunnett's test and $P<0.05$ was considered significant.

RESULTS

Yield of Mycelia Biomass and Culture Broth Extracts

There was considerable variation in the yield of extracts obtained from 70% ethanol and ethyl acetate extraction of mycelia biomass and culture broth respectively (Table 1) The methanol extract of *H. erinaceus* mycelia showed highest yield followed by *P. florida* and *M. conica.* The yield obtained from ethyl acetate extract of culture filtrate was comparatively lower than the mycelia extract. However, yield of extracts of *H. erinaceus* mycelia and culture broth recorded highest among the three mushrooms.

Table 1. Yield of extracts of mycelia biomass and culture broth.

Yield of the extracts	*P. florida*	*M. conica*	*H.erinaceus*
70% ethanolic extract (mycelia)	0.689g	0.734g	1.358g
Ethyl acetate extract (culture filtrate)	0.025g	0.044g	0.217g

Preliminary Phytochemical Analysis

The ethanolic extracts of the mycelia and the ethyl acetate extracts of the culture broth of three mushrooms showed positive reaction for steroids, flavonoids, and polyphenols but both the extracts of the three mushrooms showed negative for terpenoids (absence of yellow colour at the interface of reaction mixture in the test tubes). A wine-red colour for steroids, intense yellow colour, and bluish colour for polyphenols appeared indicating the tests were positive. The results are shown in Table 2.

Table 2. Phytochemical screening of extracts.

Extracts	Compounds			
	Steroids	***Flavonoids***	***Polyphenols***	***Terpenoids***
PEM	Present	Present	Present	Absent
PEC	Present	Present	Present	Absent
MEM	Present	Present	Present	Absent
MEC	Present	Present	Present	Absent
HEM	Present	Present	Present	Absent
HEC	Present	Present	Present	Absent

***In vitro* Antioxidant Assay**

DPPH Radical Scavenging Activity

The 70% ethanolic extract of mycelia of *M. conica* exhibited the highest scavenging activity 53.8% at 180 µg/ml concentration whereas the activities of the ethanolic extracts of *P. florida* and *H. erinaceus* were 19.68% and 31.75% at the same concentration. The ethyl acetate extracts of *H. erinaceus* showed the highest scavenging activity at 58% at 200 µg/ml whereas, the activities of the ethyl acetate extracts of *P. florida* and *M. conica* were 24.14% and 54.06% at the same concentration. Therefore, ethyl acetate extracts of *H. erinaceus*and *M. conica* culture broth showed greater scavenging activity than *P. florida* (Figures 1& 2).

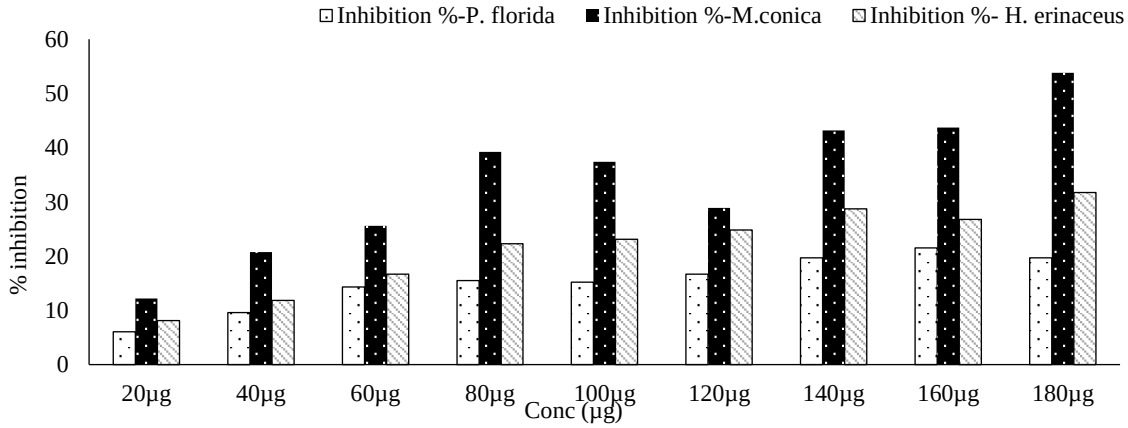

Figure 1. DPPH scavenging activity (70% ethanolic extract of Mycelia).

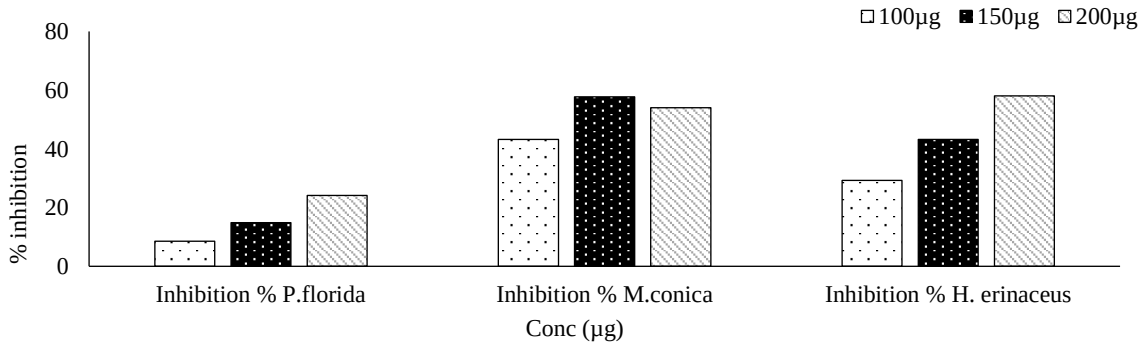

Figure 2. DPPH scavenging activity (70% ethanolic extract of Culture filtrate).

FRAP Assay

FRAP assay is used to evaluate the total antioxidant potential of the extracts. The compound's antioxidant power increases with a higher FRAP value.Our results showed that FRAP activity increased with higher concentrations of extracts. The ethanol extract of *M. conica* showed a higher reduction at 100 µg/ml whereas, the *P. florida* and *H. erinaceus* showed a reduced activity at the same concentration. Ethyl acetate extract of *M. conica* exhibited the highest reduction at a concentration of 80 µg/ml whereas, *P. florida* and *H.erinaceus* in the same concentration showed a reduced activity. Therefore, the ethyl acetate extract of both *H. erinaceus* and *M. conica* showed the highest Fe^{3+} ions reducing activity (Figures 3&4).

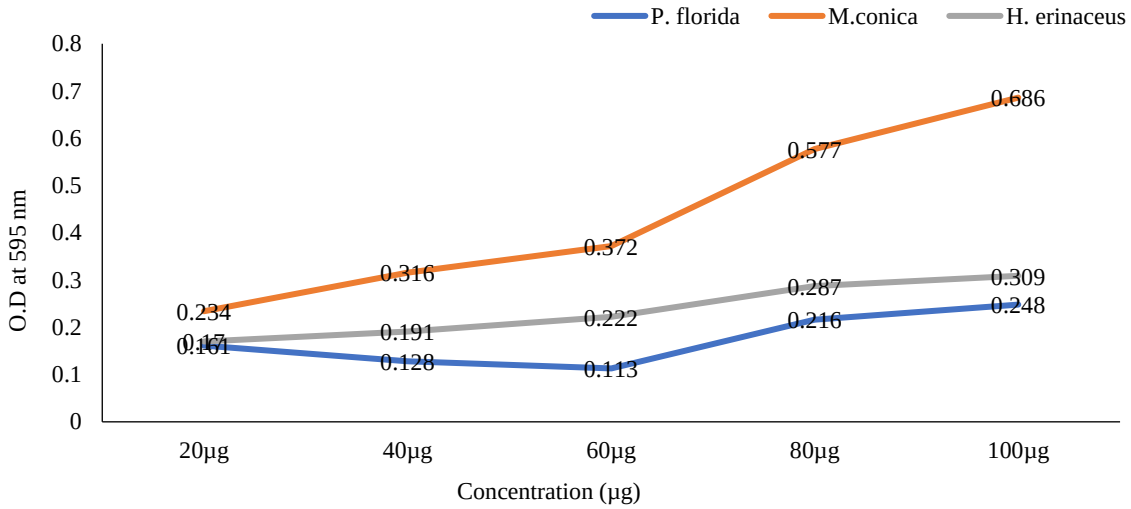

Figure 3. FRAP activity (70% ethanolic extract of Mycelia).

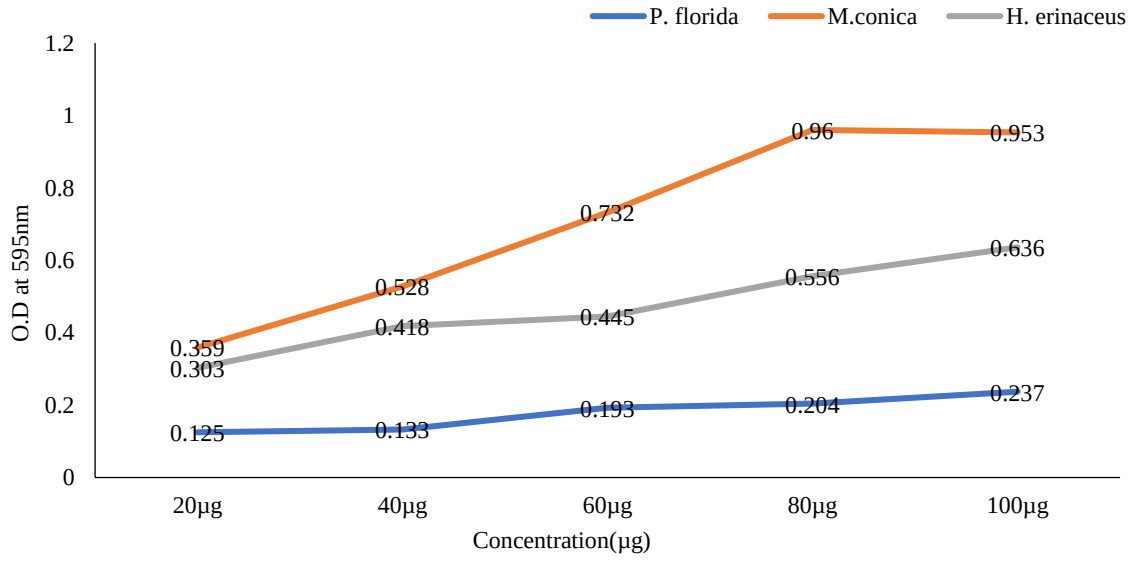

Figure 4. FRAP activity (Ethyl acetate extract of culture filtrate).

ABTS assay

Since, the radical scavenging activity of *P. florida* was lower further studies were carried out in *M.conica* and *H. erinaceus.* Ethyl acetate extract of *M. conica* showed the highest scavenging activity of 97.43% at 80 µg/ml whereas, the extract of *H. erinaceus* had an activity of 90.20% at the same concentration. The maximum scavenging activity of 97.5% was shown by the ethanolic extract of *H. erinaceus* at 100 µg, whereas the extract of *M. conica* showed an activity of 87.15% at the same

concentration. Thus, ethyl acetate extracts of *M. conica* and ethanolic extract of *H. erinaceus* are equally potent to reduce ABTS radicals (Figure 5).

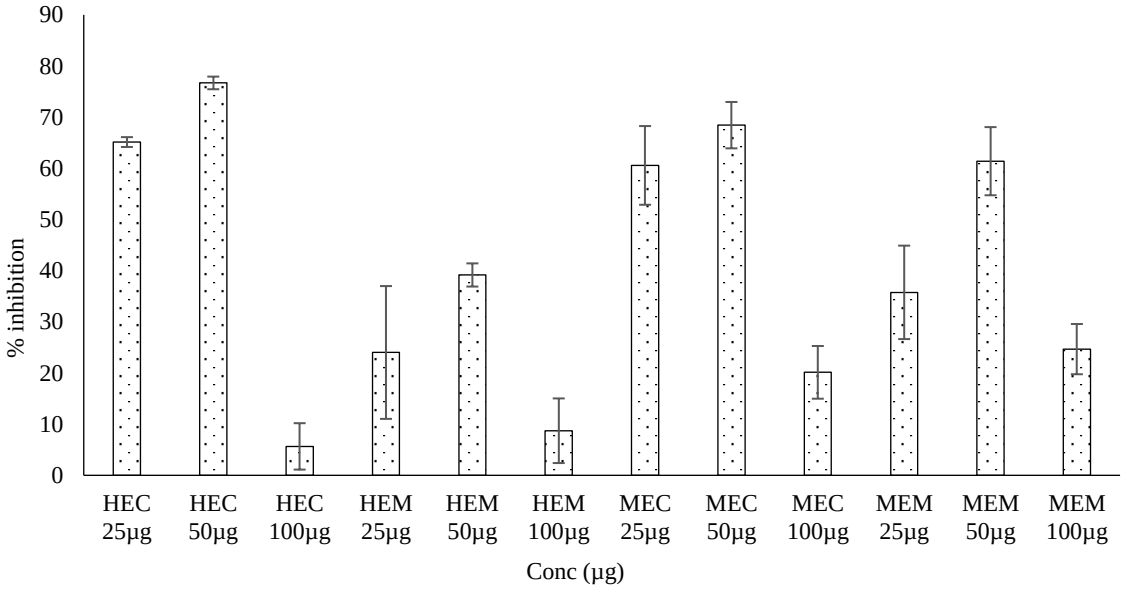

Figure 5. Lipid peroxidation assay (70% ethanolic extractand ethyl acetate extract). Values are expressed as Mean±S.D., where HEC: Culture filtrate extract of *H. erinaceus*; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

Lipid Peroxidation Assay

The ethyl acetate extract of *H. erinaceus* exhibited the highest activity as it inhibited almost 76.69% of lipid peroxidation at 50µg concentration whereas, the extract of *M. conica* at the same concentration showed an inhibition activity of 68.43%. Ethanolic extract of mycelia of *M. conica* exhibited the highest inhibition activity of 61.39% at 50µg whereas, the ethyl acetate extract of *H. erinaceus* in the same concentration showed an inhibition activity of 39.15%. Therefore, the ethyl acetate extract of culture broth of *H. erinaceus* was the most active extract capable of inhibiting lipid peroxidation (Figures 6 &7**).**

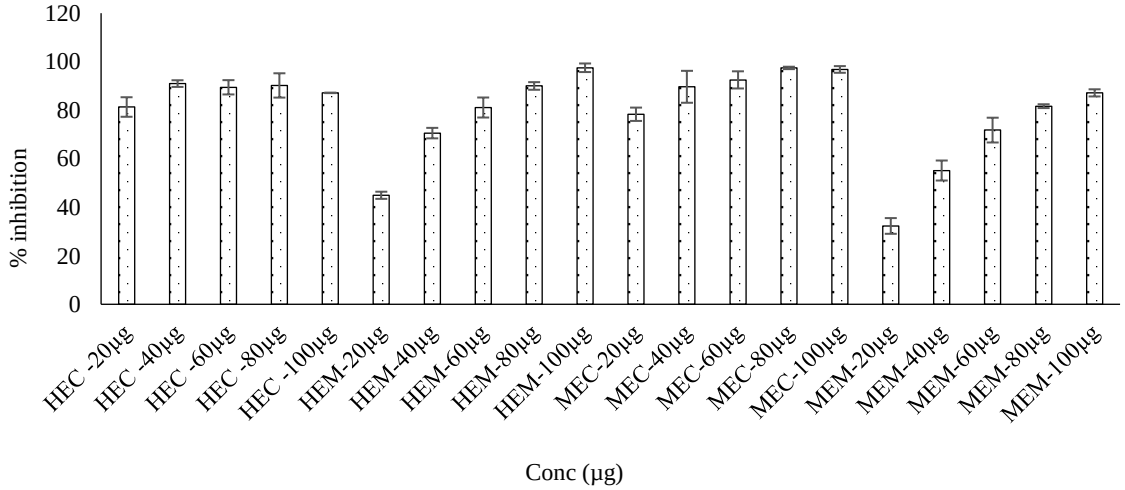

Figure 6. ABTS assay (70% ethanolic and ethyl acetate). Values are expressed as Mean±S.D., where HEC: Culture filtrate extract of *H. erinaceus* ; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

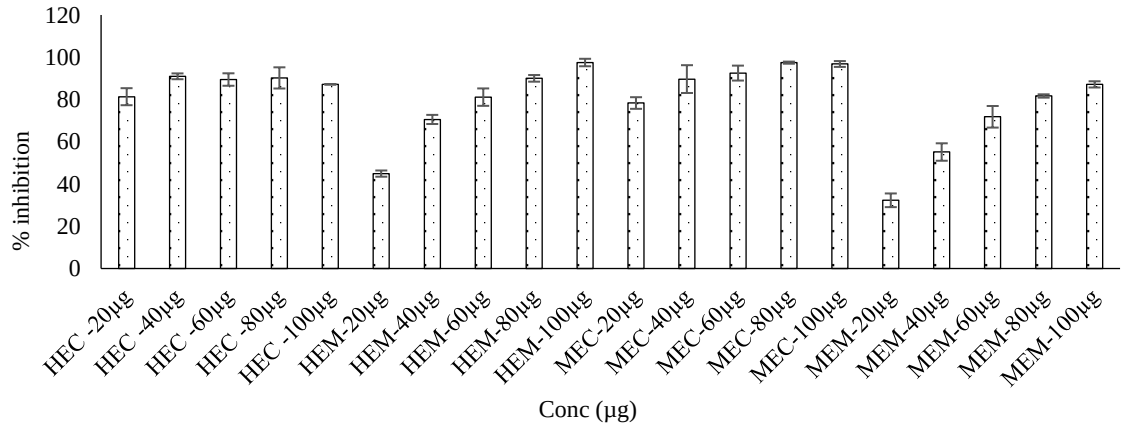

Figure 7. ABTS assay (70% ethanolic and ethyl acetate). Values are expressed as Mean±S.D., where HEC: Culture filtrate extract of *H. erinaceus*; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

In vitro cytotoxicity assay

Trypan Blue Exclusion Assay

Results shows that the extracts of *M. conica* and *H. erinaceus* had a significant cytotoxic effect against DLA cells than *P. florida*.. Ethyl acetate extract of culture broth of *H. erinaceus* at a concentration of 100 µg/ml showed the highest cytotoxic activity of 86.95% while the extracts of *P. florida*and *M. conica*at the same concentration exhibited an activity of 16.25% and 52% respectively. The ethanolic extracts of mycelia of*P. florida* and *M. conica*at a concentration of 100 µg exhibited cytotoxicity activity of 23.4% and 22.65% respectively whereas, the extract of *H. erinaceus*showed an activity of 14.3% at the same concentration.Therefore, the results indicated that the ethyl acetate extract of *H. erinaceus* hadsignificantly high cytotoxicity against DLA cells. The results are shown in Figures 8&9**.**

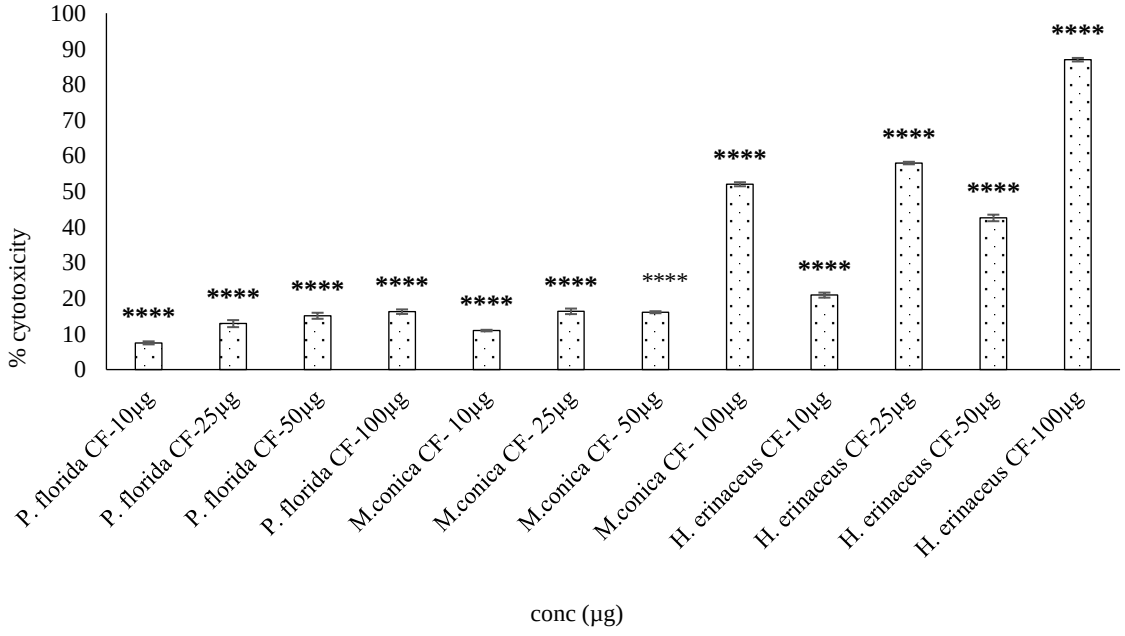

Figure 8. Trypan blue exclusion assay showing results of DLA cells treated with ethanolic and ethyl acetate extracts.Values are expressed as MEAN± SD, n=3, P<0.0001 significant compared with untreated cells.HEC: Culture filtrate extract of *H. erinaceus* ; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

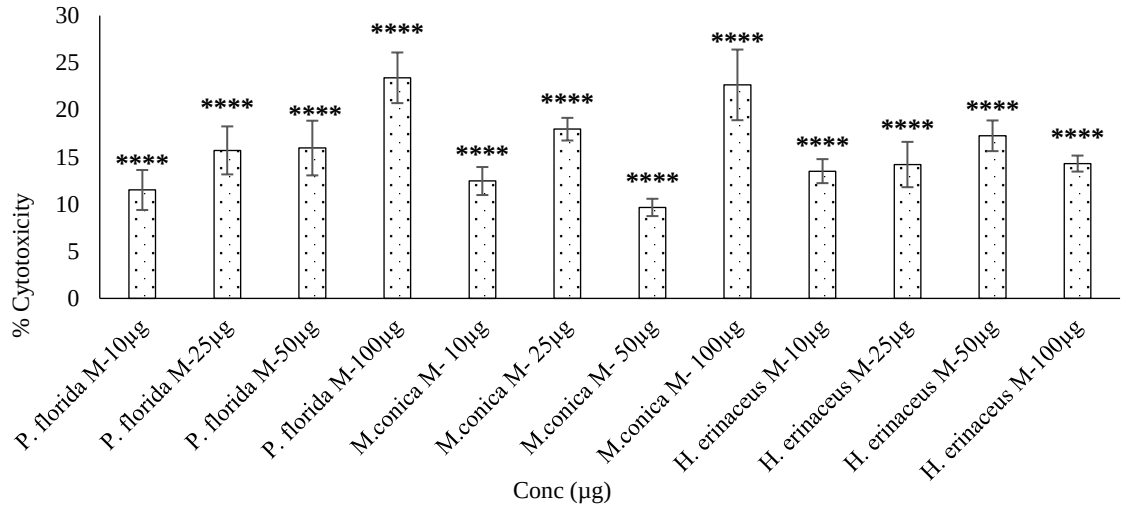

Figure 9. Trypan blue exclusion assay showing results of DLA cells treated with ethanolic and ethyl acetate extracts.Values are expressed as MEAN± SD, n=3, P<0.0001 significant compared with untreated cells.HEC: Culture filtrate extract of *H. erinaceus* ; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

MTT Assay

Due to low cytotoxic property of*P. florida* extracts it was not considered for MTT assay. The result showed that the ethyl acetate extract of culture broths of both *M. conica* and *H. erinaceus* exhibited similar cytotoxic activity of 54.86% at200 µg/ml concentration in HepG2 liver cancer cell lines. Aqueous ethanolic extract of mycelia of *M. conica* showed the highest cytotoxic activity of 35.58% at a concentration of 200 µg/ml whereas, the extracts of *H. erinaceus* exhibited an activity of 31.29% at the same concentration. MTT assay was carried out using 3T3 cells to ascertain whether these extracts cause any harmful effect to the normal cell lines. The results showed that ethyl acetate extract and ethanolic extract of *M. conica* and *H. erinaceus* had relatively low cytotoxic effect on 3T3 cells. In 3T3 fibroblast cell lines the ethanolic extract of mycelia of*H. erinaceus*had a cytotoxic activity of 10.4% at a concentration of 200µg and *M. conica* at the same concentration showed an activity of 6.74% whereas, ethyl acetate of *H. erinaceus* and *M.* conica at a concentration of 200 µg/ml exhibited cytotoxic activity of 23.6% and 6.74% respectively. It was found that the ethyl acetate extract of culture broth and ethanolic extract of mycelia of *H. erinaceus* had cytotoxic activity only at higher concentrations. Therefore, the cytotoxic effect of ethyl acetate extract and ethanolic extract of mycelia and culture broth on normal cells was far less compared to cancer cells (HepG2). The results are shown in Figures 10&11**.**

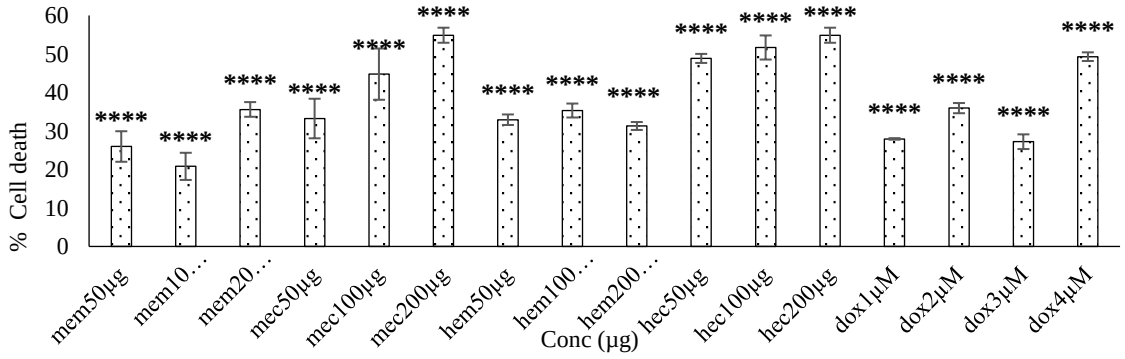

Figure 10. MTT assay showing results of HepG2 cells treated with ethanolic and ethyl acetate extracts and doxorubicin (standard). Values are expressed as MEAN± SD, n=3, P<0.0001 significant compared with untreated cells.HEC: Culture filtrate extract of *H. erinaceus* ; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica;* dox: Doxorubicin (standard drug).

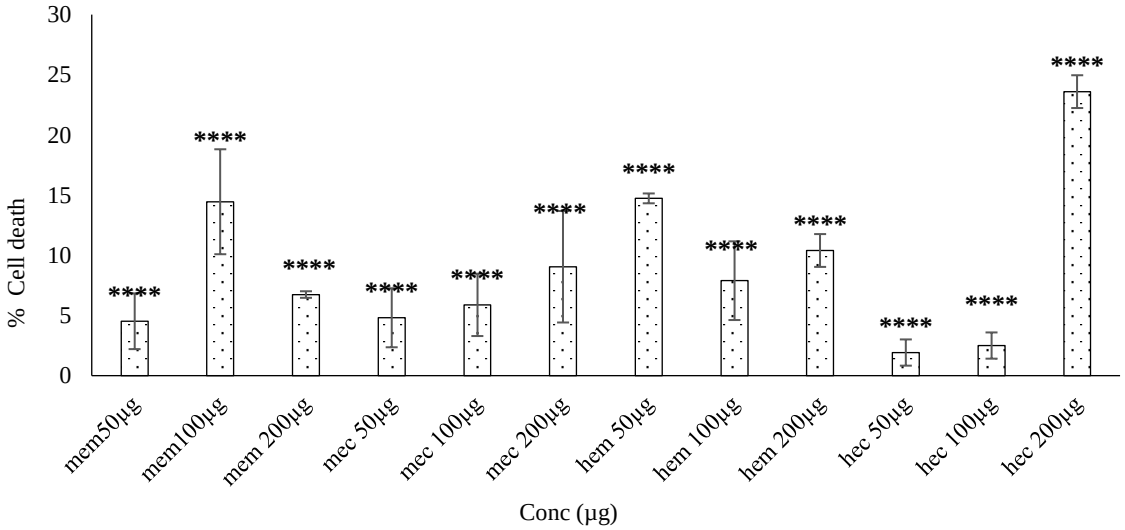

Figure 11. MTT assay showing results of 3T3 cells treated with ethanolic and Ethylacetate extracts and doxorubicin(standard). Values are expressed as MEAN± SD, n=3, P<0.0001 significant compared with untreated cells. HEC: Culture filtrate extract of *H. erinaceus* ; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica;* dox: Doxorubicin (Standard).

Effect of Mycelia and Culture Broth Extracts on Cell Morphology: Observation Under Phase Contrast Microscope

The HepG2 cells when treated with different concentrations 200µg of ethanolic extract of mycelia and ethyl acetate extract of culture filtrate showed some morphological changes as they lost the cell adherence property and most of the cells appeared in circular form whereas, the untreated cells retained with original morphology. The untreated cells appeared in epithelial-like morphology and in a polygonal shape (Figure 12 a–e).

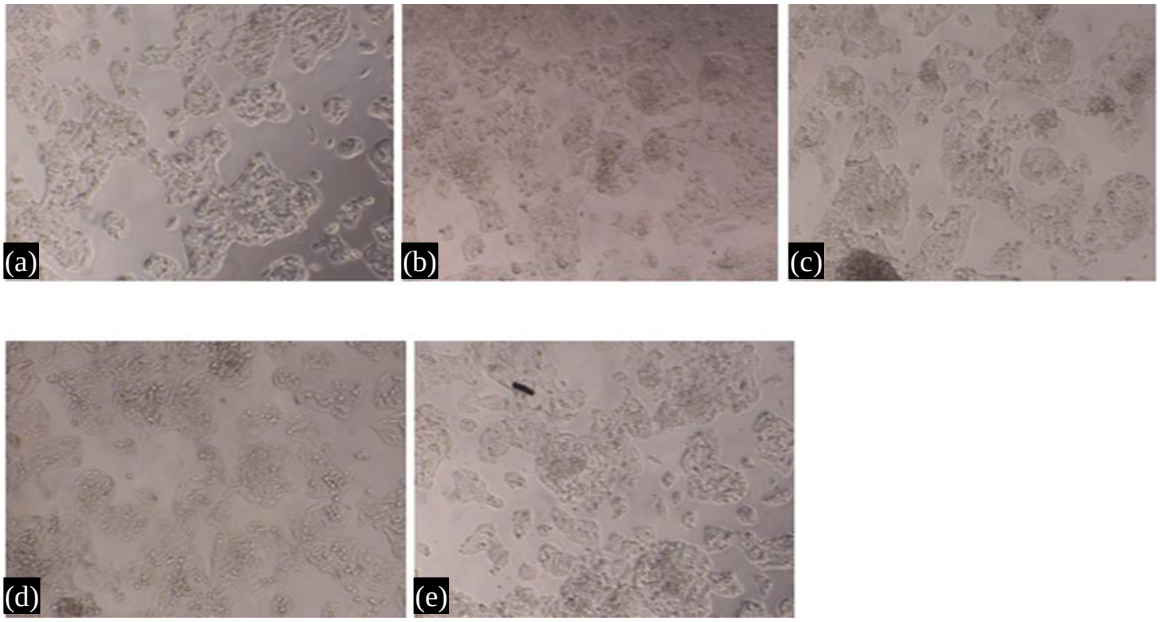

Figure 12. (a-e) This figure represents the phase contrast microscopic images of HepG2 liver cancer cell lines after drug treatment. A: Untreated cells; B: HEC 200µg; C: HEM 200µg; MEC 200µg; MEM 200µg. HEC: Culture filtrate extract of *H. erinaceus*; HEM: Mycelial extract of *H. erinaceus;* MEC: Culture filtrate extract of *M. conica;* MEM: Mycelial extract of *M. conica.*

DISCUSSION

Mushrooms are known for their culinary and therapeutic properties [9] They are loaded with bioactive compounds because of this they are widely used to treat various diseases from ancient days [23] The mycelia biomass and the culture broth of three edible mushrooms were investigated for their bioactivities. *P. florida, H. erinaceus,* and *M. conica*are edible mushrooms with significant medicinal properties. Ethanol extract of mycelia biomass grown in submerged culture and the ethyl acetate extract of the culture broths were evaluated for their antioxidant, cytotoxic and antiproliferative activities. The ethyl acetate extract of culture broth of *H. erinaceus* showed potent DPPH and superoxide radicals scavenging activity. The ethanolic extract of mycelia biomass of *H. erinaceus* showed significantly high activity to reduce ABTS radical. This indicated that antioxidant activity of mycelia and culture broth varied markedly. The ethyl acetate extract of *H. erinaceus* also showed lipid peroxidation inhibiting activity. Ethyl acetate extracts of *M. conica* had the highest power to reduce ferric ions. In this study, the extracts of *P. florida* showed low activity of DPPH radical scavenging effect and ferric reducing power (FRAP). *P.florida* also showed poor cytotoxic activity as evident from trypan blue assay compared to *H. erinaceus* and *M. conica*.

Phytochemical analysis of the extracts reveal that mycelia extracts and culture broth extracts of all the three mushrooms contain a number of bioactive compounds such as polyphenols, steroids, and flavonoids. However bioactive contents in mycelia and culture broth extracts of mushrooms varied to large extent. This may probably be due to the quantitative variation in the bioactive chemical contents.

Cytotoxicity was determined by two methods. The short-term cytotoxicity was determined by trypan blue exclusion assay using DLA cells. This helps to distinguish viable and non-viable cells. The ethyl acetate extract of culture broth of *H. Erinaceus* possessed the highest cytotoxicactivity when assayed with DLA cells. Second cytotoxic assay was done by MTT using HepG2 cells. In this assay ethyl acetate extract of culture broth of *M. conica* possessed the highest cytotoxic effect. In order to find out the toxicity in normal cells MTT assay was done in 3T3 fibroblasts cell lines and was found to be non-cytotoxic. Ethyl acetate extract of culture broth and ethanolic extract of mycelia of mushrooms showed relatively low cytotoxic activity on 3T3 cells indicating they are comparatively safe.

Cell morphology was analyzed by Phase contrast microscope and it was found that the cell of the untreated group retained the original morphology of HepG2 cells whereas, the treated group lost the property of cell adhesion, and changes in the morphology of the cells were observed. This indicate the potent effects of the mycelia and culture broth extracts on cancer cells

The present study was undertaken to examine the antioxidant and cytotoxicity activities of extracts of mycelia biomass and culture broth of three edible mushrooms, *Morchella conica, Hericium erinaceus* and *Pleurotus florida.* These mushrooms are used in traditional medicine for treating several ailments including cancer. The results of current investigation indicate that extracts of mycelia and culture broth of these mushrooms have profound cytotoxic and antiproliferative activities against cancer cell lines. This indicates the potential use of the extracts of mycelia and culture broth of these mushrooms as natural supplements for treating cancer patients. However, further studies and evaluations are required.

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