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Potential anti-cancerous activities of *Auricularia polytricha* Mont. (Sacc) – A black ear mushroom

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Abstract

The medicinal mushroom, *Auricularia polytricha* is commonly known as “black ear mushroom” and is popularly grown in China and is widely distributed from temperate to tropical regions of the world. The mushroom is known to have highly nutritious as well as pharmaceutical properties in it. Keeping view of this an experiment was conducted to exploit the anti-cancerous activities of the mushroom. The concentrations ranging from 6.25, 12.5, 25, 50 and 100 µg/ml showed decreased per cent viability of cervical cancer cells (79.34, 73.69, 66.95, 61.10 and 53.75 % respectively), colon cancer cells (87.24, 73.37, 59.0, 46.86 and 30.74%) and liver cancer cells (98.27, 92.77, 81.79, 74.50 and 53.59 percentage).

Keywords: *Auricularia polytricha*, anticancerous activities, cervical cancer cells, colon cancer cells and liver cancer cells

Introduction

Mushrooms are higher fungi with distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. They do not contain chlorophyll and are therefore eukaryotic heterotrophs which obtain food from decaying organic matter. Millions of people in many developing countries depend on wild resources to meet their food needs especially in periods of food crisis. As in all developing countries, the rapid growth in population is a great threat to natural resources. In addition, poor economy, unemployment and unfavourable climatic changes contribute to the high prevalence of extinction of natural habitat of the wild species of the edible higher fungi. Among the wild and cultivated mushroom species, *Auricularia* commonly known as ear mushroom or wood ear or Jew's ear mushroom has significant properties and is found worldwide. The fruiting body is distinguished by its noticeably earlike shape and brown colouration and it grows upon dead or rotten stumps of many trees *viz.* Mango, Coconut, Drumstick, Teak wood, *Casuarina spp.* The fungus can be found throughout the year in temperate as well as in tropical regions of the world, where it grows upon both dead and living wood.

Auricularia has been used as a medicinal mushroom by many herbalists. It was used to treat inflammations of the eye, as well as for throat problems. Preparation of a liquid extract by boiling the fruit bodies in milk, or else leaving them steeped in beer, which would then be sipped slowly in order to cure a sore throat. An attempt was made to cure jaundice when it was boiled in milk. Today, it is also used in Chinese medicine and in Ghana, as a blood tonic. Modern research into possible medical applications have variously concluded that the polysaccharides from these mushrooms may constitute a new source of compounds with action on coagulation, platelet aggregation and, perhaps, on thrombosis with possible uses regarding hypercholesterolemia, antitumor and hypoglycemic properties.

Materials and methods***In vitro* Antiproliferative Effect Determination by MTT Assay**

HeLa (cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained on Dulbecos modified Eagles medium (Gibco, Invitrogen). The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100 U/mL), Streptomycin (100 µg/mL), and Amphotericin B (2.5 µg/mL). Cultured cell lines were kept at 37 °C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope followed by MTT assay.

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Cells Seeding in 96 Well Plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10 % growth medium, 100 μ l cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37 $^{\circ}$ C in a humidified 5% CO₂ incubator.

Preparation of Extracts and Compound Stock

1 mg of ethanol mushroom extract was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 μ m millipore syringe filter to ensure the sterility.

Antiproliferative Evaluation

After 24 hours the growth medium was removed, freshly prepared samples in 5 % DMEM were five times serially diluted by two fold dilution (100 μ g, 50 μ g, 25 μ g, 12.5 μ g, 6.25 μ g in 100 μ L of 5 % MEM) and each concentration of 100 μ l were added in triplicates to the respective wells and incubated at 37 $^{\circ}$ C in a humidified 5 % CO₂ incubator.

Antiproliferative Assay by Direct Microscopic Observation

Entire plate was observed at an interval of each 24 hours up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Antiproliferative Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30 μ l of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37 $^{\circ}$ C in a humidified 5 % CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100 μ l of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm (Dinesh *et al.*, 2016)^[2].

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

Similar procedure was followed for cervical as well as liver

cancer cells.

Results and Discussion

Mushrooms can also solve most of the world's health problems because they are endowed with bioactive compounds that are of medicinal value (Chang and Miles, 2004)^[1]. Due to their good nutritious and medicinal values, mushrooms are considered ideal for vulnerable groups in the society such as children, nursing mothers, the old and the sick especially those suffering from diabetes, heart diseases, cancer and HIV/AIDS.

The results of study from anti-cancerous activities of *A. polytricha* (A1) revealed that the activity was dosage dependent and as the concentration of the sample increased, the percentage viability of cancer cells decreased. The concentrations ranging from 6.25, 12.5, 25, 50 and 100 μ g/ml showed decreased per cent viability of cervical cancer cells (79.34, 73.69, 66.95, 61.10 and 53.75 % respectively) (Plate a), colon cancer cells (87.24, 73.37, 59.0, 46.86 and 30.74%) (Plate b) and liver cancer cells (98.27, 92.77, 81.79, 74.50 and 53.59 percentage) (Plate c). Control sample showed 100 percentage cell viability in all the cancer cells tested (Table 1). The detectable changes observed in the morphology of the cells such as rounding and shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

The present study on the anti-cancerous activities of *A. polytricha* (A1) concluded that the fruiting bodies showed anti-cancerous activities against different types of cancer cell lines viz., cervical, colon and liver cancer, and the activity was found to be dosage dependent. As the concentration of sample increased, the percentage viability of cancer cells was found to be decreasing. The detectable changes in the morphology of the cells like rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were observed as indicators of cytotoxicity.

Very similar observations were made by Yu *et al.* (2014)^[5] who performed the MTT assay to measure the growth inhibitory effect of *A. polytricha* polysaccharides on human lung cancer (A549 cells) and the results showed induced cytotoxicity in A549 cells in a dose-dependent manner because when the drug concentration increased over 25 g/mL, cell viability was markedly suppressed to less than 42.25% of the control after the 48 h treatment.

Wasser and Weis (1999)^[4] reported that *Auricularia* produced many different polysaccharides, which were found to stimulate the immune system in humans or in some cases caused the production of interferon and interleukins that stopped the proliferation of cancer cells. They also reported that polysaccharides from *Auricularia* were found to have cardiovascular, hypocholesterolemic, antiviral, antibacterial and antiparasitic effects. Similar observations were also made by Stamets (2000)^[3]. However, the present study is very preliminary and requires further, elaborate research.

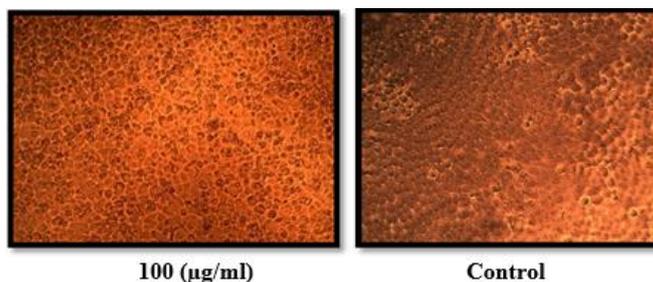


Plate a: Microscopic observation of cervical cancer cells (Inverted phase contrast tissue culture microscope) 40 X

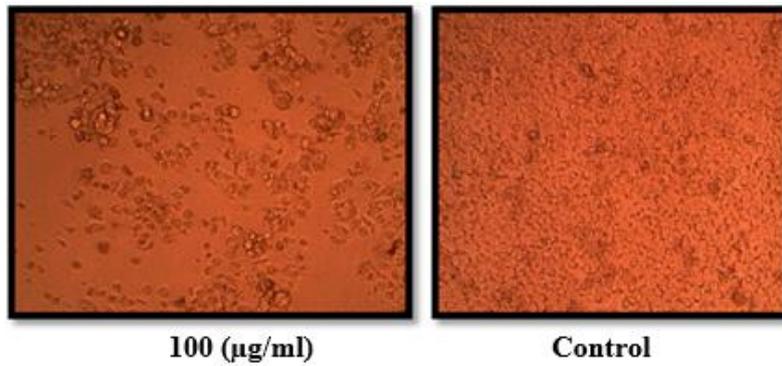


Plate b: Microscopic observation of colon cancer cells (Inverted phase contrast tissue culture microscope)

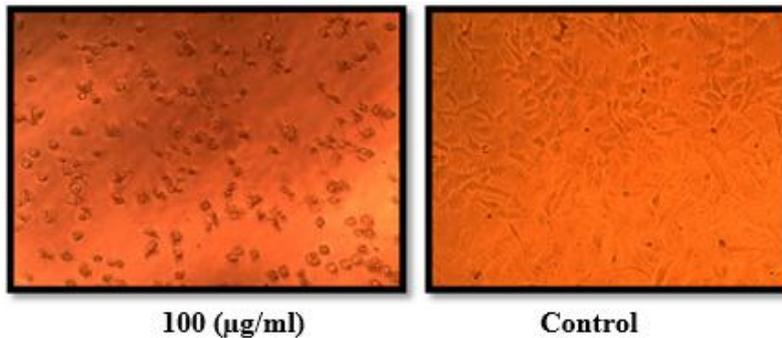


Plate c: Microscopic observation of colon cancer cells (Inverted phase contrast tissue culture microscope)

Table 1: *In vitro* antiproliferative effect of *A. polytricha* (A1) by MTT* assay (cervical, colon and liver cancer)

Sample Concentration (µg/ml)	Average OD at 540nm			Percentage Viability		
	Cervical cancer	Colon cancer	Liver cancer	Cervical cancer	Colon cancer	Liver cancer
6.25	0.72	1.90	1.78	79.34	87.24	98.27
12.5	0.67	1.60	1.68	73.69	73.37	92.77
25	0.61	1.29	1.48	66.95	59.0	81.79
50	0.55	1.02	1.35	61.10	46.86	74.50
100	0.48	0.67	0.97	53.75	30.74	53.59
Control	0.91	2.18	1.82	100	100	100

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

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