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Antiproliferative Effect of Ethanolic Medicinal Fungus Extract of *Agaricus bisporus* on HEP2 Cancer cell lines

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ABSTRACT

This study aims to determine the anti-proliferative effect of fungus *Agaricus bisporus*. Phytochemical analysis was performed and antiproliferative activity of *Agaricus bisporus* was analyzed by MTT assay The preliminary phytochemical screening of *Agaricus bisporus* revealed the presence of phenolics, carbohydrates alkaloids, flavonoids and tannins. The Antiproliferative potential of the ethanol extract was studied on Hep2 cell lines by MTT assay. The extract had an IC_{50} value of 100µg/mL which showed cell viability. Thus, the study revealed that *Agaricus bisporus* could be considered as a significant source of phytochemicals and can act as antiproliferative agent.

Keywords: Phytochemical, antiproliferative, *Agaricus bisporus*, DPPH assay, Hep2 cell line.

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1. INTRODUCTION

Mushrooms as a low-calorie high protein item with negligible starch and sugars, very high potassium and sodium ratio, low calorie and fat, make mushroom the choice of the dietician for those suffering from obesity, hypertension and atherosclerosis [1,2] Mushrooms are known to lower blood cholesterol level which possesses pronounced antiatherogenic properties. Nowadays predominant therapeutic methods such as chemotherapy and surgery are commonly used for cancer patients, but these methods have not been fully effective on many cancers [3,4] . Alternative and complementary medicine is now very popular for many disorders and is used by patients who are undergoing or have completed medical treatment for various types of cancer, and a combination of multi-therapeutic steps may effectively improve the treatment of cancer [5-7]. Some edible medicinal mushrooms have been tested against several cancers such as breast cancer, prostate cancer, liver cancer, colon cancer, lung cancer and gastric cancer. Some research has focused on the antitumor activity [8-10]. Despite all these researches with different mushroom species, there is no systemic study in the literature regarding the cytotoxic activity of *Agaricus Bisporus. Agaricus bisporus* is the most important cultivated mushroom in the world.[11] However, studies into the anticancer properties of *A. bisporus* are obviously limited, as compared to other important cultivated mushrooms. However, recent developments in the research into anticancer properties of *A. bisporus* have not been reviewed [12]. Hence, the present study is focused to evaluate the anticancer potential of *A. bisporus*

2. MATERIALS AND METHODS

2.1. Collection of fresh button Mushroom:

Fresh button mushroom *Agaricus bisporus* were purchased from local Mushroom growers. This kind of mushroom is commonly available all over Tamil Nadu and hill stations. The botanical identification and authentication was done by Dr. N.K Udhay Prakash, Mycologist and Associate Professor, Vels University, Pallavaram, Chennai. They were cut into small pieces and shade dried for few days and powdered in a stone made mortar and pestle. The finely powdered mushroom materials were packed in a soxhlet apparatus and extracted with ethyl alcohol and kept cold steeping for 24 hrs. The solvent was evaporated with the help of rotary evaporator. The dried extract was weighed and used to prepare the required volume.

2.2. Phytochemical screening: The extracts were subjected to preliminary phytochemical screening to identify the

presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids according to [13].

2.2.1. Estimation of total free phenolics: Total phenolic constituents of mushroom extracts were estimated by Folin Ciocalteau's method using Folin-Ciocalteu reagent. The estimation was done spectrometrically at 760 nm and the results were expressed as gallic acid equivalents (GAE) [14].

2.2.2. Estimation of total flavonoids: Aluminium chloride method was employed to quantify the total flavonoid content in the mushroom extracts. The results were expressed as quercetin equivalents (QE) [15]

2.2.3. Estimation of total alkaloids

Total alkaloid content of the mushroom extracts was determined according to [16]. Five gram of the sample was filtered and concentrated to one quarter of the original volume on a water bath after treatment with 200 mL of 10% acetic acid in ethanol. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide, filtered and weighed.

2.2.4. Estimation of total saponins

Powdered sample (20 g) was treated with 100 mL of 20% aqueous ethanol, heated over a hot water bath for 4 h at about 55°C with continuous stirring. The mixture was filtered and the residue re extracted. The combined extracts were reduced to 40 mL over water bath at about 90°C and the concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added to the combined extracts and washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was heated in a water bath, dried in an oven to a constant weight and the saponin content was calculated as percentage.

2.3. Thin layer chromatography

Preliminary identification of phytochemicals was made by thin layer chromatography (TLC) using silica gel plates (5gm of silica gel dissolved with 90ml of water). The extracts were eluted with chloroform: methanol: water (30:20:4) and the chromatogram was developed by spraying with solution (methanol : sulphuric acid (1:1)) and heating to 110ºC. Then Rf value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent [17].

2.4. Anticancer activity

The antiproliferative potential of the ethanol extract was studied on Hep2 cell lines by MTT assay. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay has been described as rapid, simple and reproducible method, widely used in the screening anticancer drugs and to measure the tumour cell proliferation [18-19].

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical analysis

The preliminary phytochemical screening of *Agaricus bisporus* revealed the presence of phenolics, carbohydrates, alkaloids, flavonoids, saponins, tannins and steroids in high amounts followed by glycosides, amino acids and proteins. (Table 1)

Table 1: Phytochemical Analysis.

Compound	Result
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Steroids	+
Flavonoids	+
Tannins	+

(+) Presence of phytochemicals

Table2: Quantitative phytochemical analysis: The major phytochemicals present in the selected Mushroom extracts were phenols, flavonoids, alkaloids and tannins were quantified. The results of total phenol content, alkaloids, saponins and flavonoids are given in (Table 2) [20]

Bioactive compound	Quantity
Total phenols	246.65 µg GAE/g sample
Alkaloids	1.23 mg/g sample
Saponins	0.01 mg/g sample
Tannins	1.23mg/g dry weight
Flavonoids	54.3µg Quercetin equivalent/g

3.1.2. Thin layer chromatography

The chromatogram developed with 10% ethanol in chloroform revealed the presence of five major compounds at Rf value of 0.23; 0.38; 0.46; 0.76; 0.86 as visualized under iodine vapour and UV illumination.

3.1.3. Anticancer activity

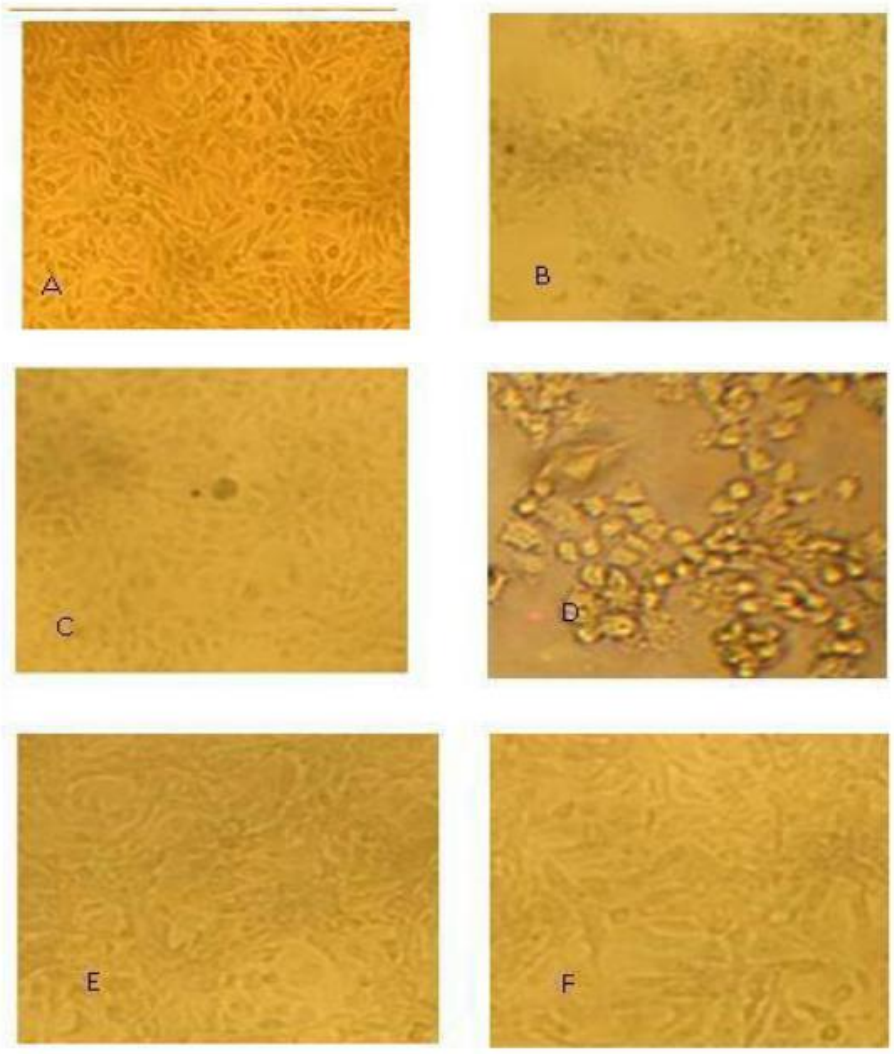
(Table: 3) MTT reduction on Hep2 cell line

Since, IC50 value for Hep2 (liver) cell line (100 µg/ml) ethanol mushroom extract was found to be effective, the reduction percentage of MTT at 48Hrs also estimated for Hep2 (liver) cells. When incubated with the extract, it induced cytotoxicity in a significant manner which implicit the damage to the member integrity of the cell when contributed with control. The cytotoxicity was minimized in the extract treated cells and near normal level was attained at various concentrations (1, 10,100 ng and 1, 10, 100µg/ ml) and maximum effect was found when treated at 100 µg/ml. From the above results, it was confirmed that ethanol *Agaricus bisporus* extract at 100 µg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies. Tryphan blue is one of the several stains recommended for use in dye exclusion procedure for viable cell counting.

Table: 2 **% MTT reduction on Hep2 cell line**

S. No	Concentration (ng)	Test	%
1	Control	0.67 ± 0.01^{c}	4.12
2	1ng	0.56 ± 0.01^{b}	23.5
3	10ng	0.53 ± 0.00^{b}	25.0
4	100ng	0.45 ± 0.02^{a}	36.6

S. No	Concentration	(µg)	%
1	1µg	$0.32\pm0.0.3^{c}$	53.6
2	10 µg	0.23 ± 0.00^{b}	69.4
3	100 µg	0.21 ± 0.01^{a}	72.2
Values are mean of three replicates. Means followed by same letters are statistically not significant at $\alpha = 0.05$ by Duncan's multiple range test.			



(Fig.:2) A: Control cells (Untreated), B: Ethanol extract 1ng/ml, C: Ethanol extract 10 ng/ml, D: Ethanol extract 100 ng/ml, E: Ethanol extract 10 µg/ml, F: Cyclophasphamide (Positive control) 100 µg/ml.

The cytotoxicity was minimized in the extract treated cells and near normal level was attained at various concentrations (1, 10,100ng and 1,10,100µg/ ml) and maximum effect was found when treated at 100 µg/ml. There has been a 22% increase in cancer incidence and mortality, with over 10 million new cases and 6 million deaths world-wide in the year 2000. Cases could further increase by 50% in the year 2020. The use of medicinal plant and mushroom extracts for cancer therapy is rapidly evolving as they are affordable, with limit or no side effects. [21] The active components present in such extracts have been shown to efficiently inhibit the processing of multistage carcinogenesis in a synergistic manner. The identification and characterization of components with potential anticancer activity derived from herbal formulation or mushroom extracts have been gaining attention [23-25] Earlier reports revealed that the antioxidant activity prevents the development of cancers. So in this context, we have examined the antiproliferative activity of *A.bisporus* extract using cancer cell line. From the above results, it was confirmed that ethanol *Agaricus bisporus* extract at 100 µg/ml seems to offer significant protection and maintain the structural integrity of the hepatocellular membrane and this active concentration was followed for further studies. The present investigation suggests that *Agaricus bisporus* possesses significant antiproliferative potential. Hence, we can conclude that with further mechanistic studies, the mushroom can be considered as an efficient source of anti-proliferative agents.

4. CONCLUSION

It was concluded that the *Agaricus bisporus* mushroom extract has a potential antiproliferative effect. Efforts should be made to find out the exact compound and its active mechanism which is responsible for the cytotoxicity and need further investigations.

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