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GANODERMA APPLANATUM (PERSOON) PATOUILLARD: AS A SOURCE OF ANTICANCER AND ANTIOXIDANT AGENT.

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ABSTRACT

The aim of the present study was to evaluate antioxidant and antitumor activities of *Ganoderma applanatum.* The methanolic extract of basidiocarps and mycelium of *Ganoderma applanatum* was analyzed. Antioxidant activity of methanolic extract of basidiocarp and mycelium was characterized using 1, 1,-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay. The EC_{50} of basidiocarp and mycelial mat of *G.applanatum* was found to be 0.67 mg/ml and 1.50 mg/ml respectively. Anticancer activity of methanolic extracts of mushrooms was determined through MTT (Tetrazolium) assay against three different cell lines, all the extract showed activity on the cell lines. This study reveals that not only basidiocarps of mushroom also vegetative mycelium from the pure culture of *G.applanatum* has potential anticancer and antioxidant activity, hence this mushroom can be exploited for its useful metabolites in food and pharmaceutical industries.

KEY WORDS: ***Ganoderma applanatum,*** **DPPH, MTT assay,**

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INTRODUCTION

The natural products and herbal medicine industry has become increasingly popular over the past three decades [1]. Many clinically useful drugs have been obtained through the screening of natural products [2]. Plants have been a major focus of investigations for novel biologically active compounds from natural resources and in recent years pharmaceutical companies have spent a lot of time developing these natural products to produce more affordable and cost effective remedies [3]. There have been a number of reviews published on the bioactive substances found in mushrooms, and their medicinal properties [4,5,6,7,8,9,10,11,12,13,14]. *Ganoderma applanatum*, a basidiomycetous fungus has been used in folk medicine for treatment of cancer. [15, 16] have also reported the antitumor activity of *G.applanatum*. [17] have reported antimicrobial activity of this mushroom. Review of literature revealed that very little work has been carried out in this regard; hence the antioxidant and anticancer activities have been determined.

MATERIALS AND METHODS

Collection of Mushrooms and identification

Basidiocarps collected from various localities in Bangalore, in rainy season the mushroom was identified using keys provided by [18] and also using molecular technique by analyzing Internal Transcribed Spacer sequence of rDNA from pure culture obtained from Basidiocarps. The basidiocarps was used to isolate the pure culture and also dried and preserved for further research.

Isolation of Pure culture of Mushrooms

The pure cultures of the mushroom was obtained by placing a portion of context of the basidiocarp aseptically on sterilized Potato Dextrose Agar in a petriplate with the help of a sterile forceps and incubated at 25±1ºC for 6-7 days for initial mycelial growth. Pure cultures obtained were maintained in PDA slants and were used for further studies.

Preparation of alcoholic extract from basidiocarps and pure cultures

In the present study, the dried basidiocarps and mycelium of *G. applanatum* were used. Mushroom material was made into fine powder. 10 grams of mushroom powder was subjected to extraction using 100 ml of methanol at room temperature for 24 hrs and filtered through Whatman No. 4 filter paper. The extracts were recovered by filtration and kept at 40ºC in a rotary vacuum evaporator [19]. The residue was collected and stored at 4 ºC for further use.

Antioxidant activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay:

The antioxidant activities of the methanol extract were measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical [20, 21]. Various concentrations of 1 ml of the test extract were added to 4 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 minutes of incubation period at room temperature, the absorbance was measured against a blank at 517 nm in spectrophotometer. Inhibition of free radical DPPH in percent (%) was calculated:

I % = (A blank – A sample / A blank) x 100

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test sample. IC_{50} was calculated from the graph plotted inhibition percentage against extract concentration.

Anticancer activity

Three different cell lines Human Cervix (HeLa), Human Liver (Hep G2) and Human Mammary gland; Epitelial; Ascites; Ductal carcinoma (ZR-75-30) procured from National Centre for Cell Sciences Pune, India were used in this study. HeLa and Hep G2 were grown in DMEM and ZR-75-30 in RPMI-1640 medium with 5% fetal bovine serum in 5% CO_2 atmosphere at 37 ºC. The cells were then transferred into microplate at the concentration of 10^2 cells/ml/well for cytotoxicity test of the mushroom extract. After 48hrs, proliferation was measured by

colorimetric MTT assay. The half maximal inhibitory concentration (IC_{50}) was calculated.

Colorimetric MTT assay

Colorimetric MTT (3-(4, 5-dimethythiazol-2yl)-2-5-diphenyl tetrazolium bromide) assay was carried out as described by [22]. Ten microliters of MTT solution (5 mg/ml) was added to wells of 96 wells microplate followed by 4 hours incubation at 37^0 C. Acid isopropanol was added to all wells to dissolve the dark blue crystals. The microplate was then read with an ELISA reader at wavelength 570 nm within 1 hour.

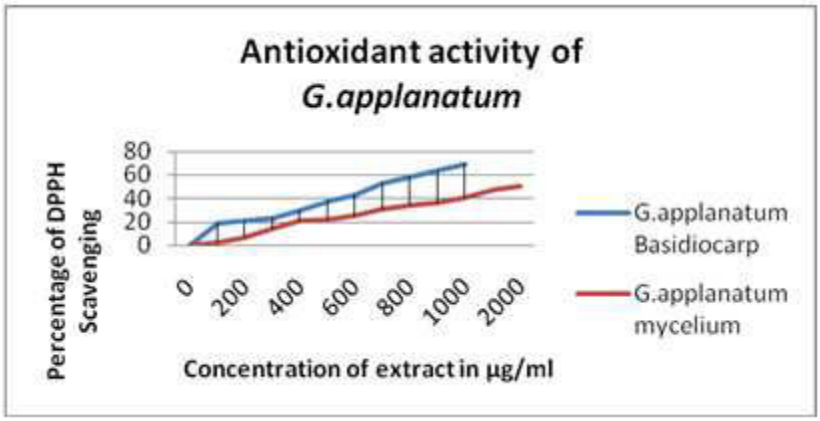
RESULTS AND DISCUSSIONS

Antioxidant Activity of G.applanatum

The methanolic extract was subjected for screening for antioxidant activity by conducting DPPH assay. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As the antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measure by DPPH assay are shown in the Graph 01. All the concentrations of the extract showed free radical scavenging activity as the concentration of the extract was increased the scavenging activity was also increased. The EC-50 (concentration of the extract required to scavenge 50% of the DPPH free radical) of the methanolic extract of Basidiocarp and vegetative mycelium of *G.applanatum* was found to be 670 µg/ml and 1500 µg/ml respectively. Hence this study reveals that *G.applanatum* has antioxidant property. [23] showed that triterpenes extracted from *G. lucidum* have anti-oxidant properties *in vitro* and can reduce oxidative damage by directly scavenging free radicals generated in the cell. [24] reported that *Ramaria flava* exhibits antioxidant activity. [25] has reported antioxidant activity of *Agaricus bisporus*, [27, 26, 28] have reported that the extracts from fruiting bodies and mycelia of *G.lucidium* occurring in South India were found to possess antioxidant activity. [29] showed that dietetic treatment using a *Ganoderma* mycelium derived polysaccharide extract can be used to suppress the formation of colonic aberrant crypt foci in rats by reducing the oxidative damage induced by ROS.

Graph 1

Antioxidant activity of Ganoderma applanatum.



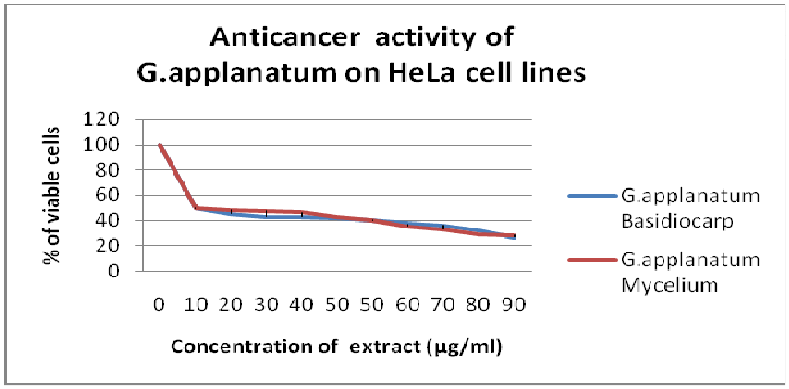
Anticancer activity of Ganoderma applanatum

The anticancer activity of methanolic extracts of *G.applanatum* was investigated using MTT assay on three human Cancer Cell lines HeLa, Hep-G2 and ZR-75-30. The cells were treated with increasing concentration of extract ranging from 0-1000 µg/ml. A mitochondrial enzyme in

living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of Formazan produced is directly proportional to the number of viable cells [22]. Graph 2, 3 and 4 shows the decrease in the percentage of the viable cells with the increase in the concentration of the extract. The viability of HeLa cells decreased to 75% and 80% when the concentration of the extract of *G.applanatum* Basidiocarp and vegetative mycelium was applied at 0.5 µg/ml. The half maximal inhibitory concentration (IC_{50}) was found to be 10±0.07 and 9.25±0.05 (Fig.05). Similarly the IC_{50} of the extract against ZH-75 was 14±0.04 and 11.9±0.08; IC_{50} of the extracts against Hep G2 was found be 16.2±0.02 and 13.9±0.06 respectively. Hence the present study implies that the potential use of *G.applanatum* extract in cancer treatment. [15, 16] have also reported the antitumor activity of *G.applanatum*. Triterpenoids from the fruiting bodies of *G. lucidum* and *G. applanatum*, and malonate half-esters from the fruiting body of *Ganoderma* sp., have shown biological activity as inhibitors against tumour promotion. [30, 31, 32] examined the cytotoxicity activity of lanostanoids from *G. tsugae in vitro* and found to exhibit significant activity against T-24, HT-3, and CaSKi cells (three cancer cell lines). Further to this, [33] isolated alanostanoid and sterol from the same fungus, which caused cell death by apoptosis and suggested that the sterol possessed the activity of cell cycle inhibition.

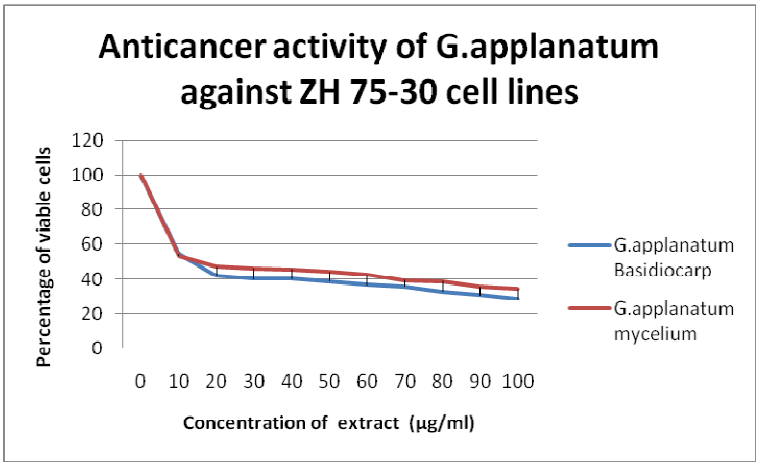
Graph 02

Anticancer activity of G.applanatum on Hela Cell line

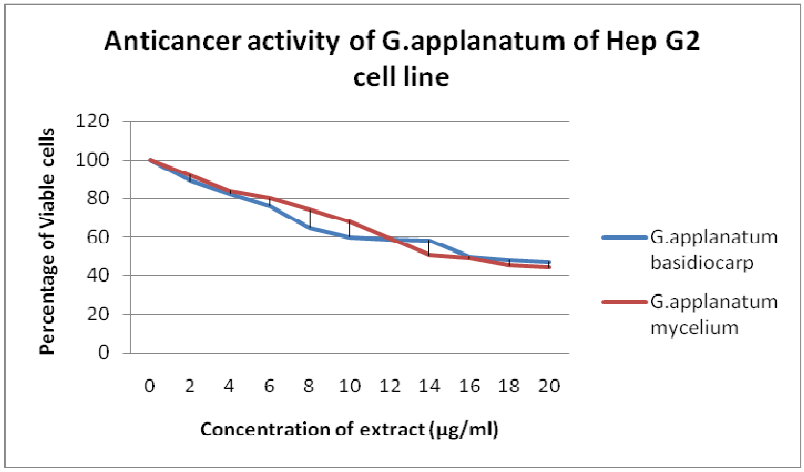


Graph 03

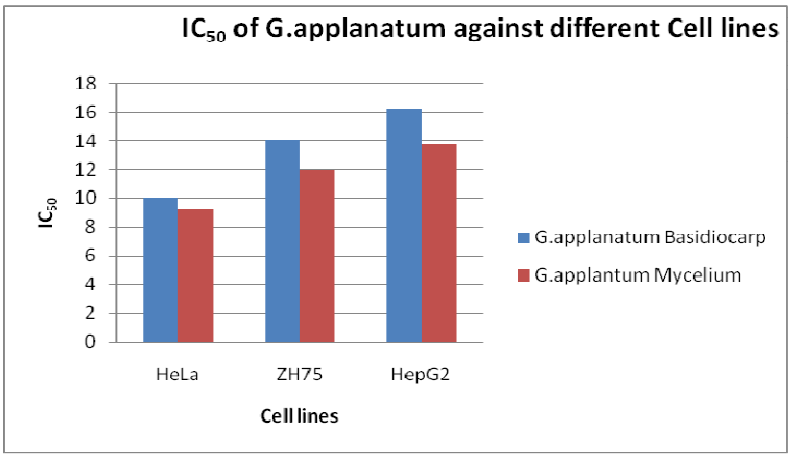
Anticancer activity of G.applanatum on ZH-75-30 Cell line



Graph 04
Anticancer activity of *G.applanatum* on Hep G2Cell line



Graph 05
IC_{50} of *G.applanatum* against different cell lines.



CONCLUSION

The present study has shown that the methanolic extract of *G. applanatum* has marked antitumor and antioxidant properties, which is important for the development of new therapeutic agents. Since the basidiocarps are seasonal, and cultivation *in vitro* condition is difficult, the vegetative mycelium could be cultured can also be used in extracting the useful metabolites. Hence further studies on isolation and identification of active compound may provide a better source for developing new drug. Hence these properties of *G.applanatum* can be exploited in food and pharmaceutical industries.

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