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Antioxidant and Anticancer Activity of *Tricholoma giganteum massee* an Edible Wild Mushroom

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Abstract: The purpose of this study was to elucidate the antioxidant capacities and anticancer activity of *Tricholoma giganteum* an edible wild mushroom. The alcoholic extract from mushroom was evaluated for antioxidant activity against DPPH radical and reducing power. Methanol extracts of basidiocarps and mycelial mat of *Tricholoma giganteum* showed 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity close to 60%. The EC_{50} of fruiting body and mycelium of *T. giganteum* showed 0.7mg/ml and 2.0mg/ml respectively. Anticancer activity of methanolic extracts of mushroom was determined through MTT (tetrazolium) assay against three different cell lines HeLa (Human; cervix), Hep G2 (Human; Liver) and ZR-75-30 (Human; Mammary gland). The IC_{50} of methanolic extract of Basidiocarps and vegetative mycelium against HeLa was found to be 9.8 µg/ml and 11.2 µg/ml, respectively. Similarly, IC_{50} against ZR-75-30 was found to be 11.0 µg/ml and 12.2 µg/ml and against Hep G2 IC_{50} was 11.4 µg/ml and 10.6 µg/ml, respectively. The findings of the present study indicated that medicinal values of *Tricholoma giganteum* methanolic extracts in terms of antioxidant and anticancer are promising.

Key words: *Tricholoma giganteum* · MTT Assay · DPPH Assay

INTRODUCTION

Mushrooms have influenced human affairs for many years, either as a direct food source or as a medicine. They are considered as rich source of food as they contain high amount of protein, sugar, glycogen, lipid, vitamins, amino acids and crude fibre [1, 2]. The medicinal values of mushrooms have been recognized all over the world [3] and several compounds have been isolated from the mushroom possessing antimicrobial activity [4-7]. Edible mushrooms are highly nutritious and could be used as medicines for treatment of cancer, heart ailments, diabetes, inflammation, hepatic damage, high blood pressure, etc [8-12]. Mushrooms were also among the best sources of other essential nutrients. Their nutritional value can be compared favourably with that of meat, eggs and milk [13, 14].

Researchers [15], included *T. titans* (as *T. cystidiosum*), *T. giganteum* and *T. praegrande* in section *Leucorigida*. *Tricholoma giganteum* is wild edible mushroom mostly seen in the tropical region during rainy season. They are robust in size and popular among the tribal people because of their gastronomic and nutritional delicacy. Literature reported [16] that *T. giganteum* resembled the morphology of *Calocybe indica* and was growing widely in summer in Indo-gangetic plains of Howrah district, Hooghly in India. *Tricholoma giganteum* showed antimicrobial against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa and Candida albicans* [17]. Investigators [18] also showed the mycelium of *Tricholoma giganteum* to have antimicrobial activity.

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The present work compares the radical scavenging capacity (RSC) and anticancer activity for methanolic extracts of both basidiocarps and mycelial of *Tricholoma giganteum*.

MATERIALS AND METHODS

The mushroom samples were collected following the standard technique during the rainy season in and around Bangalore in 2012-2013 and were identified using Singers [19] classification concept. The fresh basidiocarps samples were dehydrated in hot air oven at 40°C for a period of 4-5 days and the dried samples were stored in an air tight containers till further studies were carried out.

The pure culture was cultured on potato dextrose agar plates using the cortex region from the fresh basidiocarps of *T. giganteum*. The plates were incubated for 2-3 weeks at 28-30°C for its luxuriant growth. The mycelia obtained were then aseptically transferred into conical flask containing potato dextrose broth and were incubated for a month at 28-30°C. The mycelia obtained after the incubation were filtered using whatman filter paper No. 01 and dried in hot air oven at 40°C. The dried mycelial sample was stored in air tight container for further study.

Methanolic Extract: 10 grams of the dried and powdered basidiocarps and mycelial samples was extracted in 100 ml of methanol separately and incubated in the rotary shaker for 2-3 days. The methanolic extract was then filtered through Whatman's filter paper No. 1 and the filtrate was evaporated [20] 40°C in rotary evaporator. The residue was collected and stored at 4°C and used for further studies..

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

$$I\ \% = (A_{(blank)} - A_{(sample)} / A_{(blank)}) \times 100,$$

where $A_{(blank)}$ is the absorbance of the control reaction and $A_{(sample)}$ is the absorbance of the test sample. Extract concentration providing 50% inhibition, half maximal inhibitory concentration (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Anticancer Activity: Human; Cervix cell lines (HeLa), Human; Liver cell lines (Hep G2) and Human; Mammary gland; Epitelial; Ascites; Ductal carcinoma cell lines (ZR-75-30) were obtained from NCCL, Pune. HeLa and Hep G2 cell lines were cultured in DMEM medium and ZR-75-30 was cultured in RPMI 1640 medium. These media were supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, 1.5 g/L Na bicarbonate, 90% 1.0 mM Na pyruvate, Penicillin (100 mg/ml), Streptomycin (100 mg/ml) and Gentamycin (100 mg/ml). The cells were cultured in humidified atmosphere containing 5% CO_2 at 37°C for 24h. Cell count was determined. The MTT (tetrazolium) assay was carried out when the cell count reached the density of 2 x 10^5/ml. The cells were treated with different concentrations of the extract and the MTT assay was carried out.

MTT Assay: MTT (3-(4, 5-dimethythiazol-2-yl)- 2,5-diphenyl tetrazolium bromide) assay was carried out as described by Mosmann [22]. 10 il of MTT solution (5 mg/ml) was added to all wells of 96 wells microplate followed by 4 hrs incubation at 37°C. Acid isopropanol was added to all wells for dissolving the dark blue crystals. The microplate was then read on an ELISA reader at wavelength 570 nm within 1h after adding isopropanol.

RESULTS AND DISCUSSION

DPPH Assay: The methanolic extract of the wild mushroom was subjected to DPPH assay for their possible antioxidant activity. DPPH is a stable free radical, used to study the radical scavenging effect of the extract. Free radical scavenging capacities of the basidiocarps and mycelia extract, measured by DPPH assay are shown in Figure 1 and 2 respectively. All the concentration showed free radical scavenging activity. The 50% of inhibition for the basidiocarps methanolic extract of *Tricholoma giganteum* seem to be scavenging effect of DPPH at 750 µg/ml and seem to be fairly significant than the mycelia methanolic extract of *Tricholoma giganteum* which had the scavenging activity at 2000 µg/ml.

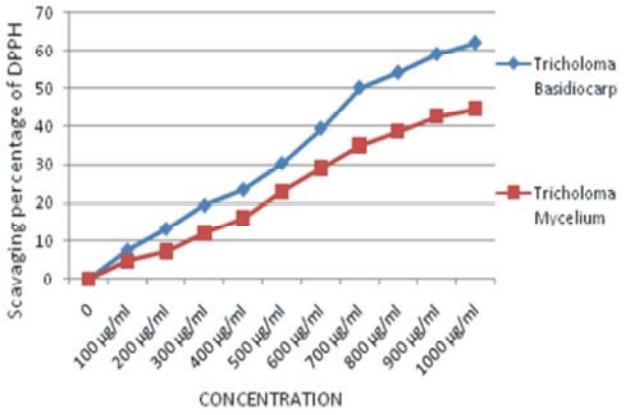

Fig. 1: Antioxidant activity- DPPH assay of *Tricholoma giganteum* basidiocarps and mycelia

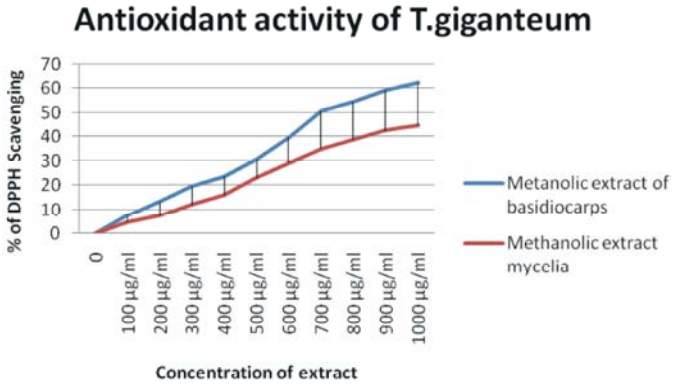

Fig. 2: Antioxidant activity of *Tricholoma giganteum*

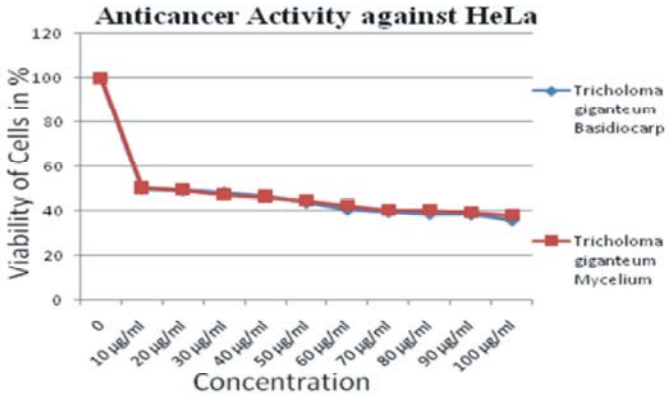

Fig. 3: Anticancer activity on HeLa by *T. giganteum* basidiocarps and mycelia

Investigators [23] similarly showed that the fractions Fa and Fc from basidiocarps of *T. giganteum* had antioxidant effect which was dose dependent and EC_{50} value was lower than 1 mg/ml. Antioxidant activity of *T. giganteum* was measured using Ferric reducing antioxidant power assay by Benzie and Strain [24]. Researchers [25] carried out the Pharmacognostic study for *T. giganteum* where in maximum yield was seen in methanol extract and lowest with acetone.

Anticancer (MTT) Assay: From the present study, anticancer activity of *Tricholoma giganteum* extract from basidiocarps and mycelia was anticipated from the cytotoxicity test against HeLa (Figure 3), HepG2 (Figure 4) and ZR-75-30 (Figure 5) cell lines. Viability of the cancer cell lines declined with the increase in the concentration of the extract. The half maximal inhibitory concentration (IC_{50}) of the basidiocarps and mycelia extract was determined for each of the cancer cell lines

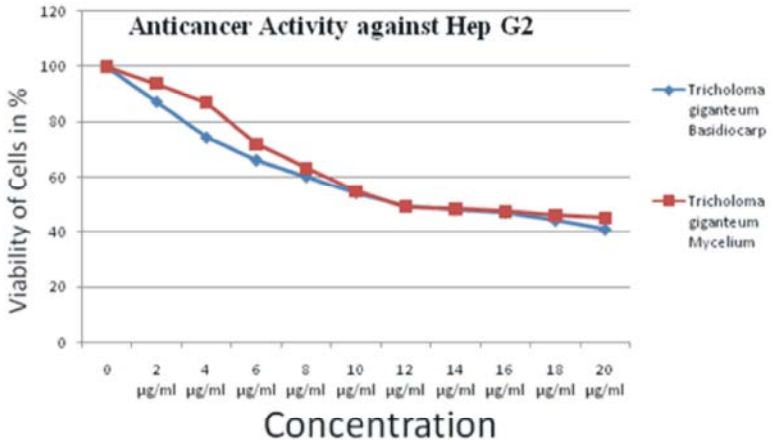

Fig. 4: Anticancer activity on HepG2 by *T. giganteum* basidiocarps and mycelia

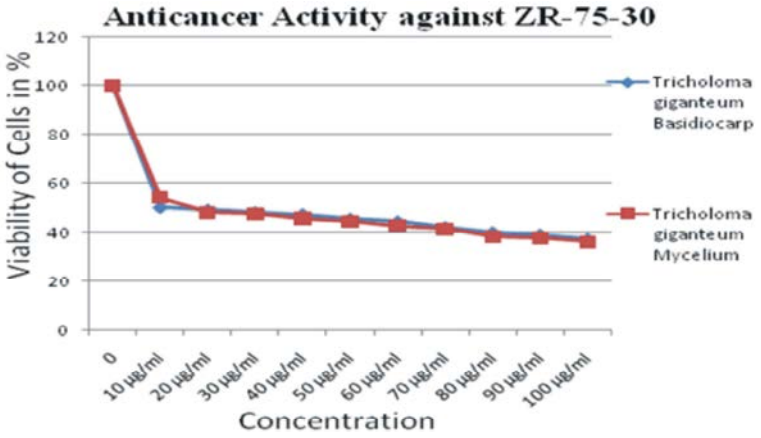

Fig. 5: Anticancer activity on ZR-75-30 by *T. giganteum* basidiocarps and mycelia

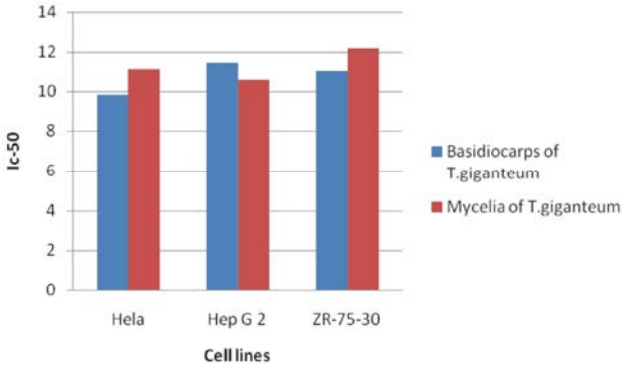

Fig. 6: IC_{50} value of *T. giganteum* basidiocarps and mycelium on cancer cell lines

(Figure 6) separately. The IC_{50} of methanolic extract of Basidiocarps and vegetative mycelium against HeLa was found to be 9.8 µg/ml and 11.2 µg/ml, respectively. Similarly, IC_{50} against Hep G2 IC_{50} was 11.4 µg/ml and 10.6 µg/ml and against ZR-75-30 it was found to be 11.0 µg/ml and 12.2 µg/ml respectively. The IC_{50} value of the cell lines are shown in Figure 6. Thus, the present investigation suggest the potential of *Tricholoma giganteum* extract in cancer treatment. Researchers [26, 27] showed that the fruiting body of *T. giganteum* has antitumor activity because of its protein-containing heteroglycan with a molecular weight of 68K. Similarly *T. lobayense* exhibits strong antitumor activity in both ICR and BALB/c mice as they possess polysaccharide-protein complex [28]. A novel polysaccharide-protein complex was extracted from cultured mycelia of

Tricholoma mongolicum [29, 30]. These complex proteins activate lymphocytes and macrophages from BALB/c mice and have proved to show no direct cytotoxic activity against fibroblasts, hepatoma cells and choriocarcinoma cells.

CONCLUSION

The present investigation proves that both basidiocarpal and mycelial methanolic extract of *T. giganteum* possess antioxidant property and antitumor activity against Human; Cervix, Liver and Ductal carcinoma cell lines. Which suggest that *T. giganteum* can be used for the development of new drugs. The elution and identification of the active compounds from *T. giganteum* can lead to a major break through in the pharmaceutical industry and can be an alternative for anticancer drug from a natural source.

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