

Evaluation of Anti-proliferative Potential and Antioxidant Activity of a Wild Edible Mushroom *Macrocybe crassa* (Sacc.) Pegler and Lodge

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ABSTRACT

Objective: This study aims to quantify the anti-oxidant activity of the methanolic extract of *Macrocybe crassa* and its anti-proliferative activity on normal and cancer cells. **Methods:** The anti-oxidant potential of the extract was determined by several *in vitro* assay system like DPPH radical scavenging activity, superoxide anion scavenging activity, percentage inhibition of lipid peroxidation and nitric oxide (NO) scavenging activity. Anti-proliferative activity was tested by MTT assay on breast cancer cell line MCF7, Human acute T lymphoblastic leukaemic cell MOLT-4 and Peripheral Blood Mononuclear Cells or PBMC isolated from a healthy donor to check its cytotoxic effect on normal cells. **Results:** Results indicated that the methanolic extract of *Macrocybe crassa* shows appreciable anti-proliferative activity against breast cancer cell line MCF7 and negligible effect on MOLT4 cells. In contrast no significant anti-proliferative effect has been observed in normal PMMCs. Moderate anti-oxidant activity was recorded in methanolic extract. **Conclusion:** Methanolic extract of *Macrocybe crassa* with moderate anti-oxidant activity and specific anti-proliferative effect on MCF7 holds a great promise can be used for isolation of bio molecules for treating Breast Cancer.

Key words: *Macrocybe crassa*, Antioxidant, MTT assay, MCF7, MOLT-4.

INTRODUCTION

Since time immemorial, edible mushrooms have been closely associated with the food habit of a large population of this world mainly belonging to ethnic and tribal groups, not only for its delicious earthy flavour but also it acts as a healthy supplement. Besides highly nutritive, mushrooms possess several chemically diverse groups of biologically active secondary metabolites mainly belong to phenolic, flavonoids,¹ β -glucan,² heteropolysaccharides,³ glycosides, saponin, terpenoids groups⁴ etc which are beneficial to health. So mushrooms can be appropriately called "Gift of the God" or "the pharmaceutical store-house of nature". Mushrooms can grow very easily during the growing season and it is cultivated also in mass amount nowadays, so they are easily available. Those novel bioactive compounds from mushrooms have shown several pharmacological activities like anti-oxidant,⁵ anti-microbial,⁶ anticancer,⁷ immuno-modulatory,⁸ anti-diabetic,⁹ anti-ulcer,¹⁰ cardio-protective¹¹ and hepato-protective¹² etc. Free radicals are extremely reactive and highly unstable molecules with an unpaired electron. Free radicals can steal or donate electrons from several sources like DNA, lipid of the cell membrane and proteins etc to get stability and overtime cause oxidative damage accumulation to the cell that is responsible for several diseases like cancer, neurodegenerative diseases, and ageing, etc. Free radicals are produced as a by-product of normal metabolic processes like respiration or from other external sources like cigarette smoking, x-rays, polluted air, and industrial chemicals, etc. To

combat the generation of oxidative stress exogenous anti-oxidant is necessary to neutralize the ROS.¹³ Cancer, one of the most potent and dangerous ancient disease. It is of several types like lung, breast, colon, skin, blood cancer etc, all of which share a common characteristic-the uncontrolled proliferation and growth of cells. According to the report of the International Agency of Research for Cancer (IARC), cancer will be rise by about 50% in the next twenty years.¹⁴ Although the rate of mortalities can vary from cancer to cancer, some of which are really fatal like lung cancer with a death rate of about 70%, followed by colon cancer (50%) and breast cancer (17%), prostate cancer (13%) in the United State in 2013.¹⁵ The aim of our present study is to access the anticancer and anti-oxidant potential of a wild edible mushroom *Macrocybe crassa* belonging to division Basidiomycotina. A lot of investigation has been already done on anticancer activity of mushrooms and the observations informed that several gifted mushrooms possess some active compounds that have immense anti-cancer properties. The role of mushrooms in cancer treatment also complement chemotherapy and radiation therapy which have many side effects like anaemia, bone marrow suppression and nausea etc. Some examples are *Phellinus linteus*, *Inonotus obliquus*, *Grifola frondosa*, *Agaricus blazei*, *Pleurotus ostreatus*, *Cordyceps militaris*, *Lentinula edodes*, *Funalia trogii*, *Hericium erinaceus*, *Ganoderma lucidum* and many more.¹⁶ Some of active anticancer substances possess by this mushrooms are hispolon, lectin, krestin, lentinan, illudin S, calcaelin, psilocybin, and grifolinect.¹⁶ Besides the polysaccharides, beta-glucan having

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the most vast and broad-spectrum activities like anticancer and immuno-stimulatory. The anticancer compounds from mushrooms possess antimetabolic activity, topoisomerase inhibitor, angiogenesis inhibitor, mitotic kinase inhibitor, etc. through which it can depress the unregulating proliferation of cancer cells.¹⁶ Besides this, the natural active compounds isolated from mushrooms also showed active proliferation of immune system cells, DNA repair activity, anti-oxidant activity and increase apoptosis of transformed cells. Many anticancer and anti-oxidant drugs are also available in the market but they cannot properly target or eliminate cancer cells and also pose several side effects in human health. Some negative side effects of chemotherapy, radiation therapy are, they depress the immune system and kill healthy cells as well as malignant cells. So researchers are trying to develop new anticancer drugs and therapeutic agents which are based on natural products with less toxicity that play an important role to inhibit the onset of cancer. The main focus and belief of alternative cancer treatment are that the immune system of the patient must be boosted to a healthy balance. Unlike conventional methods, alternative medicines seldom possess damaging agents. So many potentially bioactive compounds are used as drugs which are isolated from such naturally occurring mushrooms. The main motto should be "food should be our medicine and medicine should be our food".

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Quercetin(SRL), Sodium nitrite(Himedia), Aluminium chloride(Merck), Sodium hydroxide(SRL), 2,2-diphenyl-1-picrylhydrazyl(DPPH)(SRL), Sodium nitroprusside, Griess reagent, RPMI 1640(Sigma Aldrich), FBS(Sigma Aldrich), Penicillin and Streptomycin (Sigma Aldrich), Thiobarbituric acid, n-Butanol, Acetic acid, PMS(SRL), 0.5% TBA, MTT[3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(Sigma Aldrich), DMSO, Phosphate Buffer Saline, Gallic acid(Merck), Sodium carbonate(Merck), Folin Ciocalteu(Merck), methanol, Hexane, Petroleum ether.

Collection and preparation of methanolic extract of mushroom

The specimen *Macrocybe crassa* (Sacc.) Pegler and Lodge was collected from the coastal area of West Bengal, India in the month of August, 2017. Their colour, size, shape was recorded. Some biochemical tests were conducted and morphological and anatomical observation was authenticated. Identification of the mushroom was performed with the help of standard literature and published work.¹⁷ The voucher specimen (CUH/FN/CN/SP/04) has been deposited in Department of Botany, University of Calcutta.

The fruit bodies were then cleaned to remove residual compost and dried to remove moisture content. Then the fruit bodies were grounded into a fine powder. 20 gram of mushroom powder was weighed and mixed with 65 ml of pet-ether, to obtain pet-ether extract which kept for 48 hours at room temperature and then filtered. The residue was air-dried to evaporate out pet-ether and then added 30 ml of hexane and kept for 48 hours and filtered. The residue was air-dried to evaporate out hexane and added 60 ml of ethyl acetate and kept it for 72 hours and filtered. In the dried residue 90 ml of methanol was added and shaken continuously for 10-15 minutes at regular intervals and filtered after 96 hours. The filtrate was evaporated to form a sticky methanolic fraction. It was collected, labelled, weighed and kept in a microcentrifuge tube covered with parafilm at 4°C for future use. From this fraction a master stock solution of 5 mg/ml was prepared with DMSO, labelled, covered with parafilm and kept at 4°C for future use. The yield percentage of the extract was calculated based on the dry weight as:

$$\text{Yield percentage (\%)} = (W1 \times 100) / W2$$

Where, W1= weight of the extract after solvent evaporation

W2= weight of the minced mushroom

Cell line and culture

The cell line used for the study is MCF7, MOLT-4. Cell line was cultured at 1×10^5 cells/ml in RPMI 1640 supplemented with 2 mM L-glutamine, 10% (v/v) heat inactivated FBS, 10 U/ml penicillin and streptomycin and maintained in humidified 5% CO₂ incubator (HF90).¹⁸ MCF 7 is a breast cancer cell line isolated from a 69-years-old Caucasian woman.¹⁹ It is characterised by the presence of estrogens and progesterone receptors.²⁰ MOLT-4 is a human acute T lymphoblastic leukaemic cell line.

Determination of total phenol content of mushroom extract

Total phenol content of methanol extract was determined according to the method of Mridha *et al.*²¹ and Singleton VL *et al.*²² using folin-ciocalteu as a reagent and gallic acid as standard. The results were expressed as µg of gallic acid equivalent (GAE) per mg of the dry weight of the extract. An equal volume of 0.2 N of folin-ciocalteu and the crude extract was mixed and incubated for 3 minutes in dark. Then added 10% sodium carbonate and the mixture was vortexed and incubated for 90 minutes at 25°C. After incubation, O.D. values were taken in a spectrophotometer at 725 nm. The experiment was performed in duplicates for each concentration.

Determination of total flavonoid content of mushroom extract

According to the method of Mridha *et al.*²⁰ and Kamtekar *et al.*²³ total flavonoid content of methanol extract was determined. From the standard curve of quercetin, the flavonoid content was calculated and the result was expressed as µg of quercetin equivalent per mg of dry extract. 4 ml of distilled water and 1 ml of aliquot was mixed followed by the addition of 5% sodium nitrite. After 5 minutes of incubation, 10% aluminium chloride and 1 M NaOH was added. The volume of the mixture made up to 10 ml by addition of distilled water and absorbance was taken at 510 nm. The experiment was done in duplicates for each concentration.

Determination of DPPH radical scavenging activity of methanolic extract

DPPH radical scavenging activity was evaluated according to the method of Bloiss *et al.*²⁴ and Mridha *et al.*²⁰. 500 µl of DPPH (0.1 mM) was mixed with 500 µl of extract (concentration ranging 10,20,40,60,80,100 µg/ml). The mixture was vortexed and incubated for 30 minutes in dark at room temperature. After incubation, the absorbance was measured at 517 nm. The experiment was done in duplicates for each concentration. The percentage of DPPH scavenging was calculated according to the following formula:

$$\text{Percentage DPPH scavenging} = [\text{Absorbance (control)} - \text{Absorbance (sample)}] / \text{Absorbance (control)} \times 100.$$

Determination of superoxide anion scavenging activity of mushroom extract

According to the method of Kakkar *et al.*²⁵ and Mridha *et al.*²⁰ the superoxide anion scavenging activity was estimated. Various concentration (10, 50, 80,100 and 200 µg/ml) of methanolic extract of mushroom was taken and to each 200 µl of nitroblue tetrazolium (NBT), tris-HCl buffer, nicotinamide adenine dinucleotide (NADH), PMS was added. The reaction mixture was vortexed and incubated for

5 minutes at 25°C in dark. The absorbance was measured at 560 nm. Superoxide radical scavenging activity was measured according to the following formula:

$$\text{Percentage superoxide radical scavenging} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}] / \text{Absorbance (control)}}{\times 100}$$

Determination of percentage (%) inhibition of lipid peroxidation by mushroom extract

For determination of percentage inhibition of lipid peroxidation by mushroom extract, thiobarbituric acid-reactive species (TBARS) assay was used.²⁶ 100 µl mushroom extract and 10% (v/v) egg homogenate were added in a test tube and the volume of the mixture made up to 1 ml by adding distilled water. 0.07 M FeSO₄ was added and the mixture was incubated for 30 minutes. Acetic acid (pH 3.3), TBA in 1% SDS, was added and the reaction mixture was vortexed and heated at 95°C for an hour. After cooled down, butanol was added to the reaction mixture and the tubes were centrifuged at 3000 rpm for 10 min. The upper organic layer was separated out and the absorbance was measured at 532 nm.

Percentage inhibition of lipid peroxidation by methanolic extract of the mushroom was calculated according to the following formula :

$$\frac{[\text{Absorbance}(\text{control}) - \text{Absorbance}(\text{sample}) / \text{Absorbance}(\text{control})]}{\times 100}$$

In-vitro nitric oxide (NO) scavenging activity of mushroom extract

Nitric oxide scavenging assay of the mushroom extract was done according to the method of Marocci *et al.*²⁷ and Garrat *et al.*²⁸ Several concentration of mushroom extract (100,200,400,600,800 and 1000 µg/ml) was prepared with phosphate buffer saline along with a control set in which an equal amount of phosphate buffer present but without mushroom extract. 400 µl of sodium nitroprusside (10 mM) was added to each reaction mixture and incubated for 180 minutes at room temperature for the generation of NO. Under the aerobic condition, NO react with oxygen to produce nitrite ions, the amount of which can be measured by Griess reagent. The scavengers of nitric oxide compete with oxygen and thus reduce or inhibit the nitrite ion production. After incubation, 500 µl of Griess reagent [1% sulphanilamide in 2.5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride] was added. The absorbance of the reaction mixture was taken at 546 nm. The chromophore (azo dye) produced as a result of diazotisation of nitrite ions with sulphanilamide and subsequent coupling with ethylenediamine dihydrochloride.

Percentage of nitric oxide radical scavenging by methanolic extract of the mushroom was calculated according to the following formula:

$$\frac{[\text{Absorbance}(\text{control}) - \text{Absorbance}(\text{sample}) / \text{Absorbance}(\text{control})]}{\times 100}$$

Cell viability assay

The methanolic fraction of *Macrocybe crassa* was dissolved in DMSO. In a 96-well plate cells were seeded at 5×10^4 cells/well with four different concentration of extract-10, 40, 80 and 100 µg/ml and observed at 48 hours' time point employing MTT cell viability assay.¹⁶ In 96-well plate 5×10^4 cells were incubated in duplicate at different concentration of methanolic extract of *Macrocybe crassa* in a final volume of 200 µl for 48 hours at 37°C. MTT (5 mg/ml) solution was added to each well two hours before the completion of the incubation time, cell viability was spectrophotometrically measured at 560 nm. 100% lysis was obtained by lysing the cells in 5% SDS lysis buffer, and absorbance of 100% lysed cell sample was also measured at 560 nm.

The percentage of cell viability was calculated as mentioned below:

$$\% \text{ cell viability} = \frac{(\text{O.D. sample} - \text{O.D. 100\%lysis}) / (\text{O.D. 0\%lysis} - \text{O.D. 100\%lysis})}{\times 100}$$

RESULTS

Myco-chemical analysis

The extract was brown in colour and sticky in nature with an extractive value of 5.8%.

Total phenol content

The presence of total phenol in methanolic extract of *Macrocybe crassa* was evaluated using folin-ciocalteu as a reagent and gallic acid as standard. The results were expressed as mg of gallic acid equivalent per gram of dry extract. From the equation $y=0.0153x+0.1214$, $r^2=0.9864$ (x = gallic acid concentration, y = absorbance of *Macrocybe crassa* methanolic extract), obtained from the standard curve of gallic acid, the total phenol content is calculated to be 36.64 ± 0.19 mg of Gallic acid equivalent per gram of dry weight of the extract. (Table 1).

Total flavonoid content

The presence of total flavonoid in methanolic extract of *Macrocybe crassa* was evaluated using aluminium chloride assay and quercetin as standard. The results were expressed as mg of quercetin equivalent per gram of dry extract. From the equation $y=0.0013x+0.002$, $r^2=0.9686$ (x = quercetin concentration, y = absorbance of *Macrocybe crassa* methanolic extract), obtained from standard curve of quercetin, the total flavonoid content is calculated to be 14.99 ± 0.19 mg of Quercetin equivalent per gram of dry weight of extract (Table 1).

DPPH radical scavenging assay

DPPH is a stable N₂ centred free radical, produces violet colour in methanolic solution with a maximum absorption at 517 nm. Antioxidant molecules can quench DPPH and converted it to a colourless product i.e. 2, 2-diphenyl-1-hydrazine and as a result absorbance is decreased at 517 nm.²⁹

With different concentration of methanolic extract, the DPPH radical scavenging assay was carried out. Results show that the increase in the percentage of scavenging is linearly proportional to the increase in concentration (Figure 1a).

The activity ranged from 5.81% to 14.36% at concentration from 10 to 100 µg/ml. EC₅₀ value is the effective concentration at which the free radicals scavenging is 50%. EC₅₀ value was not obtained under the experimental concentration (Table 2).

Superoxide anion radical scavenging assay

Superoxide radicals are generated through by-products of normal metabolic processes or through addition of one electron to molecular

Table 1: Total Phenol and Flavonoid content of the methanolic extract of *Macrocybe crassa*. All the results are the mean value of three independent experiments with standard deviation.

Total Phenol Content (mg Gallic acid equivalent / gram of dry weight)	Total Flavonoid Content (mg of Quercetin equivalent / gram of dry weight)
36.64 ± 0.19	14.99 ± 0.19

Table 2: Antioxidant potential of the methanolic extracts of *Macrocybe crassa* tested in the study.

Antioxidant Assay	EC ₅₀ Value (µg/mL)
DPPH radical scavenging activity	> 100
superoxide anion scavenging activity	> 200
Lipid peroxidation scavenging activity	66
Nitric Oxide (NO) scavenging activity	< 100

oxygen. From ROS other harmful ROS can be generated that cause cellular damages.³⁰ Anti-oxidant molecules scavenge this radicals and provide protection from damaged.

The superoxide radicals scavenging activity of methanolic extract of *Macrocybe crassa*, was evaluated with several concentration of the extract (10, 50, 80, 100 and 200 µg/ml). The scavenging activity increases linearly with increasing concentration of the extract. At concentration 10, 80 and 200 µg/ml the scavenging activity were 32.7%, 40.6% and 46.3% respectively (Figure 1b). EC₅₀ value was not obtained under experimental concentration, indicating that methanolic extract of *Macrocybe crassa* is moderate potent in superoxide radical scavenging activity (Table 2).

Inhibition of lipid peroxidation assay

TBARS assay using egg yolk as a source lipid is a well-known method for determination of inhibition of lipid peroxidation. Several concentration of methanolic extract of the mushroom *Macrocybe crassa* were prepared (10, 20,40,60,80 and 100 µg/ml) and tested. Result showed an increase in inhibition that is proportional to the linear increase of extract concentration. The inhibition ranges from 10.6% to 68% at a concentration of 20-100 µg/ml. The EC₅₀ value was calculated to be 66 µg/ml (Figure 1c). Thus the assay shows that the methanolic extract of *Macrocybe crassa* have a good to moderate anti-oxidant activity in terms of inhibition of lipid peroxidation across the range of concentration studies (Table 2).

In-vitro NO radicals scavenging assay

The methanolic extract of the mushroom showed a concentration dependence reduction of nitric oxide generation as indicated by decreasing in absorbance at 546 nm. At 100 µg/ml concentration, the NO scavenging was 63.86% and at 1000 µg/ml concentration, the

scavenging was 75.56% (Figure 1d). With increasing concentration of the extract, NO scavenging is also increased as indicated by O.D values (Table 2).

Cell viability test

The methanolic fraction of *Macrocybe crassa* shows an appreciable anti-proliferative effect on the breast cancer cell line MCF7 when subjected to cell viability test at the time point of 48 hours. The data shows a concentration dependence increase of anti-proliferative activity. The IC₅₀ (inhibitory concentration) value is 34.3 ± 1.47 µg/ml. Moderate anti-proliferative effect was recorded from Human acute T lymphoblastic leukaemic cell MOLT-4 with an IC₅₀ value of 141.14 ± 3.53 µg/ml (Table 3). Negligible cytotoxic effect was seen in case of Peripheral Blood Mononuclear Cells or PBMC isolated from healthy donor (Figure 2). Hence, it can be concluded that the cytotoxic effect was cancer cell specific as no such deviation in cell viability was recorded from normal PBMC.

DISCUSSION

Cells of humans as well as other organisms are constantly exposed to a wide range of oxidizing agents. Excess production of oxidants can cause oxidative stress which leads to several chronic diseases. Therefore, increased consumption of food that contains high levels of anti-oxidants has been recommended to prevent or reduce the amount of the oxidative stress caused by free radicals. It is a well-known fact that

Table 3: IC₅₀ values of the methanolic extract of *Macrocybe crassa* in the MTT assay tested in the following cell line in this study. All the results are the mean value of three independent experiments with standard deviation.

Methanolic extract of <i>Macrocybe crassa</i>	MCF-7	MOLT-4
IC ₅₀ value (µg/mL)	34.3 ± 1.47	141.14 ± 3.53

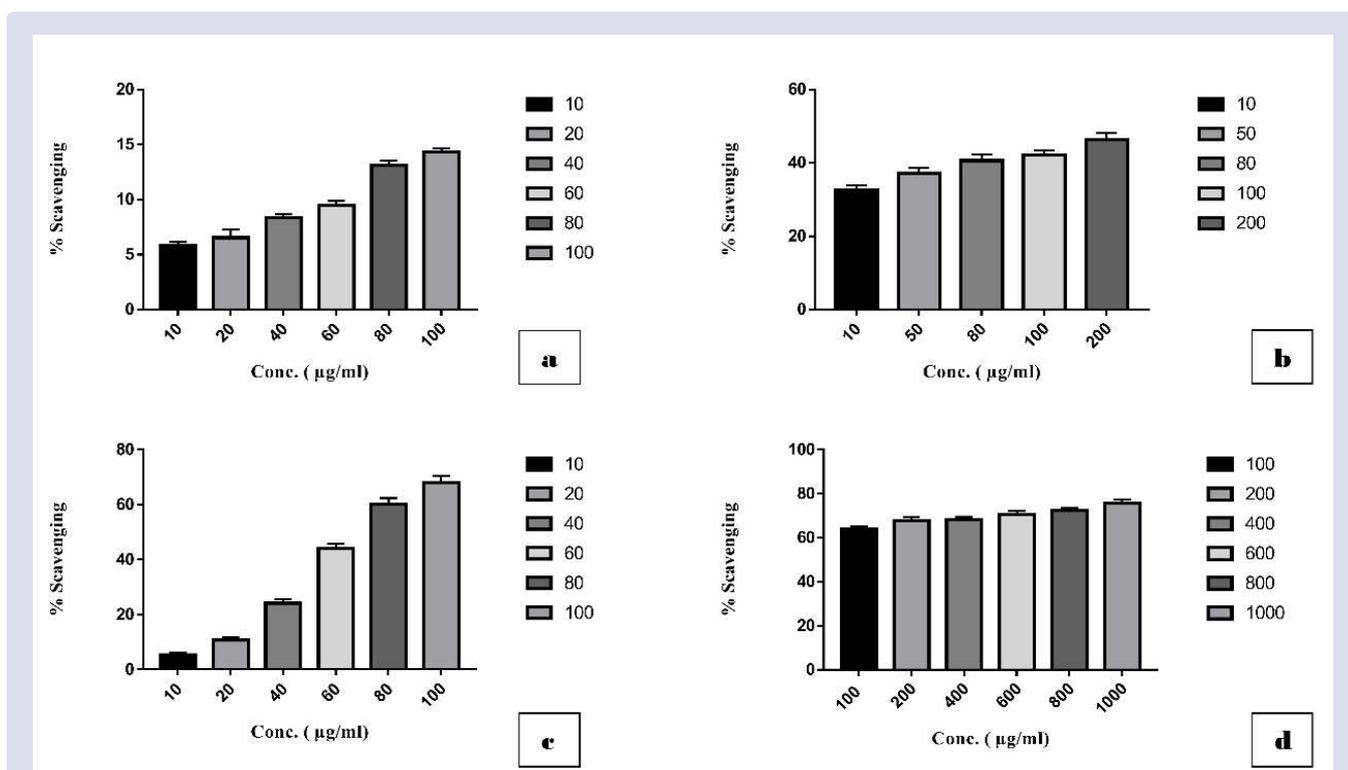


Figure 1: DPPH, superoxide, lipid peroxidase and nitric oxide radical scavenging activity of *Macrocybe crassa*.

Percentage of DPPH (a), Superoxide (b), Lipid peroxidase (c) and Nitric oxide (d) radical scavenging activity of methanolic extract of *Macrocybe crassa* of different concentration. Each column denotes mean ± standard deviation value of three independent experiments.

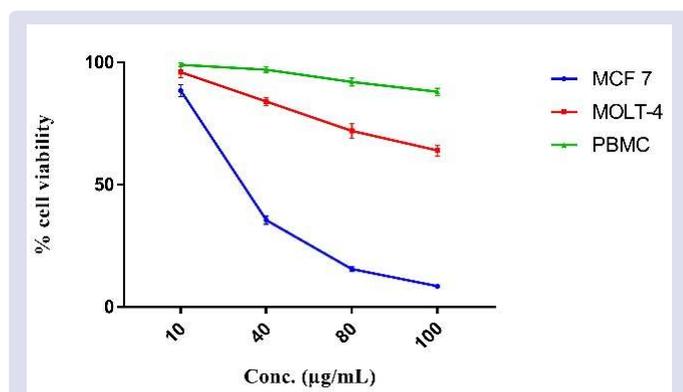


Figure 2: Effect of methanolic extract of *Macrocybe crassa* on two different cancer cell lines.

Effect of methanolic extract of *Macrocybe crassa* (10-100µg/ml) by MTT assay against MCF-7, MOLT-4 and normal PBMC isolated from healthy individual at 48h time point. Each line graph represents the Mean value \pm Standard Deviation of three individual experiments in duplicate.

mushrooms are good source of protein and other necessary nutrients but in this study our objective was to find biological activity of wild edible mushroom *Macrocybe crassa* in terms of its anti-oxidant activity as well as its anti-proliferative potential against cancer cells. Results depicts the fact that methanolic extract contain moderate anti-oxidant activity.

Phenolic compounds consists of a vast group of phytochemicals that account for most of the anti-oxidant activity. On the other hand, flavonoid compounds are the large group of naturally occurring phenolic compounds, which occurs both in Free State and as glycosides. Hence, total phenol and flavonoid content depict an overall idea about the anti-oxidant property of any extract. In this study it is also evident that moderate quantity of total phenol and flavonoid present in the methanolic extract. This biochemical data corroborate the moderate anti-oxidant activity in case of DPPH radical scavenging activity, superoxide anion scavenging activity, percentage inhibition of lipid peroxidation and nitric oxide (NO) scavenging activity as described in the result section. These results reflect the moderate anti-oxidant property of the methanolic extract which add on nutraceutical value of *Macrocybe crassa*. On the other hand it has shown robust anti-proliferative activity in human breast cancer cell line MCF7 and moderate activity on human T cell Acute Lymphoblastic Leukemia cell line, MOLT4. Anti-proliferative activity was cancer cell specific as the methanolic extract did not show any noticeable anti-proliferative activity in case of normal PBMC. Hence, the methanolic extract of *Macrocybe crassa* can be tapped to isolate and purify novel compounds in future to develop drugs to combat breast cancer.

CONCLUSION

Gradually mushrooms become an integral part of our lives. They are taken as food, used for the isolation of naturally occurring bioactive compounds. In this study the mushroom from Basidiomycotina division *Macrocybe crassa* is selected. This is a wild non-toxic, edible mushroom. In the present study anti-proliferative potential of methanolic extract of this mushroom is carried out and showed an appreciable anti-proliferative effect on the breast cancer cell line MCF7. Moderate anti-proliferative activity was observed in MOLT4 cells. The anti-proliferative effect was found to be cancer cell-specific as no such effect in cell viability was recorded from normal PBMC. Oxidative stress plays a frontier role as a link between environmental toxicity with the multistage carcinogenic process. Inside the living system, the generation of oxidative stress occurs due to imbalance of the formation of Reactive Oxygen Species i.e. ROS and their endogenous neutralization by the

process of quenching of the free radicals. Formation of ROS is an inevitable natural process. Our study show that the methanolic extract of the mushroom *Macrocybe crassa* possesses moderate anti-oxidant activity as indicated by the results of DPPH, superoxide radical and in-vitro NO scavenging assay. So it can be concluded that this extract can be tapped for isolation of potent anticancer drugs.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTION

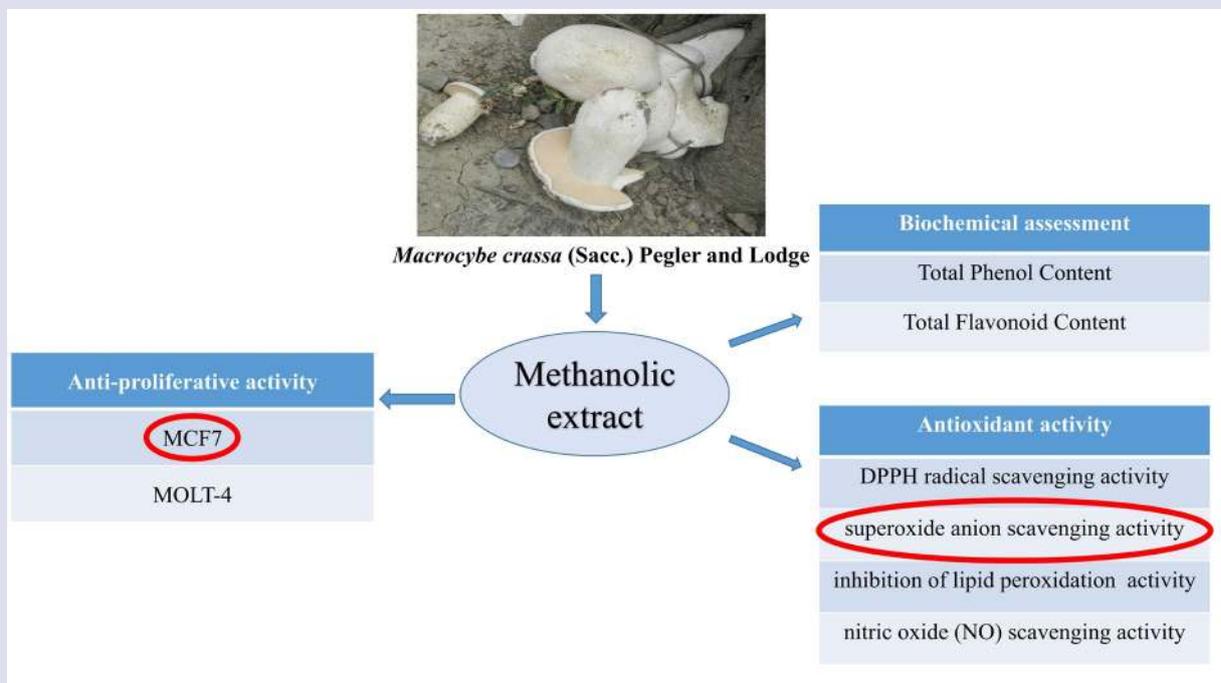
Prof. Santanu Paul design the study. Ms. Amrita Pal and Ribhu Ray collected and dried the mushroom specimen. Arpan Das prepared the methanolic extract and performed the laboratory tests with the help of Amrita Pal and Anirban Chouni. Priya K Gopal performed the cell viability assay. Prof. Santanu Paul and Arpan Das analysed data and wrote the manuscript. All authors approved the final manuscript.

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GRAPHICAL ABSTRACT



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