Plant Archives Vol. 19 No. 2, 2019 pp. 2843-2846 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IN-VITRO EVALUATION OF ANTI-CANCER PROPERTY OF FOUR EDIBLE MUSHROOMS IN COMPARISON WITH *GANODERMA LUCIDUM* : THE KING OF MEDICINAL MUSHROOMS

Agnes Jose* and D. Geetha

Department of Plant Pathology, College of Agriculture, Vellayani, Thrissur (Kerala), India.

Abstract

Preliminary trials on the anti-cancer activities of mushroom extracts of *Ganoderma lucidum* (Curtis) P. Karst., *Pleurotus florida* (Mont.), *Pleurotus djamor* (Fr.) Boedjn, *Hypsizygus ulmarius* (Bull.:Fr.)Redhead and *Calocybe gambosa* (Fr.) Singh were conducted by direct microscopic studies and MTT assay. *G. lucidum*, commonly referred as Lingzhi, is well-known for its pharmacological benefits including immune-modulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative, radical-scavenging and anti-aging effects. The white oyster mushroom, *P. florida* is a commonly available edible mushroom having excellent flavour and taste. *H. ulmarius* is an edible mushroom, also known as elm oyster mushroom or blue oyster mushroom. *C.gambosa* is a tropical milky mushroom cultivating in India which possess free radical scavenging property and thereby enhancing antioxidant capacity. The results revealed that percentage viability of cervical cancer cell lines decreased with increase in concentration of mushroom extracts. *G. lucidum* extract exhibited maximum cytotoxic effect on cancer cell lines even at lower concentration (200 μg ml^{-1}) followed by *C. gambosa*. The commercial cultivation as well as clinical studies of these medicinally important mushrooms must be undertaken in large scale.

Key words : Mushroom, anti-cancer property, *Ganoderma lucidum, Pleurotus florida, Pleurotus djamor*, *Hypsizygus ulmarius* and *Calocybe gambosa.*

Introduction

Cultivation of five mushrooms namely, *Ganoderma lucidum*, *Pleurotus florida*, *Pleurotus djamor*, *Hypsizygus ulmarius* and *Calocybe gambosa* were doneto evaluate their anti-cancerous activities. A variety of polysaccharides from mushroom varieties have been reported to enhance the immune system. These polysaccharides are mainly concentrated in the fruit bodies, mycelia and culture broth of mushrooms. The main sources of antitumor polysaccharides are cell wall components such as beta-glucan, chitin and cellulose (Chang and Miles, 2004). All of these have shown significant antitumor activity as a result of their ability to activate the host immune system rather than direct cytotoxicity. The mushroom polysaccharides appear to be well tolerated and compatible with chemotherapy and radiation therapy (Daba and Ezeronye, 2003).

***Author for correspondence :** E-mail: agnesjose3@gmail.com

The anti-cancerous drugs currently available in market possess several side effect and complications which emphasize urgent need for novel, effective and less toxic alternative. Polysaccharides and triterpenes are the most potent mushroom derived substances with anti-cancerous and immune-modulation properties. Cancer cells generate increased levels of free radicals compared to normal cells, further contributing to cancer progression. This cancer-inducing oxidative damage might be prevented or limited by mushroom derived anti-oxidants which directly react with or scavenge the free radicals (Kao *et al.*, 2013).

Materials and Methods

In vitro anti-proliferative effect determination by MTT assay

HeLa (cervical cancer) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune,

India and maintained in Dulbecos modified Eagles medium (DMEM). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with L-glutamine, sodium bicarbonate and antibiotic solution (Penicillin or Streptomycin at 100 µg ml^{-1}). Cultured cell lines were kept at 37ºC in a humidified five per cent CO_2 incubator. The viability of cells was evaluated by direct observation of cells using inverted phase contrast microscope and MTT assay method. Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 µl cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of extracts and compound stock

One mg of ethanol mushroom extract was added to one ml of DMEM and dissolved completely by cyclomixer. Then the extract was filtered through 0.22 µm millipore syringe filter to ensure the sterility.

Anti-proliferative evaluation

After 24 h of incubation the growth medium was removed and freshly prepared samples in 5% DMEM were diluted to 100 µg, 50 µg, 25 µg and 12.5 µg in 100 µl of 5 % DMEM. Each concentration of 100 µl was added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Entire plate was observed at an interval of each 24 h up to 72 h 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 was reconstituted in three ml Phosphate buffer saline (PBS) until completely dissolved and sterilized by filter sterilization. After 24 h of incubation, the sample content in wells were removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well and incubated for 4 h. After the incubation period, the supernatant was removed and 100 µl of MTT solubilisation Solution (DMSO) was added and the wells were mixed gently by pipetting in order to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (Talarico *et al*., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\text{Per cent of viability} = \frac{Mean\ OD\ Sample}{Mean\ OD\ of\ control\ group} \times 100$$

***In vitro* hepatotoxicity determination by MTT assay**

Chang Liver Cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in DMEM. Preparation of mushroom extract and seeding into 96 wells plate was done as per the procedure in anti- proliferative assay. The cytotoxicity effect of mushroom extract on liver cell line was assessed by direct microscopic observation and MTT Method (Talarico *et al*., 2004).

The percentage of growth inhibition was calculated using the formula :

$$\text{Per cent of viability} = \frac{Mean\ OD\ Sample}{Mean\ OD\ of\ control\ group} \times 100$$

Results and Discussion

In the present study, the anti-cancerous activities of ethanolic extract of *G. lucidum*, *P. florida*, *P. djamor*, *H. ulmarius* and *C. gambosa* were assessed against cervical cancer cell lines (HeLa) *in vitro* by MTT assay (table 1 and plate 1). The *G. lucidum* extract at 200 µg ml^{-1} concentration reduced the viability of cancer cells to 54.85 per cent in the current study which was the maximum inhibition of cancerous cells as compared with other mushrooms under study (fig. 2). *C. gambosa* extract at 200 µg ml^{-1} decreased the viability of cancerous

Table 1 : MTT* assay on anti-cancer property of five mushrooms.

Concentration (µg ml^{-1})	Viability of cancer cells (%)				
	G. lucidum	*P. florida*	*P. djamor*	*H. ulmarius*	*C. gambosa*
Control	100	100	100	100	100
12.5	87.823	78.60	86.928	79.842	86.660
25	82.845	76.11	80.832	76.151	78.447
50	72.809	74.83	76.521	73.421	72.027
100	62.605	69.28	73.812	67.942	69.389
200	54.850	65.62	69.477	65.891	60.927

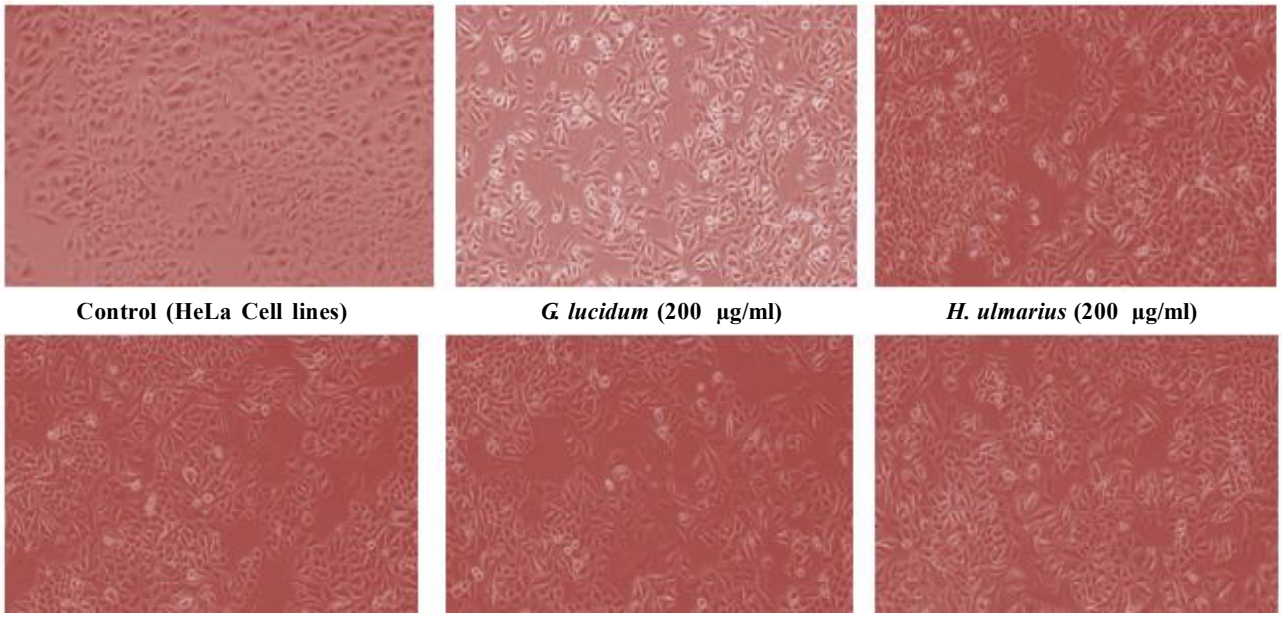

Plate 1 : Microscopic observation of cervical cancer cells (Inverted phase contrast microscope 40X).

Table 2 : Comparison of anti- cancerous activity of mushrooms at 200 µg ml^{-1} concentration.

S. no.	Mushroom	Per cent viability of cancer cells at 200 µg ml^{-1}
1.	*G. lucidum*	54.850
2.	*P. florida*	65.621
3.	*P. djamor*	69.477
4.	*H. ulmarius*	65.891
5.	*C. gambosa*	60.927

Table 3 : Cytotoxicity study of mushroom extracts on human liver cells at 200 µg ml^{-1}.

S. no.	Mushroom	Per cent inhibition of liver cells at 200 µg ml^{-1}
1.	*G. lucidum*	33.69
2.	*P. florida*	27.28
3.	*P. djamor*	25.48
4.	*H. ulmarius*	20.98
5.	*C. gambosa*	26.75

cells to 60.92 per cent. Anti-cancerous properties of *G. lucidum* were reported by Kao *et al*. (2013), who observed 50 per cent inhibition of low-grade bladder cancer cell line at 129.3 µg ml^{-1} concentration. Patel and Goyal (2011) and Gao and Zhou (2016) also reported the cancer preventive properties of *G. lucidum* extract. Sumathy *et al*. (2015) studied the anti-proliferative property of *C. indica* extract on breast cancer cell lines.

In the present study, *P. florida*, *P. djamor* and *H. ulmarius* extracts exhibited decrease in viability of cervical cancer cell lines to 65.52, 69.47 and 65.69 per cent respectively at 200 µg ml^{-1} concentration (fig. 1). Fifty per cent inhibition of human cancer cell lines by 500 µg ml^{-1} concentration of *L. edodes* extract was reported by Fang *et al*. (2006). Lavi *et al*. (2006) reported that aqueous polysaccharide extract from *P. ostreatus* induced anti-proliferative and pro-apoptotic effects on colon cancer cells. *P. pulmonarius* significantly reduced the *in vitro* and *in vivo* cancer cell proliferation and enhanced the drug-sensitivity to chemotherapeutic drug (Xu *et al*., 2012).

The cytotoxic effects in the polysaccharide extracts of mushrooms are due to the active generation of reactive oxygen species (ROS). Molecular damage caused by ROS in normal cells induces repair mechanisms, while in tumour cells ROS activate cell death processes through apoptosis (Durgo *et al*., 2013). The cytotoxicity effect of five mushroom extracts were assessed on liver cells which are more susceptible to the damage caused by toxins. In the present study, *G. lucidum* extract exhibited 33.69 per cent reduction in the viability of normal hepatic cells at 200 µg ml^{-1} concentration, which is low as compared with per cent reduction in viability of cancerous cells (table 3 and fig. 3). Popovic *et al*. (2013) reported that extracellular polysaccharides from *G. lucidum* performed high inhibition on human hepato carcinoma cell line, but also exerted certain toxicity in normal liver cell line which is in accordance with present study. *P.*

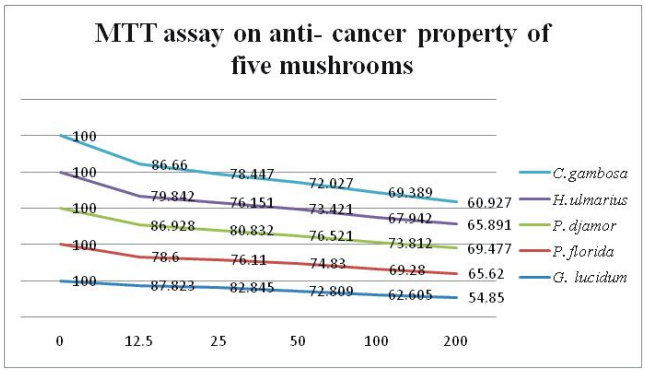

Fig. 1 : Comparison of anti-cancer properties of five mushrooms.

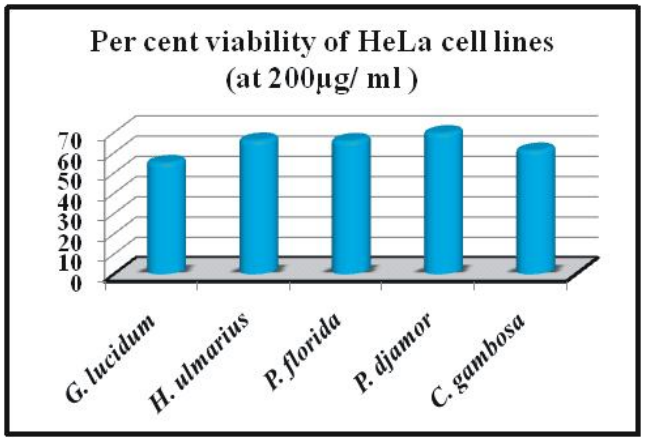

Fig. 2 : Cytotoxic effect of mushroom extracts on HeLa cell line at 200µg/ ml.

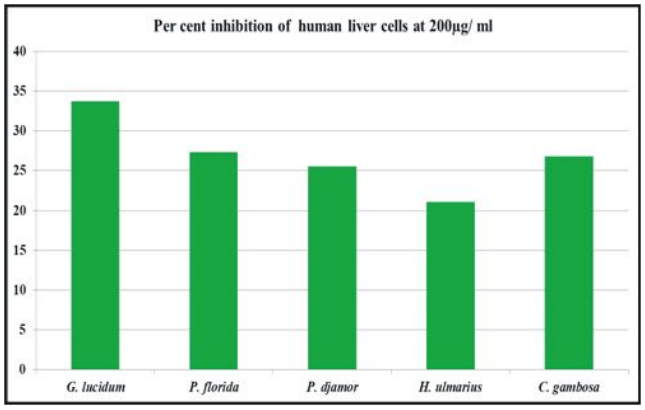

Fig. 3 : Cytotoxic effect of mushroom extracts on human liver cells at 200µg/ ml.

florida, P. djamor, H. ulmarius and *C. gambosa* extracts recorded 27.28, 25.48, 20.98 and 26.75 per cent reduction in liver cell viability respectively at 200 µg ml^{-1} concentration in the present study. Asatiani *et al*. (2018) reported that *Cordyceps militaris* did not show any cytotoxicity against normal cells.

References

Asatiani, M. D., L. Sharvit, G. S. Barseghyan, J. S. L. Chan, V. Elisashvili and S. P. Wasser (2018). Cytotoxic activity of medicinal mushroom extracts on human cancer cells. *SF J. Biotechnol. Biomed. Eng.*, **1(1)** : 1006.

Chang, S. T. and P. G. Miles (ed.) (2004). *Mushrooms-Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact* (2nd Ed.). CRC Press, Washington, pp. 64-69.

Daba, A. S. and O. U. Ezeronye (2003). Anti-cancer effect of polysaccharides isolated from higher basidiomycetes mushrooms. *Afr. J. Biotechnol.*, **2 (12)**: 672-678. *Sci.*, **7(3)**: 3066-3071.

Durgo, K., M. Koncar, D. Komes, A. Belscak-Cvitanovic, J. Franekic, I. Jakopovich, N. Jakopovich and B. Jakopovic (2013). Cytotoxicity of blended versus single medicinal mushroom extracts on human cancer cell lines: Contribution of polyphenol and polysaccharide content. *Int. J. Med. Mushrooms*, **15(5)**: 435-448.

Fang, N., Q. Li, S. Yu, J. Zhang, L. He, M. J. Ronis and T. M. Badger (2006). Inhibition of growth and induction o apoptosis in human cancer cell lines by an ethyl acetate fraction from Shiitake mushrooms, **12(2)**:125-132.

Gao, Y. and S. Zhou (2016). Cancer prevention and treatment by *Ganoderma*, a mushroom with medicinal properties. *Food rev. Int.,* **19(3)**: 275-325.

Kao, C. H. J., A. C. Jesuthasan, K. S. Bishop, M. P. Glucina and L. R. Ferguson (2013). Anti-cancer activities of *Ganoderma lucidum* : Active ingredients and pathways. *Funct. Foods Health Dis.*, **3(2)**:48-65.

Lavi, I., D. Finesem, S. Geresh, X. Hadas and B. Schwartz (2006). An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Lett.*, **244**: 61-70.

Patel, S. and A. Goyal (2011). Recent developments in mushrooms as anti-cancer therapeutics: a review. *Biotech.*, **2** : 1–153.

Popovic, V., J. Zivkovic, S. Davidovic, M. Stevanovic and D. Stojkovic (2013). Mycotherapy of Cancer: An Update on Cytotoxic and Antitumor Activities of Mushrooms, Bioactive Principles and Molecular Mechanisms of their Action. *Curr. Topics Med. Chem.*, **13** : 2791-2806.

Sumathy, R., R. Kumuthakalavalli, A. S. Krishnamoorthy and V. Balan (2015). Effect of phytochemicals and antioxidant compounds enriched extract from *Calocybe Indica* var. APK2 on proliferation of human MCF-7 breast carcinoma cells. *Der. Pharmacia. Sinica*, **6(2)** : 6-11

Xu, T. T., R. B. Beelman and J. D. Lambert (2012). The cancer preventive effects of edible mushrooms. *Anti-Cancer Agent. Me.*, **12** : 1255-1263.