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# Anticancer Potentials of Medicinally Important Mushroom Mixturs of Ganoderma Lucidum and Pleurotus Sajor-Caju

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## ABSTRACT

*Ganodermalucidum*and *Pleurotussajor-caju*are important mushrooms, which is used widely as food and medicine. One of the major concepts of siddha is food is medicine and medicine as food. To consider this the present study was conducted to assess anticancer potentials of extracts of *Ganodermalucidum* and *Pleurotussajor-caju*mixture with reference to cancer cell line cytotoxicity by MTT assay.Intracellular reactive oxygen species assay and mitochondrial membrane potential of extract and control treated cell lines were assessed by making use of standard textual procedures. Results revealed that aqueous and ethanol extracts of mushroom mixture control the growth of MCF-7 cell lines growth upto 71%, which was also evident in ROS generation and mitochondrial membrane potential assay.

**Key word:** Anticancer, *Ganodermalucidum, Pleurotussajor-caju*, fruiting body, MCF, MTT

## INTRODUCTION

Cancer can be described as an abnormal and uncontrolled proliferation of cells. Cancer cells often spread into the surrounding tissue or metastasize to distant organs through the blood or the lymphatic system (Rusciano, and Burger,1992). Cancer cells can arise in a lot of tissues and organs (Baylin and Ohm,2006). Despite advances in early detection and therapy, cancer still is a big health challenge with the highest priority for investigation (Bray, 2012). Breast cancer is a malignant tumor arising from epithelial cells of glandular milk ducts or lobules of the breast (Benson, 2009).Around two-thirds of breast carcinomas arise from epithelial cells of the ducts, called ductal carcinomaand around onethird from lobules, called lobular carcinoma (Malhotra, 2010). The mutations in expression of gene contribute approximately 5-10% among all cases of breast cancers (Natalia et al., 2013). Other known factors involved in breast cancer may include obesity, use of hormone therapies (progestin and estrogen), increased breast tissue density, alcohol use and physical inactivity (Emens and Jaffee, 2005). Normally cells undergo apoptosis after completion of their life cycle when they are not further required for body. Before apoptosis they are protected by different pathways and proteins. (Natalia et al., 2013; Peng-Yun Wang et al., 2012; Ferlay, 2012 and Matsen and Neumayer, 2013). Now people opt for multiple variable treatment methods, one among is siddha, which illustrates food is medicine and medicine as food. Scientists all over the world work on anticancer efficiency of natural food and herbs. In this study, mixture of *Ganodermalucidum and Pleurotussajor-caju* extracts are assessed for its anticancer efficiency on MCF- 7 cell lines.

## MATERIALS AND METHODS

### Collection of Ganodermalucidum

*Ganodermalucidum*and *Pleurotussajor-caju* were collected as wild from the paddy fields of Thiruvarur (Dt), Tamil Nadu and identified and authenticated in the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The selected strains were multiplied on potato dextrose agar (PDA) petriplates and slant culture was also maintained for further analysis.

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**Processing and Extraction**

*Ganodermalucidum*and *Pleurotussajor-caju* were dried completely, powdered using mechanical grinder and extracted using water and ethanol.

**Anti-cancer activity**

Human breast cancer MCF-7 cell lines were procured from the Cell repository of National Centre for Cell Sciences (NCCS), Pune, India. Dulbecco`s Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% $CO_2$ at 37°C.

**Cytotoxicity Assay**

The MCF-7 cells were placed in 96 well plates (1 X $10^5$ cells per well) and incubated in 5% $CO_2$ environment at 37°C. Once the cells placed in wells reached confluence, the prepared concentrations of extract from 100 – 1000 µg/ml were added and kept in incubator for 24 hours. Then the samples were removed from the well, and washed with phosphate-buffered saline (pH 7.4). 0.1ml of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl–tetrazolium bromide (MTT) was added to each well (100µl/well) and incubated for 4 hours. Then 1ml of dimethyl sulfoxide (DMSO) was added in all the wells to dissolve the formed formazan crystals. Each sample was placed in the cuvette; using DMSO as the blank the absorbance value at the wavelength of 570 nm was noted using Ultra-violet (UV) Spectrophotometer. The average absorbance values from three observations were taken. The percentage cell viability was calculated by determining the ratio between MCF-7 of treated cells (Agostini et al., 2011).

**Measurement of intracellular reactive oxygen species assay (Borcher et al., 1999)**

Reactive oxygen species (ROS) were distinguished by determining the fluorescence intensity of dichlorofluorescein (DCFH). When non-fluorescent DCFH-DA flows into the cell via the plasma membrane and hydrolyzed in the interior cell to DCFH. Intracellular oxidation converts DCFH into the fluorescent form, DCF. After development of the MCF-7 cells with various concentrations of extracts for 24 h, fluorescent dye DCFH-DA was added to the cells and then kept in incubator 30 min only. Finally, the cells were washed with PBS to remove the excess dye. Fluorescent intensity was prepared with excitation and emission filters set at 488 and 530 nm, respectively. Fluorescence microscopic images were taken using a blue filter (450-490 nm).

**Measurement of mitochondrial membrane potential (Itoh et al., 1994)**

The study of mitochondria and changes in the mitochondrial membrane potential (MMP) has developed an emphasis of apoptotic analysis. The possible effect of extracts in disturbing MMP was evaluated using the lipophilic cationic fluorescent probe Rh-123 for mitochondria. Rh-123 green fluorescent monomer at depolarized or a red fluorescent aggregate at hyper polarized membrane potential. Rh-123 localizes in the mitochondria of living cells owing to the somewhat high negative electric potential through the inner membrane of the mitochondria. After extracts treatment, at various concentrations Rh-123 was supplementary to attain a final concentration of 10 µg/ml and the MCF-7 cells were incubated for 30 mins at 37 °C. Then the cells were swept away with PBS and observed under fluorescence microscope using blue filter.

*Acridine orange and ethidium bromide staining*

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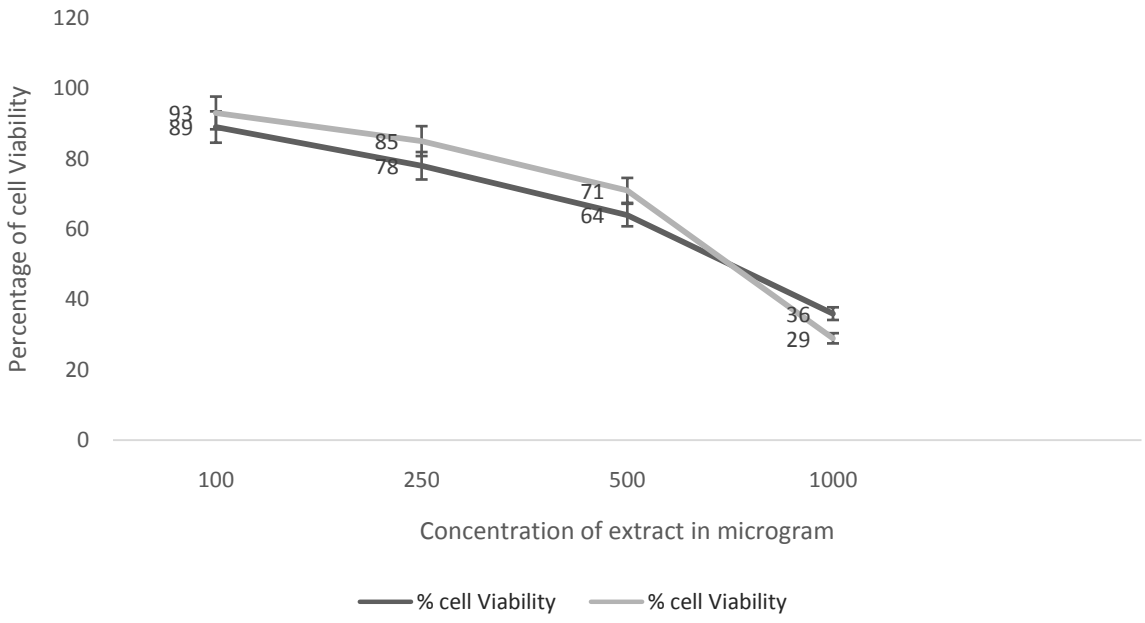
AO and EtBr staining with DNA allowed visualization of the condensed chromatin of apoptotic cells. The control and extract-treated cells were seeded in a 6-well plate (3 × 104 /well) and incubated in CO2 incubator for 48 h. The cells were fixed in extracts for 30 min at room temperature washed in PBS and stained with 1:1 ratio of AO/EtBr. Stained cells were immediately washed with PBS and viewed under a fluorescence microscope (Nikon, Eclipse TS100, Japan). The number of MCF-7 cells expressing apoptotic feature was counted and expressed as a fraction of the total number of cells present in the field.

## RESULTS

*In vitro* cytotoxic activity of mushroom mixtures wasevaluated against breast cancer (MCF-7) cell line at different concentrations. The mushroom mixtures exhibited potent cytotoxicity/anticancer activity in the tested cell lines. Results showed that at higher concentrations there is significant cell mortality. The inhibitory effect was observed after 24 h of incubation (Figure 1). Cell cytotoxicity was increased with dose dependent manner. Among the extracts aqueous extract (GPAE) produced 71% cell growth inhibition i.e., only 29% viable cells were noted. Similarly, only 36% viable cells were noted in ethanol extracts (GPEE) at 1000µg/ml concentrations. Plate I also expressed the shape and other nature of MCF 7 cell lines.

Figure 1 - Cell cytotoxicity of *Ganoderma lucidum & Pleurotus sajor-caju* mixture extracts with reference to MTT assay
Cell Line - MCF-7




The result obtained from ROS generation in MCF-7 cells exposed to mushroom mixtures for 24h is shown in Plate I. A significant induction in ROS generation was measured in MCF-7 cells exposed to mushroom mixtures at 100-1000µg/ml concentrations. The effect of extracts-induced depolarization of mitochondrial transmembrane potential was examined using JC-1 dye. Control cells emitted red fluorescence indicating intact mitochondrial membrane potential. However, mushroom mixtures treated MCF-7 cells expressed progressive and complete loss of red to green fluorescence at 24 h and 48 h respectively, due to mitochondrial transmembrane depolarization. Mushroom mixture extracts disturbed mitochondrial membrane potential and

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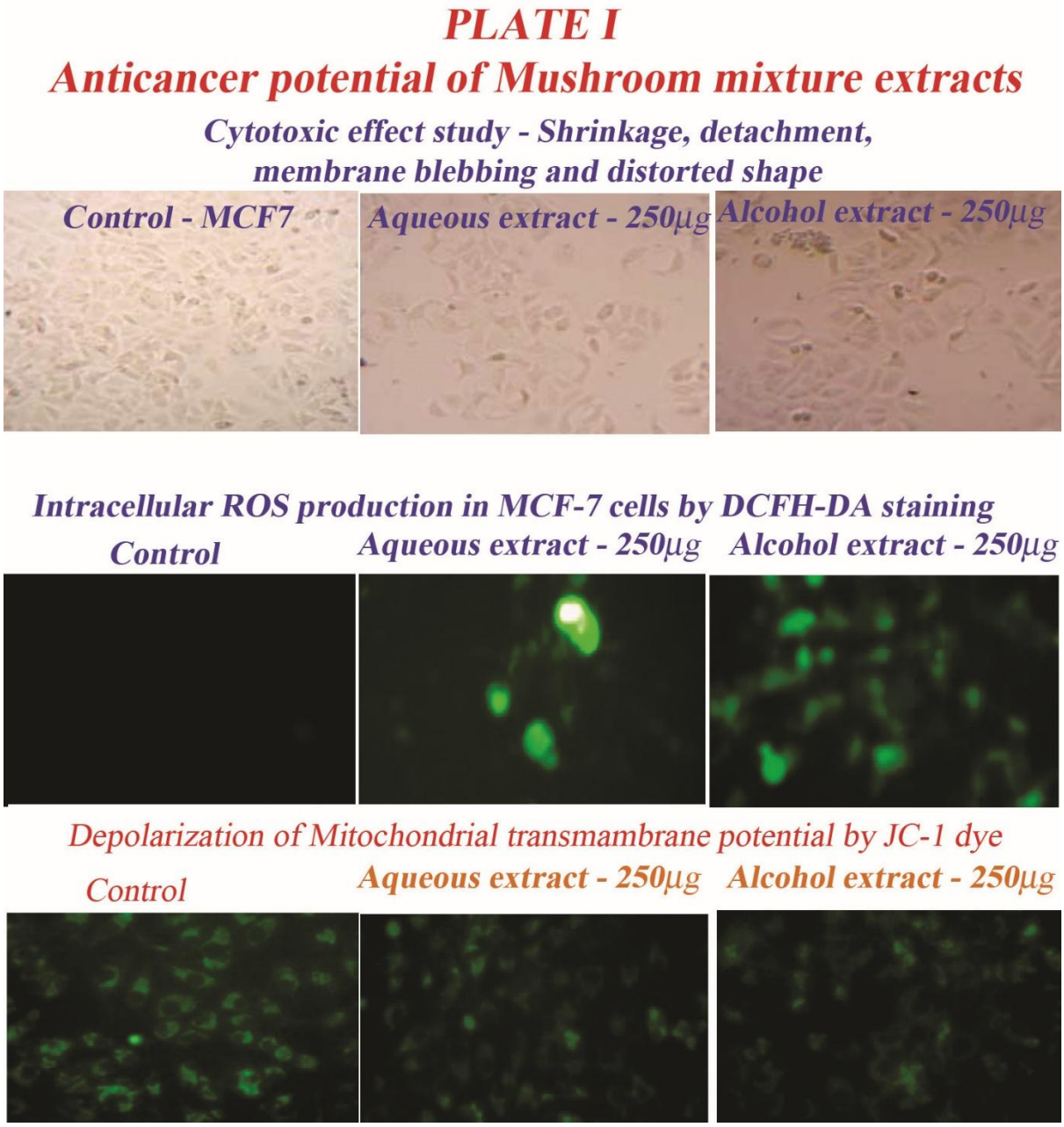
induced apoptosis in a time-dependent manner. It could ROS generation at high level leading to cellular damage by resulting in mitochondrial membrane damage and toxicity.




**DISCUSSIONS**

MTT assay is based on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product. It is frequently used as an *in vitro* model system to measure cytotoxic effects of variety of toxic substances and plant extracts against cancer cell lines. Cell treated with mixture of *Ganodermalucidum and Pleurotussajor-caju* showed cytoplasm shrinkage and loss of cell-to-cell contact. Taken together, these results indicated that *Ganodermalucidumand Pleurotussajor-caju*inhibited cancer cell growth in a dosedependent manner. Reactive oxygen species (ROS) are known to play dual role either that may be beneficial or harmful depending on their accumulation levels. Apart from its importance in physiological functions, ROS generally contributes to cell death either by apoptosis or necrosis at the levels beyond the cellular antioxidant defence mechanisms. The intracellular ROS levels was determined by measuring the intensity of a highly fluorescent derivative

2′,7′-dichlorofluorescein (DCF) which is produced from externally applied non-fluorescent substance, DCFH-DA by the cellular redox reactions. As shown in Plate I,mushroom mixturedose dependently increased the ratio of green fluorescence in MCF-7 cells suggesting that mushroom mixture stimulates production of intracellular ROS in MCF-7 cells. In addition to its metabolic functions, mitochondria play a major role in the management of other cellular functions like cell survival and death (Galluzzi et al., 2012). Intriguingly, mitochondria have several components that function as cell death regulators. Following intrinsic or extrinsic excessive stress beyond the recovery state associated with mitochondrial membrane depolarization, cells die either via apoptosis or other modes of cell death like necrosis (Zong and Thompson, 2006, Parlato et al., 2014 and Wasser, 2002). Hence, to assess the involvement of mitochondria in mushroom mixture extracts induced cell death, the mitochondrial membrane potential (MMP) was studied using a cationic fluorescent probe JC-1. It can selectively enter into the mitochondrion of cells, and reversibly change from red to green fluorescence as the mitochondrial membrane potential decreases. By measuring such a shift in the fluorescence emission by fluorescence microscopy, MMP was readily detected. As shown in Plate Imushroom mixtureextracts increased the ratio of green fluorescence in both cell lines which evidences the mushroom mixture extracts induces cell death with the involvement of mitochondrial membrane depolarization. Apoptosis is a programmed, managed form of cell death, occurs without eliciting local inflammatory response. Apoptosis is an important physiological process for the maintenance of tissue homeostasis and plays a pivotal role in the pathogenesis of various disorders (Agostini et al., 2010). In some pathological conditions, the crisis is due to abnormal apoptosis, while in others, defective apoptosis is the cause. Cancer is one of the scenarios where, defective apoptosis is also a major causative factor, in addition to uncontrolled proliferation, resulting in tumour cells that will not die (Hipfner and Cohen, 2004; Shon & Nam, 2002). Therefore, the unrestrained growth of malignant cells and deregulation of apoptosis has become the major targets for anticancer strategies (Chan et al., 2012). It was observed that mushroom mixture extractsinduces non-apoptotic cell death in MCF-7 cell lines cancer cells (Plate I). The
extracts of two mushroom mixture induce apoptotic morphological changes in MCF-7 cells such as nuclear shrinkage, DNA condensation, fragmentation, membrane blebbing and formation of apoptotic bodies. The JC1 staining result also suggested that the extracts of mushroom mixture triggered apoptosismediated cell death in MCF-7. Mitochondrial swelling is often associated with loss of transmembrane potential and it is considered as an important phenomenon, which occurs as an early event of apoptosis. Loss of mitochondrial membrane potential followed by the release of cytochrome C from mitochondria to the cytosol leads to subsequent activation of caspases resulting in apoptosis.In the present study, the loss of mitochondrial membrane potential was observed in extracts-treated cells and analysed using JC1 dye. These staining results suggest that the extracts oftwo mushroom mixture triggered apoptosis-mediated cell death in MCF-7 cell lines.

## REFERENCES

1. Agostini, M., Tucci, P., Chen, H. 2010. p73 regulates maintenance of neural stem cell. Biochem. Biophys. Res. Commun., 403, 13-17.
2. Agostini, M., Tucci, P., and Melino, G. 2011. Cell death pathology: perspective for human diseases. *Biochem. Biophys. Res. Commun*., 414: 451–455.
3. Baylin, S.B., and Ohm, J.E., Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? *Nat. Rev. Cancer*, 6(2): 107-116.
4. Borchers, A.T., Stern, J.S., and Hackman, R.M., 1999. Mushrooms, tumours, and immunity. *In:* Proceedings of the Society for Experimental Biological Medicine. 221: 281-293.

5. Bray, F., Jemal A, Grey N, Ferlay J, Forman D. 2012. Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. *Lancet Oncol.*, 13(8): 790-801.
6. Chan, K.S., Koh, C.G.. and Li, H.Y., 2012. Mitosis-targeted anti-cancer therapies: where they stand. *Cell Death Dis.*, 3: e411.
7. Emens, L.A., and Jaffee, E.M., 2005. Leveraging the activity of therapeutic cancer vaccines with cytotoxic chemotherapy. *Cancer Res.*, 65: 8059-8064.
8. Ferlay, J., *et al.*, 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer*, 136(5): 359-386.
9. Galluzzi, L., Kepp, O., Trojel-Hansen C., and Kroemer G. 2012. Mitochondrial control of cellular life, stress, and death. *Circ. Res.*, 111: 1198–1207.
10. Hipfner, D.R., and Cohen, S.M., 2004. Connecting proliferation and apoptosis in development and disease. *Nat. Rev. Mol. Cell Biol.*, 5: 805–815.
11. Itoh, H., Ito, H., Amano, H., Noda, H. 1994. Inhibitory action of a (1, 6)-beta-D-glucanprotein complex (F III-2-b) isolated from AgaricusblazeiMurill. (Himematsutake) on Meth A fibrosarcoma- bearing mice and its antitumor mechanism. Jpn. J. Pharmacol., 66, 265-271.
12. Malhotra, G.K., 2010. Histological, molecular and functional subtypes of breast cancers. *Cancer Biol. Ther.*, 10(10): 955-960.
13. Matsen, C.B., and Neumayer, L.A., 2013. Breast cancer: a review for the general surgeon. *JAMA Surg*., 148(10): 971-979.
14. Matuszewska, A., Jaszek, M., Stefaniuk, D., Ciszewski, T., and Matuszewski, Ł., 2018. Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus *Cerrena unicolor*. PLoS ONE, 13(6): e0197044.
15. Natalia, B., Sonja, H., and Thilo, D., 2013. Hereditary breast cancer: ever more pieces to the polygenic puzzle. *Hered Cancer ClinPract.*, 11: 12.
16. Parlato, M., Souza-Fonseca-Guimaraes, F., Philippart, F., Misset, B., Captain Study Group. Adib-Conquy, M., and Cavaillon, J.M., 2014. CD24-triggered caspase-dependent apoptosis via mitochondrial membrane depolarization and reactive oxygen species production of human neutrophils is impaired in sepsis. *J. Immunol*., 192: 2449-2459.
17. Peng-Yun Wang, Xiao-Ling Zhu, and Zhi-Bin Lin. 2012. Antitumor and Immunomodulatory Effects of Polysaccharides from Broken-Spore of *Ganodermalucidum. Front Pharmacol., 3:* 135.
18. Rusciano, D., and Burger, M.M., 1992. Why do cancer cells metastasize into particular organs? *Bioessays*, 14(3): 185-194.
19. Shon, Y.H., and Nam, K.S., 2002. Cancer chemoprevention inhibitory effect of soybeans fermented with basidiomycetes on 7,12-dimethylbenz[*a*] anthracene/12-*O*tetradecanoylphorbol- 13-acetate-induced mouse skin carcinogenesis. *Biotechnol. Lett.*, 24: 1005–1010.
20. Wasser, S.P., 2002. Medicinal mushrooms as a source of antitumor and immunemodulating polysaccharides. *Applied Microbiol and Biotechnol.*, 60(3): 258-274.
21. Zong, W.X., and Thompson, C.B., 2006. Necrotic death as a cell fate. *Genes Dev*., 20: 1–15.