

Inhibition of *in vitro* cytotoxic effect evoked by *Alpinia galanga* and *Alpinia officinarum* on PC - 3 cell line

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Abstract :

Plants have been a source of medicine and a major resource for health care since ancient times, with some traditional herbal medicines having been in use for more than 2,000 years. Herbs and spices are recommended for prevention and cure of various diseases including cancer. *Alpinia galanga* and *Alpinia officinarum*, botanical cousin to ginger was recognized superior in many ways and has been employed in medicine for over a thousand years. Prostate cancer is the most common form of cancer in males and the second leading cause of cancer related death. PC-3 cell line was derived from adenocarcinoma of human prostate. This was assayed for MTT assay on treatment with ethanolic extract of *Alpinia galanga* and *Alpinia officinarum*, where in inhibition of the cell growth was noticed. This study was supported by DNA fragmentation where a characteristic DNA laddering was noticed in treated tumor cell line and not in the control.

Keywords : Cytotoxicity; MTT assay; *Alpinia galanga*; *Alpinia officinarum* ; DNA fragmentation.

Introduction

The human prostate gland, a male sexual accessory tissue involved in seminal fluid production, has a remarkably high incidence of neoplastic disease. Prostate cancer remains the most common non-cutaneous malignancy in the developed world and is the second-highest cause of cancer death in males¹. As local prostate cancer rarely causes symptoms, 38 %-51 % of patients present with locally extensive or metastatic disease at the time of diagnosis. Between 10 % and 50 % of clinically localized cases inevitably progress and the patients die from metastatic disease^{2,3}.

Prostate cancer mortality results from metastases to the bones and lymph nodes and progression from androgen-dependent to androgen-independent disease. Although androgen ablation was found to be effective in treating androgen-dependent prostate cancer, no effective life-prolonging therapy is available for androgen-independent cancer⁴. Therefore the androgen independent cell line (PC3) have been used as a model for the present work.

A wide number of traditionally important medicinal plants are still used by Indian traditional practitioners for the treatment of cancer. Herbal drugs play an important role

in health care programmes world wide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including cancer. Medicinal herbs and extracts prepared from them are widely used in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite ⁵.

Alpinia galanga (greater galanga) and *Alpinia officinarum* (lesser galangal) are ancient and highly revered medicinal agent in Ayurvedic, Traditional Chinese and Thai folk medicine. This botanical cousin to ginger was recognized to be superior in many ways and has been employed in medicine for over a thousand years. It has a pungent, hot and spicy taste with an aromatic, ginger-like odor. In traditional Chinese Medicine it is said as “warm the middle” and alleviate pain, being especially good for abdominal pain, vomiting, hiccups and diarrhea ⁶. It is also recognized as a powerful dispersing agent with analgesic properties that make it an appropriate and effective intervention for rheumatic pains and other inflammatory disorders ⁷. The present study was thus designed to find the anticancer effect of *Alpinia galanga* and *Alpinia officinarum* against prostate cancer cell line (PC3).

Materials and Methods

Collection of plant material: Dried root of *Alpinia galanga* and *Alpinia officinarum* were collected from Ayurvedic pharmacy, Coimbatore and authenticated by the botanist Dr. Arumugaswamy, Department of Botany, Kongunadu college of arts and science, Coimbatore, and the voucher specimen was preserved in Department of Biochemistry, Dr N.G.P Arts and Science college, Coimbatore, India.

Preparation of Plant extract: Dried root of *Alpinia galanga* and *Alpinia officinarum* were

made into coarse powder using mixer grinder. The powder obtained was successfully extracted in petroleum ether (60-80°C), chloroform, ethanol and distilled water by using soxhlet extractor. The extracts were concentrated in a rotar evaporator under reduced pressure. A pilot study revealed that ethanolic extract evoked the maximum activity. It has been found that IC₅₀ value was noted to be 37.6 for *Alpinia galanga*, and 41.45 for *Alpinia officinarum*.

Cell culture PC-3, derived from adenocarcinoma of human prostate, was purchased from the National Centre for Cell Sciences, Pune, India. The cells were grown in Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/liter sodium bicarbonate (90%) supplemented with 10% fetal bovine serum, as recommended by American Type Culture Collection. The cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

Preparing Cancer Cell Lines: Prostate Cancer Cell Line (PC-3) was grown at 37° C. in Ham's F12K Media. The cells were grown until confluent and then washed once with PBS and aspirated. The cells were then washed in Trypsin, Ham's F12K medium was added, and the cell solution was aspirated in the test tube and centrifuged for 5 minutes at 6000 rpm. After centrifugation, the supernatant was discarded and the pellet was resuspended in 2 ml of Ham's F12K medium . 0.5 ml of this solution was combined with 15 ml of Ham's F12K medium and placed in a cell culture tube. The cells were counted by placing 20 µl of the cell suspension in a Hemocytometer, diluting as necessary for calculation. The cell concentration was kept at approximately ten thousand per µl for testing against the extracted compounds.

MTT Assay: The cells were seeded in 96 well plate (1X10⁶ cells/well) and allowed to attach by incubating at 37°C for 24 hours. The cells were treated with the drug (10,

25, 50, 100 µg of the ethanolic extract of *Alpinia galangal* and *Alpinia officinarum* respectively) and incubated the wells for 24hrs. The medium was removed and 100 µl of MTT reagent added to the well, and after incubation for 24hrs at 37°C, the medium was removed completely. 100 µl of SDS has then added to each well and incubated at 37°C for 4 hours, the well plates were read at 570nm using ELISA reader. Each assay was performed in triplicate⁸.

DNA Fragmentation Analysis., PC-3 cells were seeded in 6 well plates and allowed to adhere overnight. After treatment with the durg (ethanolic extract of *Alpinia galanga* and *Alpinia officinarum*) respectively as per the same schedule described above, cells were harvested and then lysed in a solution containing 100 mM NaCl, 10 mM Tris (pH 7.4), 25 mM EDTA, and 0.5% SDS. After the centrifugation, the supernatants were incubated with 300 mg/ml

proteinase K for 5 h at 65°C and extracted with phenol-chloroform. The aqueous layer was treated with 0.1 volume of 3 M sodium acetate, and the DNA was precipitated with 2.5 volumes of 95% ethanol. After treatment with 100 mg/ml RNase A for 1 h at 37°C, the sample was electrophoresed on a 2% agarose gel and stained with ethidium bromide⁹.

Results and Discussion

Cancer has been scourging on the human population since long. Although numerous advances have been made in prevention, diagnosis and treatment of the disease, it still continues to torment mankind^{10, 11}. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases, inflammation and problems caused by cell and cutaneous aging¹².

Cell viability (MTT) Assay

S.No	Concentration of Extract in(µg)	Percentage inhibition	
		<i>Alpinia galanga</i>	<i>Alpinia officinarum</i>
1.	10	28±2.36	25 ± 1.46
2.	25	44± 1.53	41 ± 3.15
3.	50	55±1.82	52 ± 2.63
4.	100	69±3.91	63 ± 2.80

Values represented as mean ± SD(N=3)

Fig 1 MTT CYTOTOXICITY ASSAY OF *Alpinia galanga*

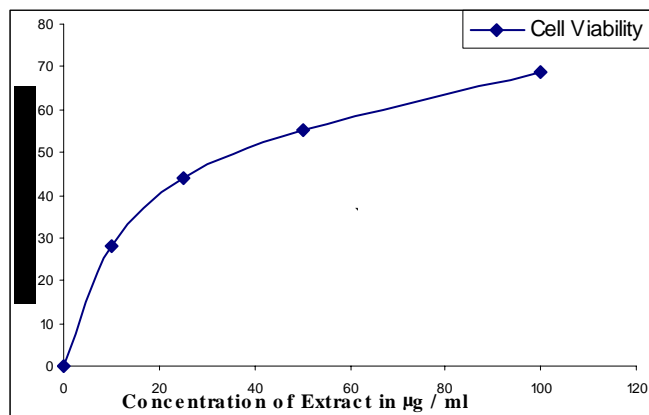
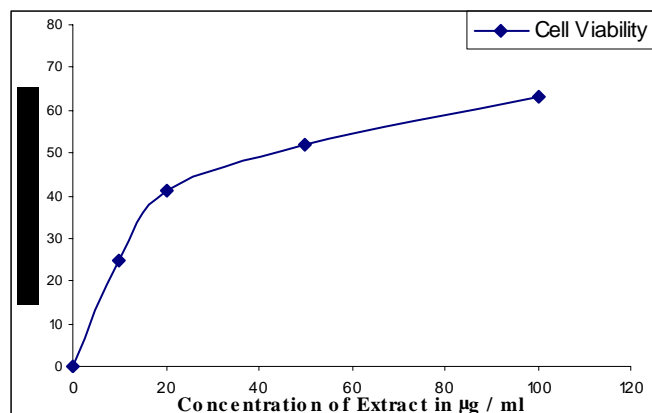


Fig 2 MTT CYTOTOXICIRY ASSAY OF
Alpinia officinarum



4.5.2 DNA Fragmentation Assay

The concentration of the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum* which showed 50 % inhibition of Tumor cell line growth as obtained from the MTT assay was used for the DNA fragmentation assay. Fragmentation of genomic DNA to high molecular weight (180kb-200kb) fragments is a characteristic of the early event in apoptosis and may represent the key step of the process. Apoptosis or programmed cell death represents a physiological form of cell death that occurs during development and in the mature animal.

Agarose gel electrophoresis was used to evaluate whether high molecular DNA fragmentation was a feature of the cellular response of the tumor cell to the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum*. The internucleosomal DNA was detected on agarose gel as a ladder of DNA fragment (figure 2) and this confirmed that the cytotoxic effect of the *Alpinia* extracts were mediated via apoptosis. It is emphasized that there was a close relationship between apoptosis and proliferation in malignant neoplasm cells. A characteristic DNA laddering was noticed only in tumor cell line treated with the ethanolic extract of *Alpinia galanga* and

Alpinia officinarum. This pattern was not observed in the control cells. The data reported here demonstrated the lethal injury to carcinoma cells via activation of the cell death by both the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum* (fig3).

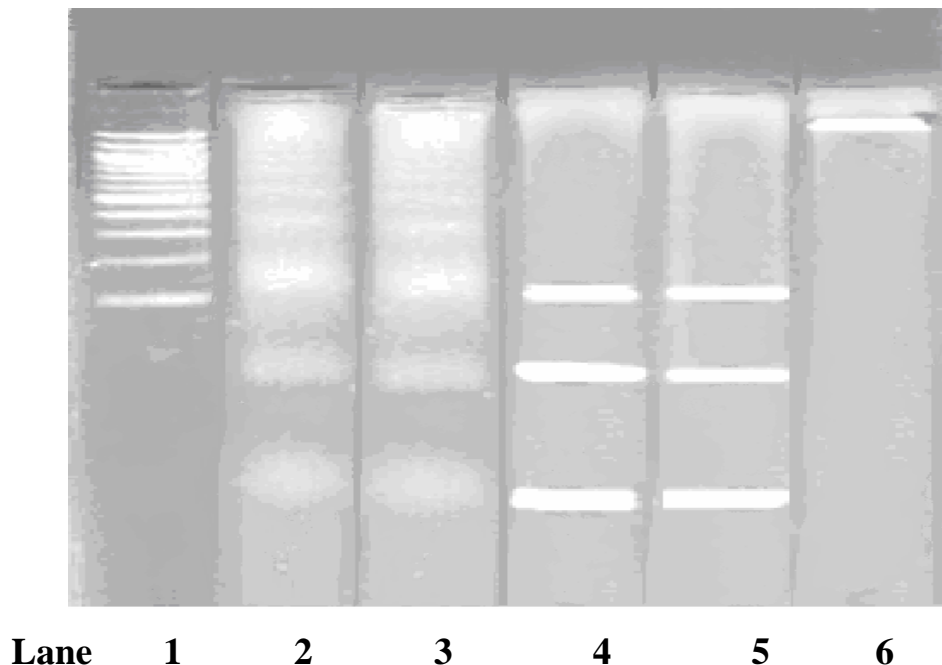
Emerging research suggests that *Alpinia* extracts may have potent anticancer activity, for both breast and prostate cancer. Research shows that 30 to 35% of all cancers can be prevented by eating well, being active and maintaining a healthy body weight (Canadian Cancer Society). However, much more research, including human studies, needs to be done before any specific recommendations can be made.

The specificity of an anticancer compound against cancer cell depends on the proliferation rates of the treated cells. Cancer cells proliferate more rapidly than normal cells, which make them more sensitive to drug treatment. Plant-derived anticancer agents act by entering with cancer cell proliferation or by the induction of apoptosis in tumor cells¹³. The advantage of apoptosis inducing anticancer drugs is that the apoptotic bodies formed can be

scavenged by the human body's immune system without inducing an inflammatory response¹⁴. In addition to apoptosis induction, another common effect shared by many anticancer drugs is the introduction of cell cycle arrest. Cell cycle arrest has been found in cells exposed to a variety of

stimuli, including irradiation, microtubule stabilizing agents and topoisomerase inhibitor¹⁵. Lee C.C. & Houghton P. (2005) has established that extracts from *A. officinarum* can exhibit cytotoxicity against human cancer cell-lines¹⁶

Fig. 3 GEL Electrophoresis



Based on the above study the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum* were able to inhibit the growth and induce apoptosis of cancer cells *in-vitro*.

Conclusion

The MTT analysis carried out in the prostate cancer cell line (PC-3) revealed that the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum* could effectively reduce the growth and multiplication of the tumor cells and ultimately suppress the growth of

the tumor cells. This study was supported by DNA fragmentation analysis which proved that characteristic DNA laddering was noticed only in tumor cell line treated with the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum*. This pattern was not observed in the control cells. The anticarcinogenic effect of the ethanolic extract of *Alpinia galanga* and *Alpinia officinarum* may be due to rich antioxidant content and the presence of secondary metabolites like flavonoids present in them.

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