

## Study on the Apoptotic properties of methanolic extracts of *Peltophorum pterocarpum*, *Cassia auriculata*, *Cassia alata* and *Lamprachaenium microcephalum*

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### ABSTRACT

*Peltophorum pterocarpum* (Tam: Malai porasu), *Cassia auriculata* (Tam: Avaram), *Cassia alata* (Tam: Seemaiganthi) and *Lamprachaenium microcephalum* (Tam: Brahmadandi) were chosen for the study to induce apoptosis. The methanolic extracts of the leaves were dried and dissolved in DMSO in concentration of 100mg/mL for treating MCF-7 (Breast cancer) cell line grown in RPMI- C medium with 10% Foetal Bovine Serum at pH 7.1, temperature 37°C and 5% CO<sub>2</sub>. The cytotoxicity of extracts on MCF-7 was determined by MTT Assay and found to be as 3mg/ml for *Peltophorum pterocarpum* and *Cassia auriculata* and 1mg/ml for *Lamprachaenium microcephalum* and *Cassia alata*. The sublethal concentrations of all the four extracts were treated and the morphology of cells after AOEB/Hoechst 33258 staining was visualized under Epifluorescence. Cells treated with *Lamprachaenium microcephalum* and *Cassia alata* showed resemblance to Apoptotic death while that of *Peltophorum pterocarpum* and *Cassia auriculata* resembled much of Necrosis.

**Key words:** *Peltophorum pterocarpum*, *Cassia auriculata*, *Cassia alata*, *Lamprachaenium microcephalum*, MTT Assay, Apoptosis, AOEB, Hoechst 33258

### INTRODUCTION

Cancer is one of most dreadful diseases of 20<sup>th</sup> and 21<sup>st</sup> century. Although people of all ages develop cancer, most types are more common in people over the age of 50. Cancer usually develops gradually over many years, the result of a complex mix of environmental, nutritional, behavioral, and hereditary factors. Apoptosis is a fundamental physiological process in mammals in which cells die by activating an Intrinsic Suicide Mechanism<sup>1</sup>. Defects in apoptotic signaling pathways play critical roles in a multiplicity of pathophysiological status including cancer. The highly regulated systematic nature of apoptosis lends itself to distinct morphological and biochemical criteria including selective and tightly controlled activation of

proteolytic cascades that result in an ordered disassembly of cells<sup>2</sup>.

Modern surgery had significantly reduced the cancer mortality rate, chemotherapy and radiation therapy were reducing the death rate not more than 5% and they are producing numerous side effects. But majority of tumor cells were resistant to these therapies therefore search for the alternative agent which can cure tumor without any side effects is under way. Among them, herbal plants are used as complementary and alternative medicine. Use of herbal medicines in Asia represents a long history of human interactions with the environment. According to World Health organization (WHO) more than 80% of the World's population relies on traditional medicine for their

primary health care. These medicinal herbs were used for long time to cure illness and some of these plants were believed to promote resistance against infection. Recently great emphasize is given towards the complementary and alternative medicine treatment. Ayurveda is one of the best complementary medicinal treatments for cancer therapy. Ayurveda describes cancer as inflammatory or non-inflammatory swellings<sup>3</sup>.

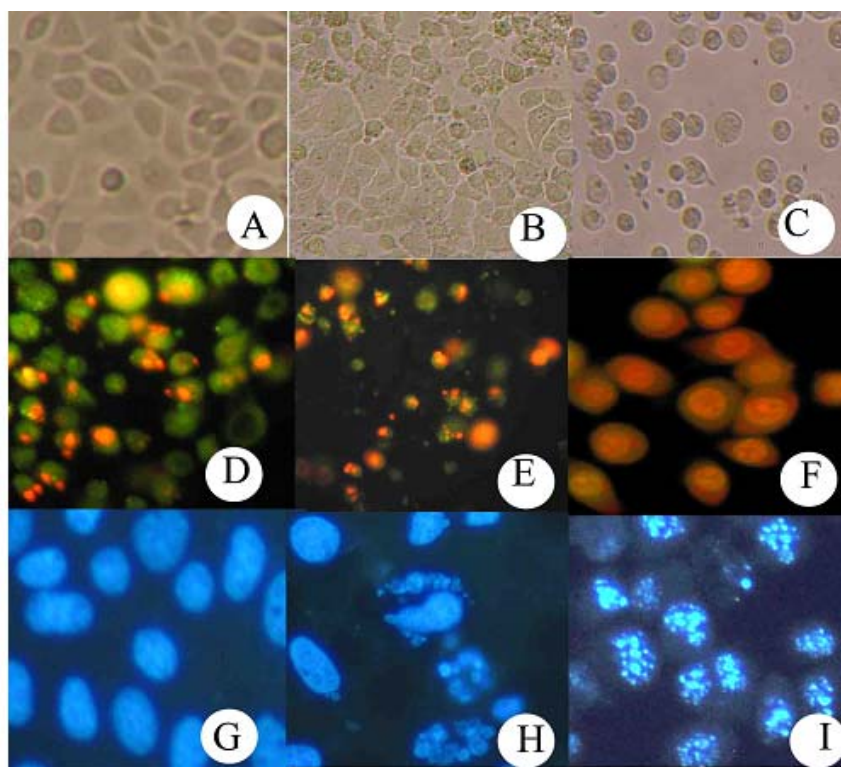
Herbal decoctions consisting of multiple herbs each possessing tremendous potential for a cancer cure are commonly used in Ayurveda. These formulations are reported to work on multiple biochemical pathways and are capable of influencing several organ systems simultaneously. The benefit of a herbal decoction is that it can nourish the body as a whole by supporting various organ systems.

In the present study, methanolic extracts of the leaves of *Peltophorum pterocarpum*, *Cassia auriculata*, *Cassia alata* and *Lamprachaenium microcephalum* have been tested for the ability to induce cell death in cancer cell line by apoptosis as determined by AOEB / HOESCHT 33258 staining and morphological examination.

## MATERIAL AND METHODS

### Plant Collection and Methanolic Extraction

Plants such as *Cassia auriculata*, *Cassia alata*, *Peltophorum pterocarpum* and *Lamprachaenium microcephalum* were collected from places around Tiruchirappalli District and identified by the Postgraduate and Research Department of Plant Biology and Plant Biotechnology, St. Joseph's college (Autonomous), Tiruchirappalli. Leaves of the plants were dried



A. D. G. - MCF Cell Line (Control)  
 B. E. H. - MCF Cell Line after 24 Hours of Treatment  
 C. F. I. - MCF Cell Line after 48 Hours of Treatment.  
 A. B. C. - MCF Cell Line (Phase Contrast Microscopic View)  
 D. E. F. - AOEB Stained MCF Cell Line (Fluorescent Microscopic View)  
 G. H. I. - Hoechst 33258 Stained MCF Cell Line (Fluorescent Microscopic View)

**Fig. 1: Microscopic views of MCF - 7 cell lines treated with *Lamprachaenium microcephalum***

under shade, powdered and extracted using methanol as solvent by hot extraction method with Soxhlet apparatus. The extracts were collected, dried and used for treating the cells at various concentrations<sup>4</sup>.

#### Cell lines

MCF-7 Cell line was derived from National Centre for Cell Science, Pune. The Cell line was cultured in RPMI- C medium with 10% Foetal Bovine Serum. The cell line was cultured at pH 7.1, temperature 37°C with 5% CO<sub>2</sub>. All reagents and media were purchased from Himedia, Mumbai, India.

#### RPMI Complete Medium (RPMI-C)

RPMI – 1640 was supplemented with 10% heat – inactivated foetal Bovine serum (FBS), 2 mM L- Glutamine, 20mM HEPES Buffer, 100µg/ml penicillin, 150µg/ml streptomycin and 50µM 2-mercapto-ethanol.

#### MTT Assay

MTT (3 - 4,5 - dimethyl thiazol-2-yl) 2-5, diphenyl tetrazolium bromide), a pale yellow substrate is converted into a formazan, a violet compound by the activity of succinate dehydrogenase of mitochondria. Since the conversion takes place in living cells, the amount of formazan is directly correlated with the number of viable cells present. MTT assay was done following the method of Mosmann with slight modification<sup>5,6,7</sup>. In brief, cells (1×10<sup>5</sup> per well in 200µl medium) were seeded in 96 well plate and allowed to adhere for 24 hours at 37°C with 5% CO<sub>2</sub> in air. Medium was aspirated and replaced with medium containing methanolic extracts in DMSO in concentrations ranging from 1, 10, 100, 1000 and 10000mg/ml for 24 hours at 37°C with 5% CO<sub>2</sub> in air. Then 10µl of MTT (5mg/ml stock solution) in PBS was added to each well containing 100 µl of cell suspension and re-incubated for 4 h at 37°C. The reaction mixture was carefully taken out and 200 µl of DMSO was added to each well and mixed thoroughly. After 10 minutes, the color was read at 530nm using multi-well microplate reader (BIORAD, USA). The untreated sets were also run parallel under the identical conditions and served as 'Control'.

#### AOEB Staining

MCF-7 cells were cultured in RPMI-C at 37°C in a humidified atmosphere of 5%CO<sub>2</sub> in air for 24 or 48 h in the presence or absence of each methanolic extracts of the leaves mentioned above. After treatment, 100µl of these cell suspensions (1×10<sup>5</sup> cells/ml) in RPMI-1640 were collected, centrifuged at 200g for 1 minute and mixed with 1µl of dye mix (1µg/ml acridine orange plus 1µg/ml Ethidium bromide in PBS). Observation was carried out at 400x using an epi-illumination microscope (Olympus, Japan) with a filter combination suitable for fluorescein visualization. At least 200 cells were counted and the number of cells with fragmented nuclei, increased cytoplasm and condensed chromatin, which reliably indicate apoptosis, were determined as previously described<sup>8,9,10</sup>.

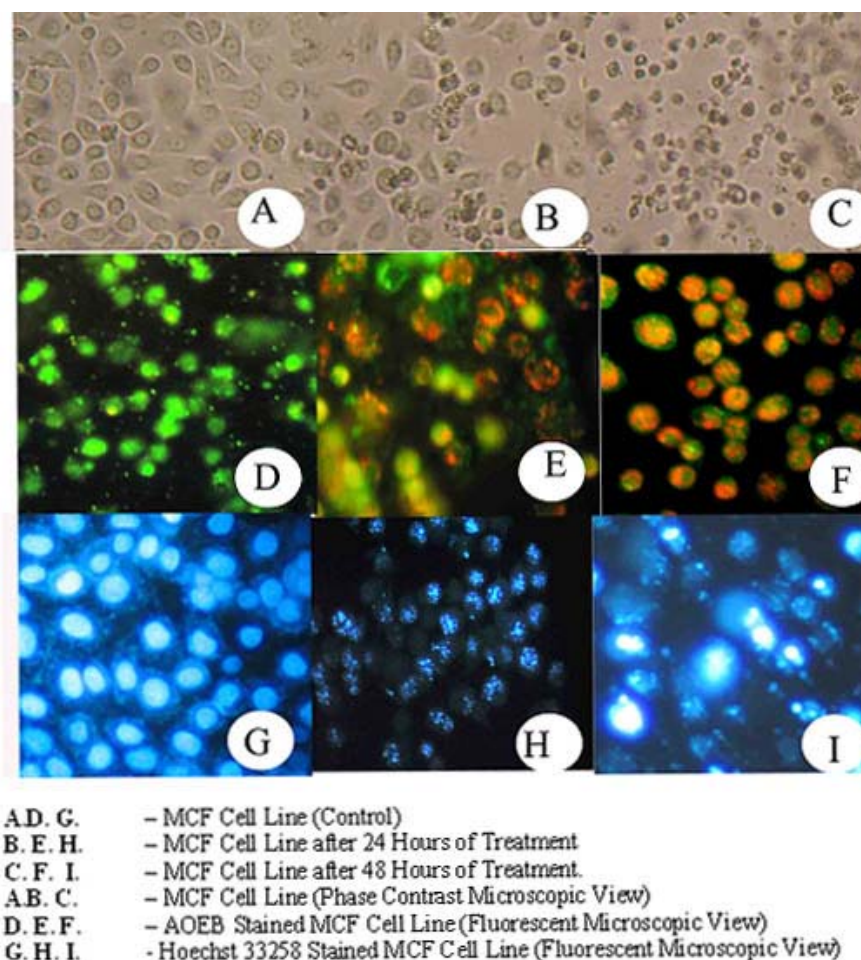
#### Hoechst H-33258 Staining

About 1×10<sup>5</sup> cells were washed twice with PBS and then re-suspended in 500 µl of Ice cold methanol and placed at 40C for 5 minutes. Cells were washed once with PBS and resuspended in a solution of Hoescht H-33258 nuclear stain (Sigma) (1/100 dilution of Hoescht H-33258 in PBS) and placed for 5 minutes at 4°C (Light protected). Samples were subsequently washed twice with PBS and finally the pellet was resuspended 20µl of PBS. Cells were spread on microscope mounting plate, covered with a cover slip and analyzed under a Fluorescence Microscope (Olympus, Japan) with emission at 492 nm with excitation at 356 nm. Nuclei exhibiting fragmentation and disintegration were observed.

## RESULTS AND DISCUSSION

#### Cell Viability Assay

As determined by MTT assay, the methanolic extracts of the leaves of *Peltophorum pterocarpum*, *Cassia auriculata*, *Cassia alata* and *Lamprachaenium microcephalum* strongly affected the survival of the MCF-7 cell line in time and dose dependent manner. MCF-7 cell line is a breast cancer cell line being very sensitive to cytolytic agents and is easily maintained. In the study, the cells were treated with various concentrations of the extracts ranging from 1, 10, 100, 1000 and 10,000 mg/ml and the cell viability was measured



**Fig. 2: Microscopic views of MCF - 7 cell lines treated with *Cassia alata***

by MTT assay. The inhibition of the cell viability was clearly observed in a dose-dependent manner. The  $IC_{50}$  value was 3mg/ml for *Peltophorum pterocarpum* and *Cassia auriculata* and 1mg/ml for *Lamprachaenium microcephalum* and *Cassia alata* for MCR-7 after 24 hours (Table 1).

#### Analysis of Apoptosis

Acridine orange (AO) - Ethidium bromide (EB) and Hoechst staining were performed to evidence possible changes in cellular morphology. The control cells appeared green and blue respectively (Fig.1 and 2), whereas methanolic

**Table 1:  $IC_{50}$  of methanolic extracts at 24 and 48 Hours**

S. No.	Plant Name	$IC_{50}$ 24 hours		$IC_{50}$ 48 hours	
		$\mu\text{g}/200\mu\text{L}$	$\mu\text{g}/1\text{mL}$	$\mu\text{g}/200\mu\text{L}$	$\mu\text{g}/1\text{mL}$
1	<i>Cassia auriculata</i> & <i>Peltophorum pterocarpum</i>	200	1000	30	150
2	<i>Lamprachaenium microcephalum</i> & <i>Cassia alata</i>	300	1500	60	300

extract - treated cells were shown to possess abnormalities. Microscopic images of the cells treated with *Lamprachaenium microcephalum* and *Cassia alata* revealed nuclear alterations typical of the apoptotic process. Both the stains revealed cytoplasmic blebbing, presence of apoptotic bodies, marginalization of chromatin and innumerable micronuclei in cells treated with both the methanolic extracts of *Lamprachaenium microcephalum* and *Cassia alata* (Fig 1 and 2).. On the other hand, death pattern of cells treated with *Cassia auriculata* and *Peltophorum pterocarpum* was much similar to necrosis.

### CONCLUSION

Results from this study allow us to demonstrate that it is mainly the extracts of

*Lamprachaenium microcephalum* and *Cassia alata* to inhibit tumor cells. Such inhibition may be mediated by Apoptosis. It is also further implicated from the study that the methanolic extracts of *Lamprachaenium microcephalum* and *Cassia alata* can be re-extracted with other solvent such as Chloroform, ethyl acetate and water for further analysis into the suspected apoptosis mediated cell death.

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