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IN VITRO ANTICANCER ACTIVITY OF Carica papaya (FRUITLESS TYPE) EXTRACT AGAINST HUMAN MCF-7 CELL LINE

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ABSTRACT

Breast cancer is the most common cancer in women worldwide. It is a type of cancer where cells in the breast divide and grow without normal control. MCF-7 is breast cancer cell lines isolated in 1970 from a 69-year-old Caucasian woman and are useful for in vitro breast cancer studies. Hence, the present study aims to investigate the Carica papaya for their anticancer activity against human breast cancer cell line viz. MCF-7. The cell growth inhibition of the Carica papaya (Fruitless Type) extract tested against MCF-7 cell line at different concentrations (25, 50, 100, 200 and 400 µg/ml). The cell growth inhibition of the Carica papaya (Fruitless Type) extract revealed that the concentrations increases there is an increase in the cell growth inhibition but is found to be lowest growth inhibition was 9.38 % at 25 µg/ml and highest growth inhibition was 71.76% at 400 µg/ml. The IC_{50} value was more than 233.01µg/ml. The results of the study revealed that the Carica papaya extract possesses anti-cancer activity.

Keywords: Breast cancer, Carica papaya, Michigan Cancer Foundation-7, Anti-cancer activity

I.INTRODUCTION

Plants have long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. For example, Hartwell has collected data on about 3 000 plants, those of which possess anticancer properties are subsequently used as potent anticancer drugs (Graham et al., 2000; Cragg and Newman, 2003; Shoeb, 2006). Plant secondary metabolites and their semi-synthetic derivatives continue to play an important role in anticancer drug therapy (Indap, 2006; Cragg et al, 2009). These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Several promising new agents are in clinical development based on selective activity against cancer related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources (Pan et al., 2010; Shoeb et al, 2006).

Use of plants for medicinal remedies is an integral part of the Indian cultural life and this is unlikely to change in the years to come. Many traditional healers and herbalists in the Tamil Nadu state of India have been treating cancer patients for many years using various medicinal plant species (Jain and Jain, 2010). Hence, an attempt has been made to screen some medicinal plants used for the prevention and treatment of cancer in Chhattisgarh state, India. It is generally known that ethnomedical data provide substantially increased chance of finding active plants relative to random approach (Cordell et al., 1991; Kintzios, 2006). Uncontrolled proliferation is a universal property of tumor cells. Investigation of the cellular growth control mechanism has contributed to the understanding of carcinogenesis and to the identification of compounds with specific antitumoral activity (Kang et al., 2000; Ruffa et al., 2002). In this study, we have explored *Carica papaya* (Fruitless Type) extract for their cytotoxic activity on the human MCF-7 cell line.

II. MATERIALS AND METHODS

2.1. Collection of Plant Material

The plant material was collected from the Kolli Hills. This is a fruitless type species.

2.2. Preparation of Extract

The leaves were dried at room temperature for 7 days. Then the leaves were ground well. The plant extract was prepared by dipping 20 g powder sample in 200 ml methanol for 24 Hours. The extract was prepared by cold perculation method. The extract was obtained by filtering.

2.3. In Vitro Abticancer Activity (MTT assay)

The Cytotoxicity of samples on MCF-7 was determined by the MTT assay (Mosmann, 1983; Monks et al., 1991). The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of 1x105 cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO_2, 95% air and 100% relative humidity. Aliquots of 100 µl of different concentrations of sample A, B, C and D extracts (25, 50, 100, 200 and 400µg/ml) dissolved in DMSO (1%) were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations for 48h at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 48h of incubation, to each well 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) phosphate- buffered saline solution was added and incubated at 37°C for 4 h. Then, 100µl of 0.1% DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for inhibition Concentration (IC_{50}) was determined graphically. The absorbance at 570nm was measured with a UV- Spectrophotometer. The medium without samples served as control and triplicate was maintained for all concentrations. The effect of the samples on the proliferation of MCF-7 was expressed as the % cell viability & % Cell growth inhibition using the following formulas:

% Cell viability = Abs 570 of treated cells / Abs 570 of control cells × 100%.

% Cell growth inhibition = [100- Abs (sample) /Abs (control)] x100.

III. RESULTS

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line and useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen via estrogen receptors. The cell growth inhibition of the *Carica papaya* (Fruitless Type) extract tested against MCF-7 cell line at different concentrations (25, 50, 100, 200 and 400 µg/ml) (Plate 1). The cell growth inhibition of the *Carica papaya* (Fruitless Type) extract revealed that the concentrations increases there is an increase in the cell growth inhibition but is found to be lowest growth inhibition was 9.38 % at 25 µg/ml and highest growth inhibition was 71.76% at 400 µg/ml (Plate 4.1). The IC_{50} value was more than 233.01µg/ml (Table 1 and Fig 2-3).

TABLE 1 Percentage cell growth inhibition of Carica papaya extract on MCF 7cell line by MTT assay

S.No.	Concentrations (µg/ml)	Absorbance (Optical density)	Cell Viability (%)	Cell growth inhibition (%)
1.	25	0.331	90.61	9.38
2.	50	0.291	79.59	20.40
3.	100	0.233	63.66	36.33
4.	200	0.177	48.360	51.63
5.	400	0.103	28.23	71.76
6.	Cell Control	0.366	100	0
Half Inhibition Concentration (IC_{50})				233.01µg/ml

FIG. 1: Percentage cell growth inhibition of Carica papaya extract on MCF 7cell line by MTT assay

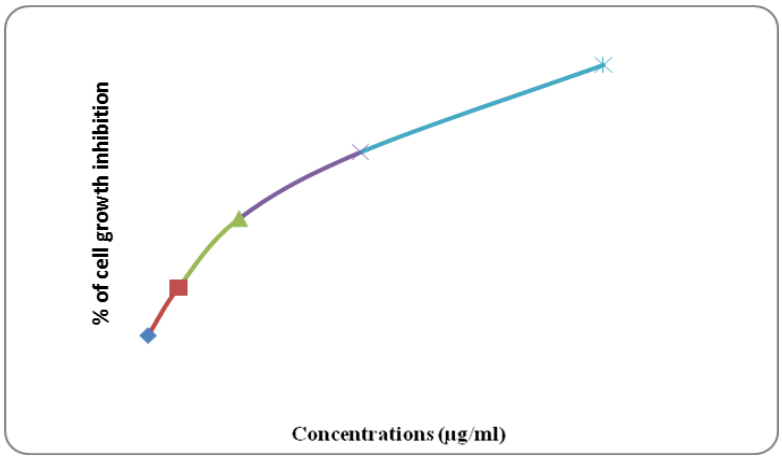


FIG. 2: Percentage of cell viability of *Carica papaya* extract on MCF 7cell line by MTT assay

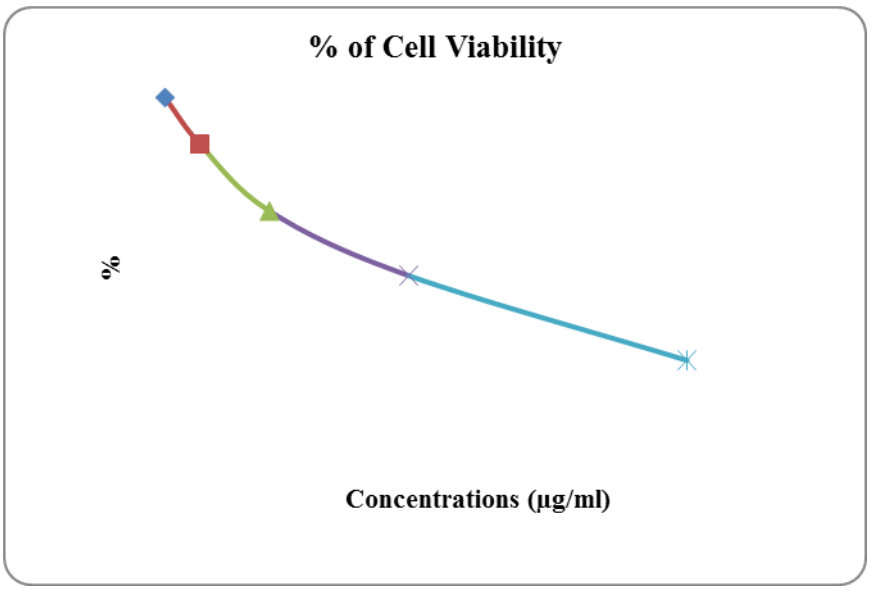
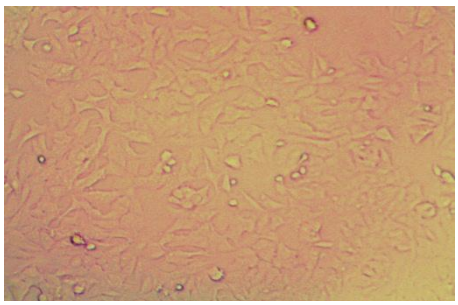
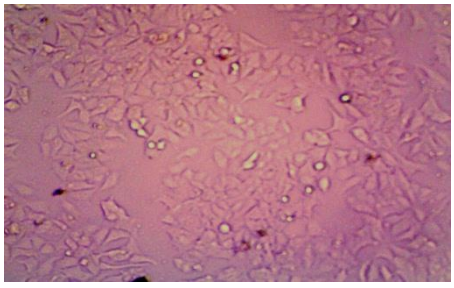


FIG 3: Plate 1 Photomicrograph of MCF-7 cell line of Carica papaya

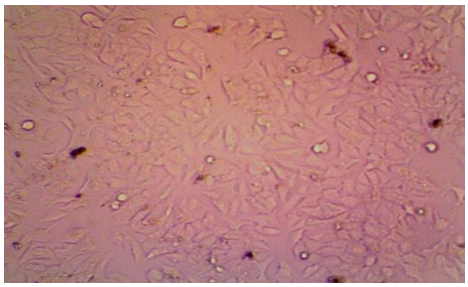
Control



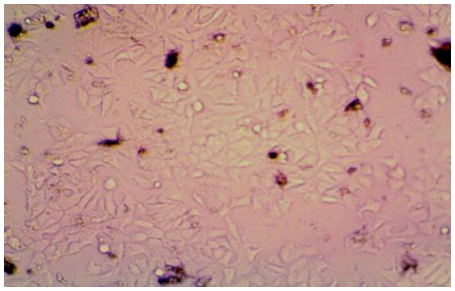
25µg/ml



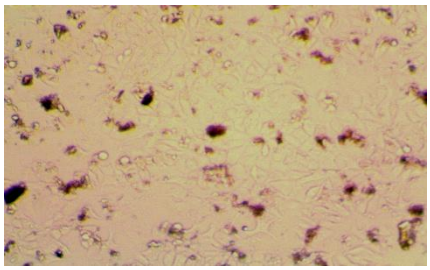
50µg/ml

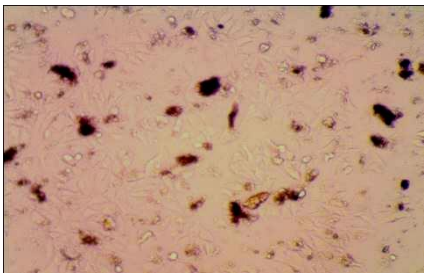


100µg/ml



200µg/ml **400µg/ml**





- Normal cells showing surface architecture
- Cytotoxic cells shows the cells became rounder, shrunken and showed signs of detachment from the surface of the wells denoting cell death (Block spot).

IV. DISCUSSION AND CONCLUSION

Breast cancer is the most common cancer in women worldwide. It is a type of cancer where cells in the breast divide and grow without normal control. The incidence of breast cancer has doubled during the past 30 years. Between 50 and 75 per cent of breast cancers begin in the ducts, 10 to 15 per cent begin in the lobules and a few begin in other breast tissues (Dillon et. al., 2010). Fortunately, the mortality rate from breast cancer has decreased in recent years with an increased emphasis on early detection and more effective treatments (Http://www.imaginis.com/general-information-on-breast-cancer/what-is-breast-cancer-2). Several commonly used herbs have been identified by the National Cancer Institute as possessing cancer-preventive properties. Those include members of the Allium sp. [garlic, onions and chives], members of the Labiatae family [basil, mints, oregano, rosemary, sage, and thyme], members of the Zingiberaceae family [turmeric and ginger], members of the Umbelliferae family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley). In addition, many herbs contain a variety of phytosterols, triterpenes, flavonoids, saponins, and carotenoids, which have been shown from studies of legumes, fruit, and vegetables to be cancer chemoprotective (Jaikumar and Jasmine, 2016). Hence, the present study aims to investigate the *Carica papaya* for their potential anticancer activity against human breast cancer cell line viz. MCF-7.

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers (Mosmann, 1983) The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute (Nagamine *et al*., 2009).

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line that was first isolated in 1970 from the malignant adenocarcinoma breast tissue of a 69-year old woman. MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen via estrogen receptors. MCF-7 cells are also sensitive to cytokeratin. When grown *in vitro*, the cell line is capable of forming domes and the

epithelial like cells grow in monolayers. Growth can also be inhibited using tumor necrosis factor alpha (TNF alpha) (Son et al., 2009).

The morphological changes of the cell lines treated cells with various concentrations of *Carica papaya* extract was incubated for 24 h and compared with the untreated cells. Compared to control cells after the incubation period, morphology of the *Carica papaya* extract treated cancer cells significantly changed. The extract treated cells appeared less uniform with the loss of membrane integrity, although still intact at lower concentrations. Whereas at higher concentrations the extract treated cells showed remarkable difference with the control group. The significant changes such as loss of intact membrane, karyopyknosis, cell detachment from the plate and change of morphological features were evident when compared to untreated cells.

The most identifiable morphological features of apoptosis were observed by inverted light microscopy in the extract treated cells. The treated cells appeared like cells undergoing apoptosis with prominent features such as detaching from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighbouring cells (Monga *et al*., 2013). However the untreated cells appeared normal and were confluent.

The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death (Plochmann *et al*., 2007). The main objective of this assay is to check the cytotoxicity brought about by the extract and find the toxicity levels in terms of IC_{50} dose when live and dead cell percentages are equal, which is considered as the optimum dose for the various assays. It has been shown that the *Carica papaya* extract possesses anti-cancer activity at higher concentration. Chemoprevention and dietary modification studies are underway to identify promising candidates for reduced cancer risk. It is concluded that the extract of *Carica papaya* had anticancer properties against breast carcinoma MCF-7 cell line.

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