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Cytotoxic activity of *Acalypha indica* L. hexane extract on breast cancer cell lines (MCF-7)

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ABSTRACT

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Research advances over the past two decades have changed the landscape of breast cancer care. Genetic testing, targeted treatments and more precise surgical techniques have helped boost survival rates in some cases while helping to support breast cancer patients' quality of life. The herb *Acalypha indica* which belongs to Euphorbiaceae family has multiple medicinal properties which include anti-fungal, anti-bacterial, anti-oxidant, anti-inflammatory, anti-helmintic, anti-ulcer, anti-venom, anti-cancerous, and neuro-protective activity. The present study emphasises cytotoxic activity of hexane leaf crude extract of *Acalypha indica* Linn. On mcf-7cell lines by MTT (3-(4, 5-Dimethylthiazol-2)-2,5-Diphenyltetrazolium Bromide) assay method using Cisplatin as a positive control. Hexane Crude extract of different concentrations (10µg/ml, 25µg/ml, 50µg/ml and 100µg/ml) were treated with cell lines, Out of these concentrations 50µg/ml showed maximum inhibitory effect (IC_{50} value). Hence the present study is taken up to systematically evaluate the anti-cancer properties of the extracts and also to isolate and characterize the active principles of *Acalypha indica*.

Keywords: Cancer, MTT reagent, Cytotoxic activity, Trypsinization, Cisplatin, IC_{50} values.

INTRODUCTION

Cancer starts from the primary tumour. Cancer sometimes spread to other parts of the body resulting in secondary tumour or a metastasis. Cancer and its treatments affect body systems, such as the blood circulation, lymphatic and immune systems, and the hormone system. Cancers are divided into groups according to the type of cell they start from. They include Carcinomas, Lymphomas, Leukaemia's, Brain tumours, Sarcomas. There are many types of cancers effecting man and women. Unlikely women get easily affected to cancers compared to men. One of such cancers which women easily get affected is breast cancer. Breast cancer is the most common invasive cancer in females worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. 18.2% of all cancer deaths worldwide, including both males and females, are from breast cancer Breast cancer usually occurs in the inner lining of lobules (or milk ducts) that supply them with milk. A malignant tumour can spread to other parts of the body. A breast cancer that started off in the lobules is known as lobular carcinoma, while one that developed from the ducts is called ductal carcinoma. Breast cancer rates are much higher in developed nations when compared to developing countries. There are several reasons for this, breast cancer is more common in elderly women. The different lifestyles and eating habits of females also contributory factors, experts believe. According to the National Cancer Institute, 232,340 female breast cancer. MCF-7 is a breast cancer cell line isolated from a 69-year-old Caucasian woman, in a year 1970 [1]. MCF-7 is named after Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-worker. It is 45 years since a pleural effusion from a patient with metastatic breast cancer led to the generation of the MCF-7 breast cancer cell line. MCF-7 is the most studied human breast cancer cell line in the world, and results from thiscell line have had a fundamental impact on breast cancer research and outcomes of patients [2]. Prior to MCF-7, it was not possible for cancer researchers to obtain a mammary cell line that was capable of living longer than a few months. Other breast cancer cell lines, named T-47D and MDA-MB-231, account for more than two-thirds of all abstracts reporting studies on breast cancer cell lines, concluded from a Medline-based survey. 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, in the form of estradiol, via estrogen receptors in the cell cytoplasm. This makes the MCF-7 cell line an estrogen receptor (ER) positive control cell line. Study of ER alpha lead to the

generation of ER positive and ER negative breast cancer cell lines [3]. Identification of estrogen receptor 'alpha'(ER) expressed by the MCF-7 cell line is one of the most important contributions for the breast cancer. The MCF-7 Cell Line is a best model for the study of Apoptosis and it is the best investigative tool for the study of breast cancer cell lines. Distinctive MCF 7 cell lineages have been evolved after the several experimentation done by different independent labs under different situations [4]. Expression of ER on the cell lines resulted in the generation of ER positive and ER negative MCF 7 cell line breast cancer. The pS2 gene in MCF-7 cells represents a unique example of a human gene whose transcription is directly controlled by estrogen. [5]. MCF-7 cells continue to serve as an excellent in vitro model for studying the mechanisms of chemo resistance as it relates to susceptibility in apoptosis [6]. Medicinal plants represent a rich sources of anti-cancer, anti-fungal, anti-oxidant, and antimicrobial agents [7]. Medicinal plants have been of age long remedies for human diseases because they contain chemical components of therapeutic value [8]. Medicinal herbs are moving from flounce to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemical compounds [9]. The derivation of plants to produce drugs is basically bound to a principle which describes the potential of breaking plants down, separating their active components and producing powerful medicine in standard form [10]. *Acalypha indica* Linn. (*Euphorbiaceae*) also known as 'kucing galak' is widely distributed throughout South Africa tropical Africa and, Sri Lanka and India, as well as Pakistan and Yemen. It is a monoecious plant with a weedy nature, annual to sometimes short-lived perennial herb that can grow up to 1.5 to 2.5 m tall [11]. *Acalypha indica* has been extensively used in Ayurvedic system of medicine for different ailments. It is deciduous and mixed-monsoon forests throughout greater parts of India, is widely used in traditional medicinal system of India has been reported to possess antitussive, antifungal and anti-inflammatory, used also check wounds healing and antibacterial [12]. Plants produce a diverse range of bioactive compounds, making them a rich source of various types of medicines. The most important of these bioactive compounds of plants are tannins, flavonoids, alkaloids and phenolic compounds. These substances are usually found in several parts of plants like root, leaf and shoot [13]. This present study on in vitro anticancer activity of leaf extract of *Acalypha indica* would be useful in future for isolating the respective active constituent and for yielding a potential drug to mankind in treating cancer without inauspicious effects [14].

Taxonomic Classification

Kingdom : Plantae
Class : Magnoliopsida
Order : Euphorbiales
Family : Euphorbiaceae
Genus : Acalypha
Species : *Acalypha indica* Linn.

MATERIALS AND METHODS

Preparation of crude extract

Collection of Plant Sample

Different plant materials of *Acalypha indica* were collected from CIMAP (Central Institute of Aromatic and Medicinal Plants) and authenticated by department of Botany, Osmania University, Hyderabad, Telangana. The leaves were separated and were washed in a tray and shade dried for 3-5 days. After three days this shade dried leaves were milled to obtain a fine powder. Always shade drying is preferred as it prevents denaturation of important phytochemical when compared to sun drying.

Extraction from the plant powder using soxhlet apparatus

This fine powder was subjected to extraction using soxhlet apparatus. About 250ml of hexane solvent and 50 grams of dried fine plant powder was taken for extraction. The whole setup of soxhlet extraction unit was subjected to continuous extraction for 72 hours at 65ºc (Boiling point of hexane) hexane plant extract was obtained in the round bottom flask. Remove the solvent from hexane crude extract with rotar vapour. This hexane crude compound was used for the to perform the Cytotoxic activity on breast cancer cell lines.

Materials required for cell culture and trypsinization

MCF-7 Cell Lines 1 x 10^6 cells /mL in DMEM Media (Dulbecco's Modified Eagle's Medium), PBS (Phosphate buffered saline), T-25 Flasks, Trypsin, FBS (Fetal Bovine Serum), Antibiotics solution, MTT Reagent, Centrifuge tubes, Micropipette (100ul and 1ml), Micro Tips (100ul and 1ml).(All chemicals and Media procured from HIMEDIA)

Equipments Required: Inverted Microscope (Make: EVOS (Life Technologies)), Centrifuge (REMI CM-8 PLUS) and CO_2 Incubator (Make: IKS International)

Materials required for MTT Assay

96-Well Plate, MTT Reagent, DMSO, PBS, Centrifuge tubes, Micropipette (100ul and 1ml), Micro Tips (100ul and 1ml).

Equipments Required: Microplate Reader (Make: BIO RAD) Centrifuge (REMI CM-8 PLUS) and CO_2 Incubator (Make: IKS International)

Methods

Cell culture and trypsinization

Preparation of complete media for cells

500ml of DMEM (Dulbecco's Modified Eagle's Medium) media was taken and to this 5ml of antibiotic solution was added. This makes the whole volume to 505ml and from this 45 ml of volume is taken and to this 45ml, 5ml of FBS was added making a total of 50ml of complete media. Plain media is direct usage of DMEM only.

Trypsinization

MCF-7 Cells from mother cultures, which are initially stored at -80ºC, are sub cultured into T-25 flask with complete media. After 6 hours, the media used by the cells (spent media) is discarded and cells are washed with 3ml of PBS. Then PBS is discarded and 2ml of trypsin is added and the cells in the flask are incubated at 37ºC in CO_2 Incubator for 3 minutes. The cells in the flasks are detached and digestion of extracellular matrix (which holds cells together) is done by trypsinization. Then the cells are visualized under inverted microscope. 2ml of fresh complete media is added and contents in the T-25 flask are transferred into centrifuge tubes and centrifuged at

2000 rpm for 2 minutes. The supernatant (which contains media and trypsin) is discarded. The pellet which contains is washed with 3ml PBS and PBS is discarded. To the Pellet with cells, 3ml of complete media is added.

MTT Assay

To the 96 well plate, 100ul of media with pellet cells is added to 12 wells (12 wells are working wells, with three for compound addition and three for control and the work is done for triplet hence a total of 15 wells). This was subjected to 6 hours of incubation in CO_2 Incubator at 37°C. Three concentrations of plant extracts 10µg/ml, 25µg/ml and 50µg/ml were prepared by dissolving the crude leaf extract, obtained after rotary evaporator, in PBS.To the 1st triplet (1st three vertical wells), 100ul of 10µg/ml plant extract was added. Similarly to the 2nd and 3rd triplets 100ul of 25µg/ml and 100ul of 50µg/ml of leaf extract was added. Only PBS was added to the control wells without leaf extracts and cisplatin as a possitive standerd. Now this 96 wellplate is subjected to 21 hours of incubation in CO_2 Incubator at 37°C. After 21 hours of incubation in CO_2 Incubator at 37°C, 20ul of MTT reagent was added to all the wells was incubated in CO_2 Incubator at 37°C for 3 hours. The concentration of MTT used is 0.5µg/ml. After 3 hours of incubation, 100ul of DMSO was added to all the wells. O.D values were noted using ELISA reader at 570nm. The percentage growth inhibition was calculated using the following formula

$$\% \text{ Growth Inhibiton} = \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

RESULTS

The anticancer activities of A. indica extracts were investigated. The samples were tested against cancerous cell lines: MCF7-Breast Cancer, using the MTT Assay. Triplicated determinations were performed. The present study Cytotoxic activity done by *Acalypha indica* L. leaf extract on breast cancer cell lines (mcf-7) was shows more inhibitory concentration (IC_{50}) values 50 µg/ml. less Cytotoxic activity shows 10µg/ml, 25 µg/ml and 100 µg/ml. Results are presented in Table 3 and graph 2 while percentage of cell viability more in 10 µg/ml, (table 2 and graph 1).

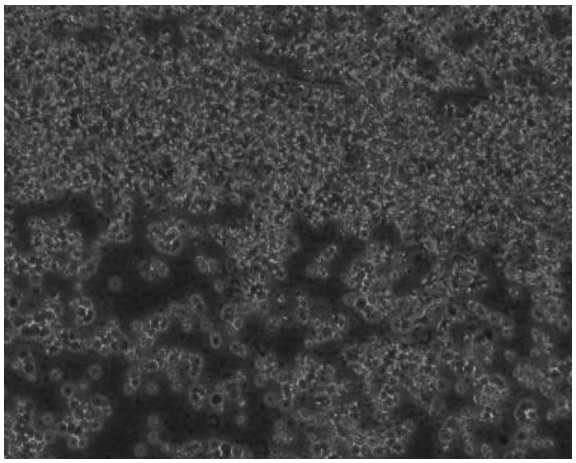


Figure 1: Normal mcf-7 Cells

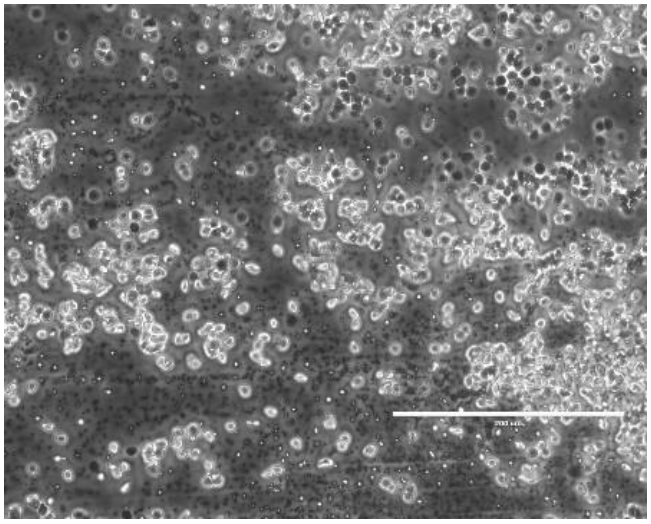


Figure 2: Trypsinised mcf-7 Cells

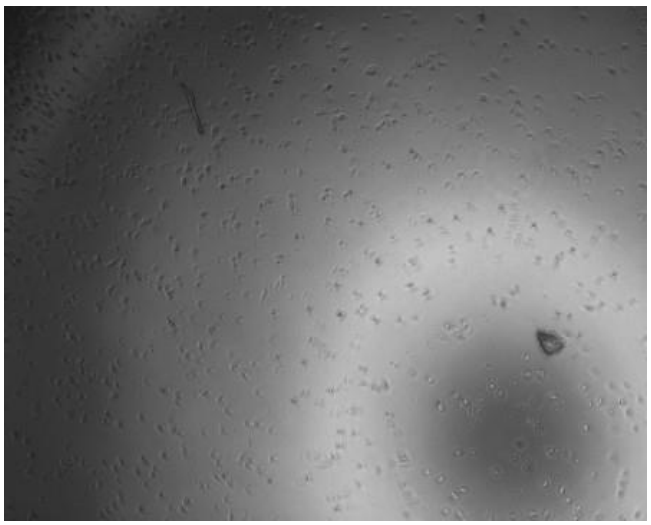


Figure 3: Addition of drug to the mcf-7 cells

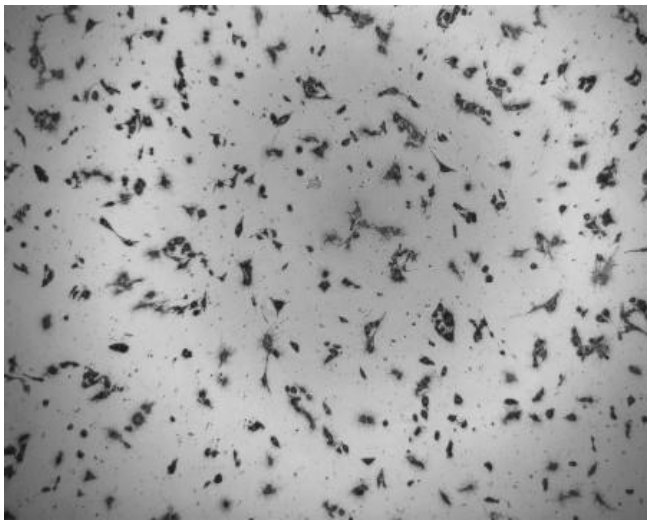


Figure 4: Addition of MTT reagent to the mcf-7 cells

Following are the Mean and Standard deviation values obtained for Hexane solvent extract at 570nm

Table 1

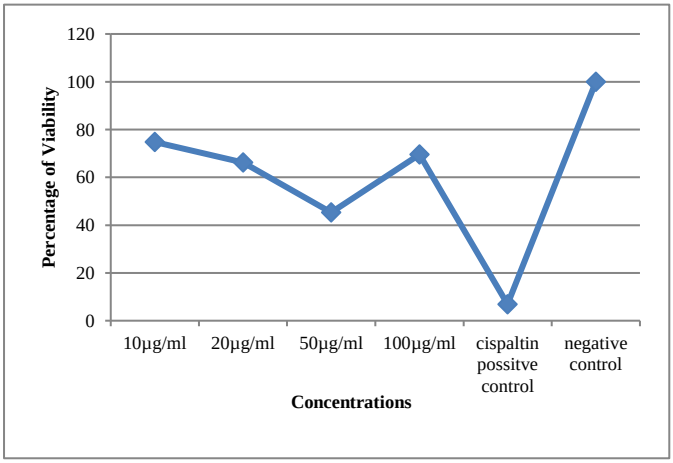
Concentrations	Mean ±Standard deviation
10µg/ml	0347 ±0.010
25µg/ml	0.307±0.010
50µg/ml	0.210±0.040
100µg/ml	0.323±0.012
Cisplatin Positive control	0.032±0.004
Negative Control	0.464±0.009

Table 2

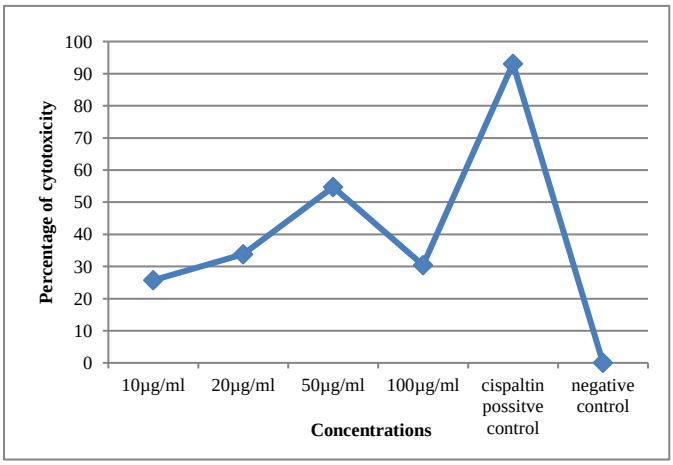
Sample	10µg/ml	25µg/ml	50µg/ml	100µg/ml	Cisplatin	Control
% of Cell Viability	74.78	66.16	45.25	69.61	6.89	100

Table: 3

Sample	10µg/ml	25µg/ml	50µg/ml	100µg/ml	cisplatin	Control
% of inhibitory conc. (IC_{50} Values)	25.72	33.84	54.75	30.39	93.11	0



Graph 1: (table: 2) Representing the percentage (%) of Cell Viability of Hexane extract



Graph 2: (table: 3) Representing the Percentage (%) of Cell cytotoxicity of Hexane extract

DISCUSSION

The use of traditional medicinal plants in most developing and developed countries for the maintenance of good health has been widely observed. *A. indica* is a traditional medicinal plant in India, used as antibacterial, anti-fungal, antioxidant and anti-inflammatory activities. It is observed that this plant has anti-cancer properties. It is also used to treat diabetes and other many diseases and disorders. The hexane extract showed significant anticancer activity against MCF7-Breast Cancer with an IC_{50} of 50µg/ml and lowest cytotoxicity 10µg/ml at 570 nm. Cisplatin and 0.5% DMSO were used as positive and negative controls, respectively.

CONCLUSSION

Based on the results of this study, it is possible to conclude that Acalypha indica consists of numerous important biological activities including antioxidant, antidiabetic and antimicrobial activities. The extraction technique and the solvent should be carefully chosen according to the desired bioactivity. The hexane extract has very prominent cytotoxic effects to be used in many pharmacological as well as biological actions. The hexane extracts of *Acalypha indica*, plant traditionally used in Ayurvedic medicine was screened for cytotoxic activity in this study However and further studies are suggested to investigate these effects for the isolated and identified pure compounds from *A. Indica.*

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