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IN VITRO ANTICANCER ACTIVITY OF ETHYL ACETATE EXTRACT OF PREMNA LATIFOLIA AGAINST MCF-7 CELL LINE

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ABSTRACT

The aim of the present study is to evaluate the effect of in vitro anticancer activity of the ethanolic extract of Premna latifolia against mCF-7 human breast cancer cell line using MTT assay showed a percentage of cell viability of 49% at 25µg/ml which decrease with increase in concentration of extract. Anticancer activity of ethyl acete extract of premna latifolia on Mcf-7 human cancer cell line showed potent cytotoxic activity. The inhibition percentage with regard to cytotoxicity was found to be 52% at 75µg/ml which was comparable to the control Cyclophosphamide that showed a cytotoxicity of 55%. Therefore, the minimum effective concentration of ethyl acetate extract of Premna latifolia was toxic to Mcf-7 cells was recorded at a concentration of 25µg/ml of the ethyl acetate extract of premna latifolia.

KEY WORDS

Premna latifolia, Ethyl Acetate, Mcf-7 and Invitro Anticancer.

INTRODUCTION

Premna latifolia belongs to the family verbenaceae, widely distributed in tropical and subtropical and coastal areas. The leaves are diuretic in nature and is used as a folk medicine for treating dropsy[1]. *Premna latifolia* possesses anti-inflammatory activity in the animal models[2]. *Premna latifolia* bark is applied to cure boils[3]. Traditionally it has been used in the treatment of hepatic disorders[4], antioxidant[5] and anticancer activity[6]. Plant derived agents are being used for the treatment of cancer. Several anticancer agents from plants include, taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodo phyllotoxin are in clinical use all over the world. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents. Scutellaria baicalensis was used as a component of PCSPES, an herbal mixture that showed efficacy in laboratory trials for prostate cancer, small-cell lung cancer and acute myeloid leukemia[7-12]. The *in vitro* antitumor potential of the total alkaloid extract from Tabebuia rosea leaves against the human leukemic cells (MOLT-4) and the extract showed cytotoxic activity in a dose and time dependent manner[13]. The different extracts of hexane, chloroform, ethyl acetate and Crude methanol extract fractions of *Debregeasia salicifolia* stem for anticancer activity against MCF-7 cancer cell line and revealed that MCF-7 showed minimum inhibition of 25.31% at the concentration of 10µg/ml and maximum inhibition of 99% was observed at the concentration of 500 µg/ml[14]. The invitro anticancer activity of *Sansevieria roxbhurginia* against HepG2 liver cell and compared with normal 3T3 cells and showed a percentage of cell viability of 92.2% at 125µg/ml which decreased with increase in concentration[15].

In vitro anticancer activity of Rubia cordifolia against Hela and Hep2 cell lines and exhibited a significant cytotoxic activity in human cervical cancer cell line when

compared to human larynx carcinoma[16]. The ethanolic extract of Argemone mexicana, *Polyalthia longifolia*, Terminalia bellarica and Terminalia chebula were evaluated for anticancer activity against Hela-B75, Hep 3B and PN-15 cell lines and revealed that *P.longifolia* was found to be more potent against the Hela cell lines[17].

MATERIALS AND METHODS

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

Media and Cell lines

Mcf-7 cells were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 µg/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Collection of Plant material

Premna latifolia (includes leaf, stem and root) were collected from in and around Chinnapaliyampattu Thiruvannamalai district, the voucher specimen were kept in the Department of Zoology, Chennai, Tamil Nadu, India and used for this study.

Preparation of extract

The 25 g of dried powder of *Premna latifolia* was mixed with 100ml of ethyl acetate solvent and kept in rotary shaker at 100 rpm overnight and filtered with Whatmann no.1 filter paper and concentrated to dryness at 400c. until further use. Different concentration of the ethyl acetate extracts (25µg/ml, 50µg/ml, 75µg/ml) were prepared in 5% Di-Methyl Sulfoxide (DMSO) for determining cytotoxicity.

Cell viability assay on Mcf-7 cell lines

The Cytotoxicity of samples on MCF-7 was determined by the MTT assay. Cells (1 × 105/well) were plated in 100 µl of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/isopropanol was added. Viable cells were determined by the absorbance at 450 nm. Measurements were performed and the concentration required for a 50% inhibition of viability was determined graphically. The absorbance at 450 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of cells was expressed as the % cell viability, using the following formula:

% cell viability =

A_{450} of treated cells / A_{450} of control cells × 100%.

Cell viability on Mcf-7 cell lines

The anticancer activity of ethyl acetate extract of *Premna latifolia* was performed on Mcf-7 cell lines obtained from NCCLS Pune, India. The cell viability was measured using MTT assay as described above. Controls were maintained throughout the experiment. The assay was performed in triplicates for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract. Cells and % viability was plotted against concentration of the plant extract. The maximum concentration of the plant extract that was toxic to Mcf-7 cells was recorded as the effective drug concentration.

RESULTS AND DISCUSSION

The nontoxic dose of the ethyl acetate extract of premna latifolia on Mcf-7 cell line showed that the percentage with regard to viability of cells was found to be 49% at a concentration of 25µg/ml which decreased with increase in concentration (Table 1). The extract showed a potent cytotoxic activity against Mcf-7 cancer cell line (Table 1). Cyclophosphamide served as positive control and 45% cancer inhibition was observed (fig.1). The concentration of ethyl extract of *premna latifolia* at 75µg/ml showed an inhibition of 47% compared to that of positive control. Ethyl acetate extract of *premna latifolia* at 25µg/ml, 50µg/ml and 75µg/ml showed cytotoxic activity of 49%, 48% and 47% respectively (fig.3). Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control (fig.2). These observations may be due to the presence of active

biological compounds. Therefore, the minimum effective concentration of ethyl acetate extract of premna latifolia that was toxic to 50% Mcf-7 breast cancer cells was recorded (IC50) at a concentration of 25µg/ml of the plant extract.

Table *1*: Cell viability assay on Mcf-7 cell liine

Concentrations(µg/ml)	% of Viability	% of Toxicity
Control	100	100
25µg	49.23	50.76
50µg	48.09	51.90
75µg	47.03	52.96
Positive control	45	55

Fig 1: Cyclophosphamide control

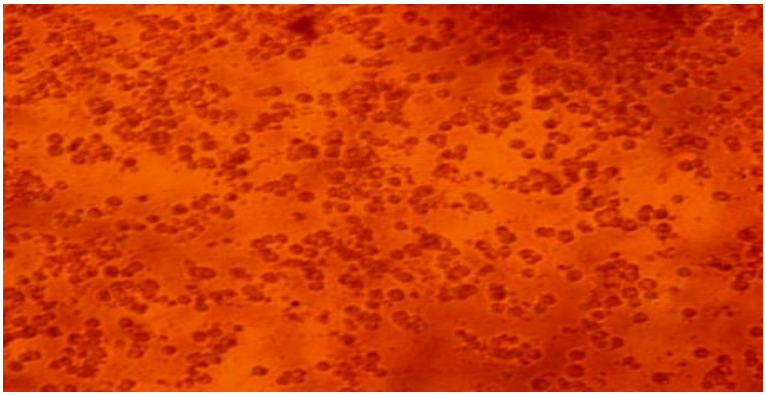


Fig 2: Ethyl acetate extract of Mcf-7 cell line

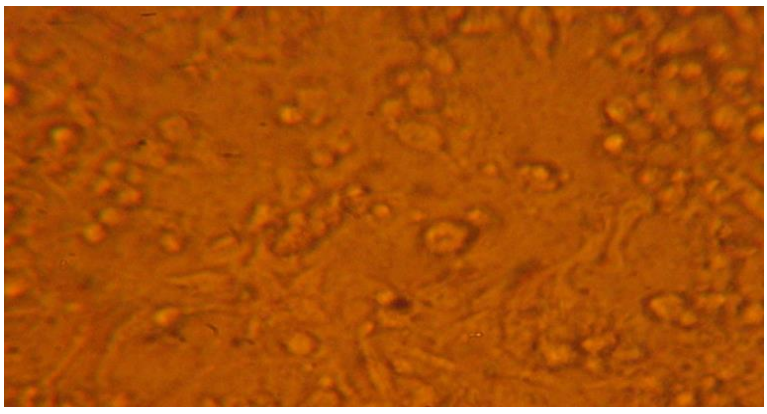
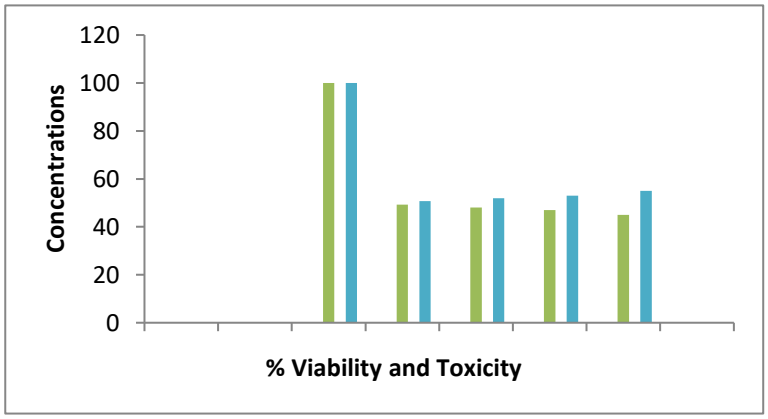


Fig 3: Percentage viability of Mcf-7 cell line



CONCLUSION

The results of this study support the efficacy of *premna latifolia* as an anticancer agent for breast cancer cell line. From the present study it has been revealed that ethyl acete extract of *premna latifolia* shows 50% anticancer activity in Mcf-7 breast cancer cell line at the concentration of 25µg/ml. It acts a potential adjuvant treatment to current chemotherapeutic agents and can be used in the treatment of Mcf-7 breast and a further research has to be done. From this it is said that due to the presence of some phytocomponents18, which shows 50% activity. In future the components present on *premna latifolia* may act as a drug, further in-vivo studies should be carried out. Considerable works have been done on the medicinal plants to treat cancer, and some plant products have been marketed as anticancer drugs. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues. Several reports describe that the anticancer activity of these plants is due to presence of antioxidants.

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