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In vitro anti-cancer activity *Tecoma stans* against human breast cancer yellow elder (*Tecoma stans*)

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Abstract

Phytochemicals are the chemicals its present naturally in plants. Now days these phytochemicals become more popular due to their countless medicinal uses. *Nature has been a powerful source of enormous medicines for thousands of years and number of modern drugs has been extracted and exploited from natural sources, for its use in traditional medicine. The present study has been performed experimentally by in vitro method to examine the anti-cancer activity of bark of tecoma stance* The human breast cancer cell line (Michigan Cancer Foundation-7 (MCF 7)) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified tmosphere of 5% CO₂ at 37 °C. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. *Over 50% of all modern scientific drugs are natural products that play an important role in drug development in pharmaceutical industries*

Keywords: Anticancer activity, breast cancer cell line (Michigan Cancer Foundation-7 (MCF 7)), pharmacological actions etc.

1. Introduction

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell ^[1]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders ^[2]. Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor, they are not totally free from side effects ^[3].

Yellow elder (*tecoma stans*) is a shrub or small tree having a wide natural distribution in tropical and subtropical America from Mexico to Argentina. It is also found in the Caribbean and the Bahamas. Yellow elder is the territorial flower of the Bahamas and the U.S. Virgin Islands. Perhaps because of its extensive range, its origin within the Western Hemisphere remains uncertain. The species is characteristic of rocky slopes, often limestone outcrops, but also of alluvial soils as long as there is excellent drainage. It is common mainly in deforested and other disturbed sites and along roadways. Yellow elder has naturalized in much of tropical and subtropical Africa, Asia, the Pacific Islands and Australia. It has been evaluated using the IFAS Assessment of the Status on Non-Native Plant in Florida's Natural Areas and is not considered a problem species in the state ^[4].

Tecoma stans is not a toxic herb because the same is used in Latin America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico. Despite the traditional use of *Tecoma stans*, its pharmacological properties on smooth muscle have not been carried out. The plant is extensively used traditionally by the tribal people as Anthelmintic Therefore, The aim of the present study was to investigate the anthelmintic effect of aqueous, alcoholic and hydro-alcoholic and methanolic extract (sohxlet) of leaf extract of *Tecoma stans* (*L.*) on adult Indian earthworms (*Pheretima posthuma*). ^[5]

2. Materials and Methods

2.1 Collection of Flowers

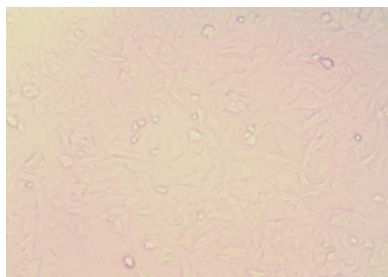
Fresh flowers of *Tecoma stans* were collected from Poondi village, thanjavur district, Tamil Nadu, India, during the month of May and identified by Dr.S.John Britto, Director, St.Joseph's College (Campus), Trichy. Tamil Nadu, India.

2.2 Preparation of extracts

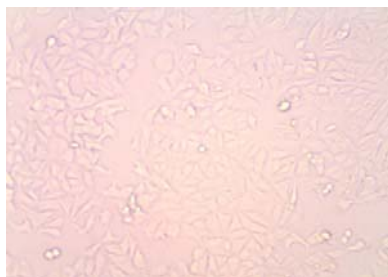
Fresh bark (1kg) of *Tecoma stans* were collected from Poondi village, Tamil Nadu, 1 g of bark powder extracted with 250ml of ethanol and water using the soxhlet extractor for 9-10 hours. The plant extract bath at 100 °C for 2 hours. The extract was filtered through whatman No.1 filter paper to remove all un wanted matter and final extract was used in anti-cancer activities also. Different concentrations of (12.5, 25, 50, 100, 200 and 400 µg/ml) leaf and bark extracts were prepared. These concentrations were used for Antiproliferative activity.

2.3 MTT Assay method

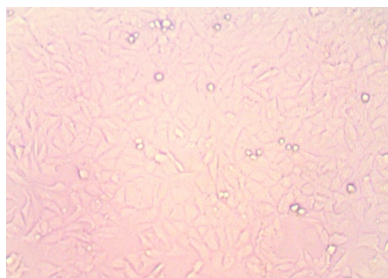
2.3.1 Photomicrograph of MCF-7 cell line of plant extract



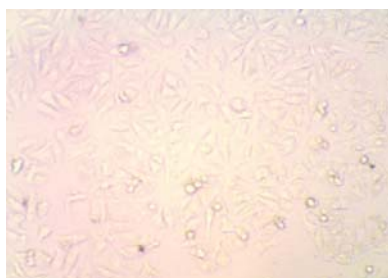
Control



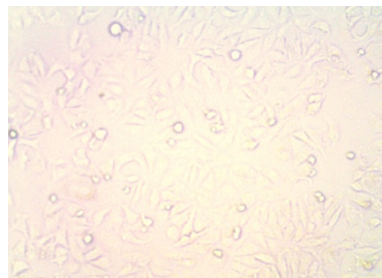
25µg/ml



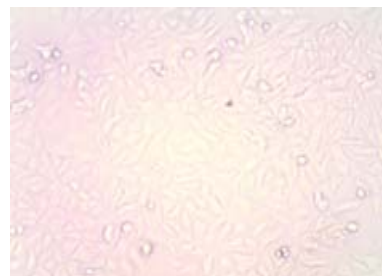
12.5µg/ml



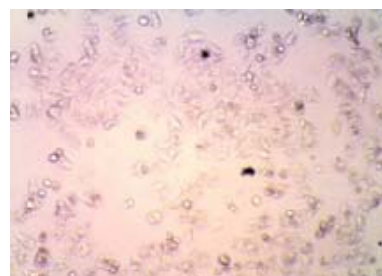
50µg/ml



100µg/ml



200µg/ml



400µg/ml

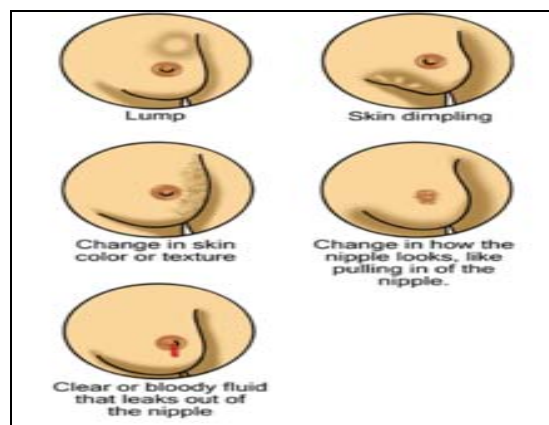
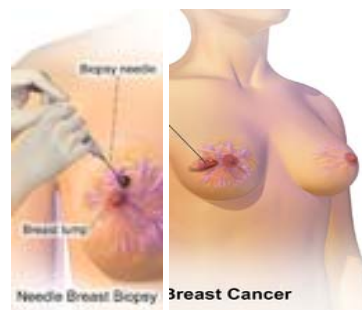


Fig 1-7: Effect of the compound of tecama stance against human breast cancer MCF-7 cell line in different concentrations.

3. Result and Discussion

Table 1: Percentage cell growth inhibition of extract on MCF 7cell line by MTT assay

S. No.	Concentrations ($\mu\text{g/ml}$)	Absorbance (Optical density)	Cell Viability (%)	Cytotoxicity (%)
1.	12.5	0.356	93.94	6.05
2.	25	0.342	90.07	9.92
3.	50	0.311	81.91	18.08
4.	100	0.224	59.17	40.82
5.	200	0.124	32.74	67.25
6.	400	0.072	19.05	80.94
	Cell Control	0.379	100	0
Half Inhibition Concentration (IC_{50})				196.61 $\mu\text{g/ml}$

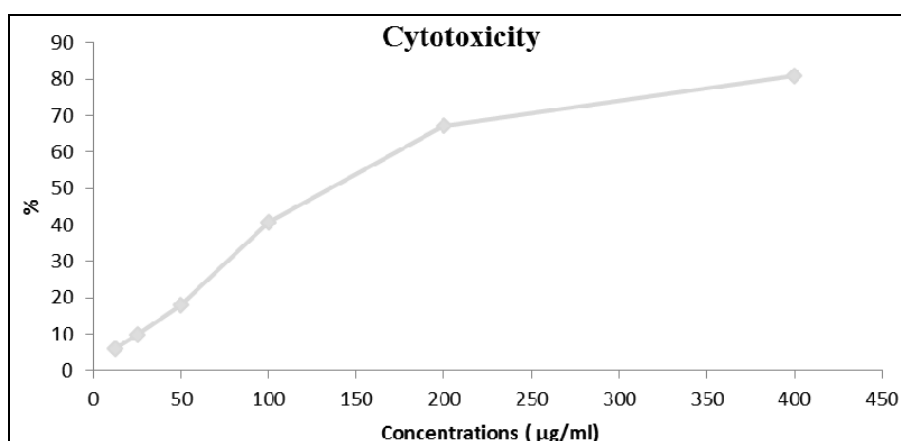
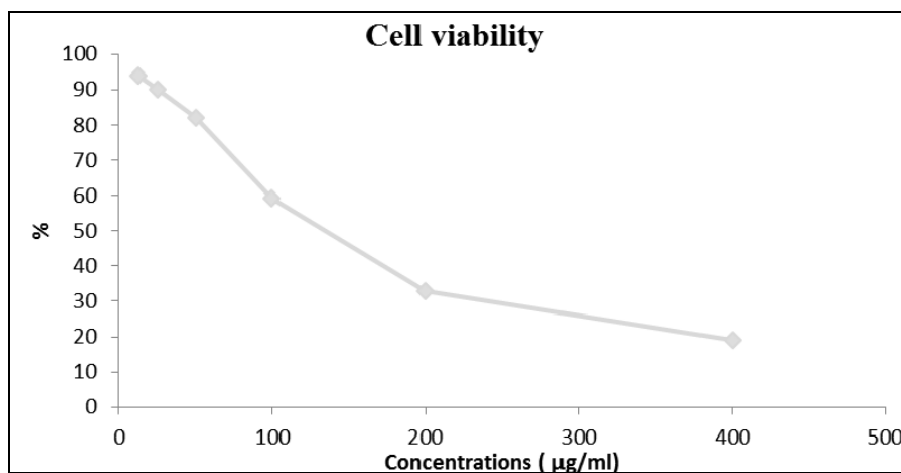


Fig 1: Percentage of cell growth inhibition of extract on MCF 7cell line by MTT assay



Graphical representation of the CTC50 values of the of Tecama stance bark against human breast cancer MCF 7cell line by MTT assay Cell line.

Fig 2: Percentage of cell viability of extract on MCF 7cell line by MTT assay

3.1 Cell line and culture

The human breast cancer cell line (Michigan Cancer Foundation-7 (MCF 7)) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 $\mu\text{g/ml}$) in a humidified atmosphere of 5% CO_2 at 37 °C. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

3.2 Reagents

Minimum Essential Medium (MEM) was purchased from Hi Media Laboratories FBS was purchased from Cistron

laboratories Trypsin, 3-(4,5-dimethyl-2-thiazolyl)-2,5-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.

3.3 Anticancer assay

Anticancer assay was evaluated by the MTT reduction assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] (Mosmann, 1983; Monks *et al.*, 1991). The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediamine tetraacetic 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 1×10^5 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% CO₂, 95% air and 100% relative humidity. Aliquots of 100 µl of different concentrations of leaf and bark extracts (12.5, 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% CO₂, 95% air and 100% relative humidity. After 48h of incubation, to each well 20 µl/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 (IC₅₀) 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: ^[6-7]

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

% of Cytotoxicity = [100 - Abs (sample) /Abs (control)] × 100.

4. Conclusion

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediamine tetraacetic 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. it shows that all concentrations are having anticancer activity. The sample concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml and 50 µg/ml show 25 µg/ml, 1 CTC50 value against the human breast cancer MCF 7 cell line by MTT assay Cell line. Respectively. These concentrations were able to induce apoptosis on human breast cancer cell lines and its anticancer activity was found to be precise. Further work is required in order to establish the identity of the chemical component responsible for anticancer activity. Studies are in improvement in our laboratory to explain the molecular structure. This contributes towards the development of potent anticancer drug.

5. Acknowledgements

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