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Research Article

# *INVITRO* ANTICANCER ACTIVITY OF *TECOMASTANS* (L.) ETHANOLIC LEAF EXTRACT ON HUMAN BREAST CANCER CELL LINE (MCF-7)

**M. Thirumal*, G. Kishore, R. Prithika, Sampa Das and G. Nithya**

Department of Pharmacognosy, Jaya College of Pharmacy, C.T.H.Road, Thiruninravur, Chennai, Tamilnadu, India.

**ABSTRACT**

*Tecomastans* (L.) (Family:Bignoniaceae) is called as yellow elder in english.Traditionally flowers and bark are used for treatment of various cancers. In current study Crude ethanolic leaf extract of *Tecomastans*(L.)leaves were examined for their anticancer activity. To determine *invitro* anticancer activity, different concentrations of crude extract were tested on MCF- 7 cancer cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Tecomastans*leaf extract showed a significant antiproliferative activity and a dose dependent effect was observed. Minimum inhibition of 14.6 % was shown by extract at concentration 7.8 µg/ml and maximum inhibition (95.9%) was observed at 1000 µg/ml. The plant extract showed activity in potential range for further investigation on cancer cells.

**Keywords:** *Tecomastans,* MCF- 7 cancer cell line, MTT assay, anticancer activity.

## INTRODUCTION

Cancer is the second leading cause of death all over the world[1] is a multifactorial, multifaceted and multimechanistic disease requiring a multidimensional approach for its treatment, control and prevention[2]. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation[3]. Age is also a primary risk factor for most cancers, with about 77% of all cancers diagnosed among people aged 55 or older[4]. Breast cancer is the most common form of cancer in women worldwide[5]. According to an estimate, 50% of breast cancer and 37% of prostate cancer patients use herbal products[6]. Several chemopreventive agents are used to treat cancer, but they cause toxicity that prevents their usage[7]. In this context, the natural products derived from medicinal plants have gained significance in the treatment of cancer[8].

*Tecomastans* (L.) belongs to the family Bignoniaceae are distributed worldwide, mostly occur in tropical and sub tropical countries. However a number of temperate species also grow in North America and East Asia[9] .*T.stans* is a shrub or small tree, 5-7.6 m in height. Bark is pale brown to grey and roughens with age. Leaves are compound and imparipinnate with 2 to 5 pairs of leaflets and a larger single terminal leaflet. Leaflets are lanceolate, up to 10 cm long, with serrated margins, mid-green above and soft to the touch. Flowers occur in clusters at the ends of the branches and are trumpet shaped with 5 rounded lobes, 6 cm long, pale to bright yellow, with faint orange stripes at the throat.

Fruits are narrow, slightly flattened to pointed capsules, up to 20 cm long, containing many winged seeds; green when young, pale brown on ripening and remain on the tree in untidy clusters for many months[10].Traditional use of leaves of *T.stans* in throughout Mexico and central America for diabetes and urinary disorder control[11,12,13]. Roots are used as diuretic and vermifuge[14]. Traditionally flowers and bark are used for treatment of various cancers. The stem barks showed better antimicrobial activity[15]. Its Leafs shows Anthelmintic Activity[16] ,Antispasmodic effect[17], Antibacterial activity[18] , Anticancer Activity[19], Wound Healing property[20]. Flower shows Antidiabetic Activity[21]& anticancer activity[22] while roots shows Antibacterial Acitivity[23]. Aerial Parts shows Antioxidant Activity[24]. Bark shows Wound Healing property[25].

## MATERIALS AND METHODS

### Plant Collection and Extraction

The leaves of *Tecomastans*were collected from local area of Avadi (west Chennai) in the month of march. The plant was identified and authenticated by Dr. P. Jayaraman (PARC, chennai), bearing a voucher Reg.no of PARC/2012/1141. The plant material was air dried at room temperature, coarsely powdered and stored in air tight container and used for further extraction. The dried powder (50gm) was extracted successively with ethanol (60°C) by using a Soxhlet apparatus for 8 hrs.

### Phytochemical screening

Qualitative chemical tests were carried out using extracts from plant to identify the phytochemicals[26].

### Cell line and Culture

Breast cancer- MCF-7 cell lines was obtained from National centre for cell sciences, Pune (NCCS). The cells were maintained in Minimal Essential Media (MEM**)** supplemented with 10% Fetal bovine serum **(**FBS**)**, penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml $CO_2$ at 37 °C.

### Reagents

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.

### *In vitro* assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on MCF-7 was determined by the MTT assay[27]. Cells (1 × $10^5$/well) were plated in 100µl 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 (pH 7.4), 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. After 4h incubation, 0.04M HCl/ isopropanol was added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for aIinhibition Concentration (IC50) was determined graphically. The absorbance at 570nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of MCF-7 was expressed as the % cell viability & % Cell death using the following formulas:

**% cell viability = A 570 of treated cells / A 570 of control cells × 100%.**

**% Cell death = (Control OD –Sample OD)/Control OD x 100.**

## RESULTS

Phytochemical screening reveals the presence of carbohydrates, proteins, saponoins, flavanoids, alkaloids, tannins, phenolic compounds.

Preliminary reports have attributed the leaves of *T. stans* with *in vitro* Anticancer activity (Table.1, Fig.1 & 2). The Photomicrograph of MCF-7 cell line at various concentrations are shown in Fig.3. The IC50 was found to be 64.5µg/ml. Accumulating evidence clearly indicates that apoptosis is a critical molecular target by dietary bioactive agents for the prevention of cancer.

## DISCUSSION

Plants are storehouse of "pre-synthesized" molecules that act as lead structures, which can be optimized for new drug development. In practice, a large number of cancer chemotherapeutic agents that are currently available in the market can be traced back to their plant source. Plant derived compounds; in particular have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use includes paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor from water soluble derivatives. Obviously natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modellingdesign, none of them can replace the importance of natural products in drug discovery and development[28,29]. Literature data prove that triterpenes and flavonoids are biologically active against different strains of bacteria and many human cancer cell lines[30,31,32]. The present study shows a dramatic *invitro*anticancer activity of Ethanolic Leaf extract of *Tecomastans*(L.) on human breast cancer cell line (MCF-7) at increasing concentrations. Inhibitory concentration (IC50) was found to be 64.5µg/ml.

**Table 1: *In vitro*Anticancer effect of *Tecomastans*leaf extract on MCF-7 cell line**

| S. No. | Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell viability (%) | Cell death (%) |
|---|---|---|---|---|---|
| 1 | 1000 | Neat | 0.02 | 4.1 | 95.9 |
| 2 | 500 | 1:1 | 0.09 | 18.7 | 81.3 |
| 3 | 250 | 1:2 | 0.13 | 27.3 | 72.7 |
| 4 | 125 | 1:4 | 0.19 | 39.5 | 60.5 |
| 5 | 62.5 | 1:8 | 0.24 | 50.0 | 50.0 |
| 6 | 31.2 | 1:16 | 0.29 | 60.4 | 39.6 |
| 7 | 15.6 | 1:32 | 0.36 | 75.0 | 25.0 |
| 8 | 7.8 | 1:64 | 0.41 | 85.4 | 14.6 |
| 9 | Cell control | - | 0.48 | 100 | 0 |

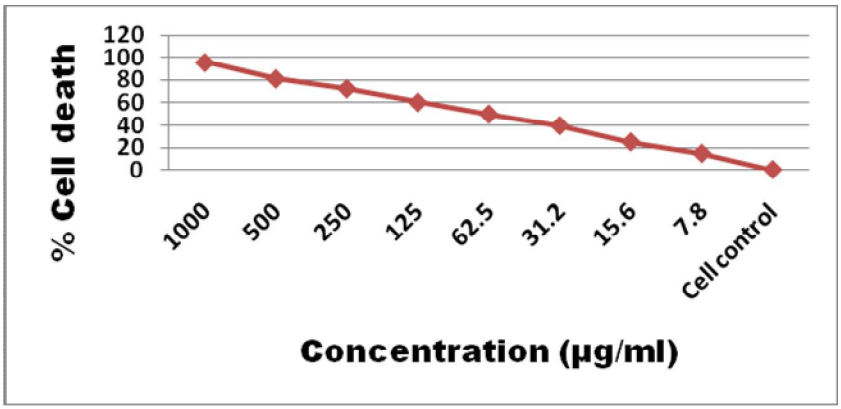


**Fig. 1: Effect of ethanolic extract of *Tecomastans leaf extract* on MCF-7 cell death**

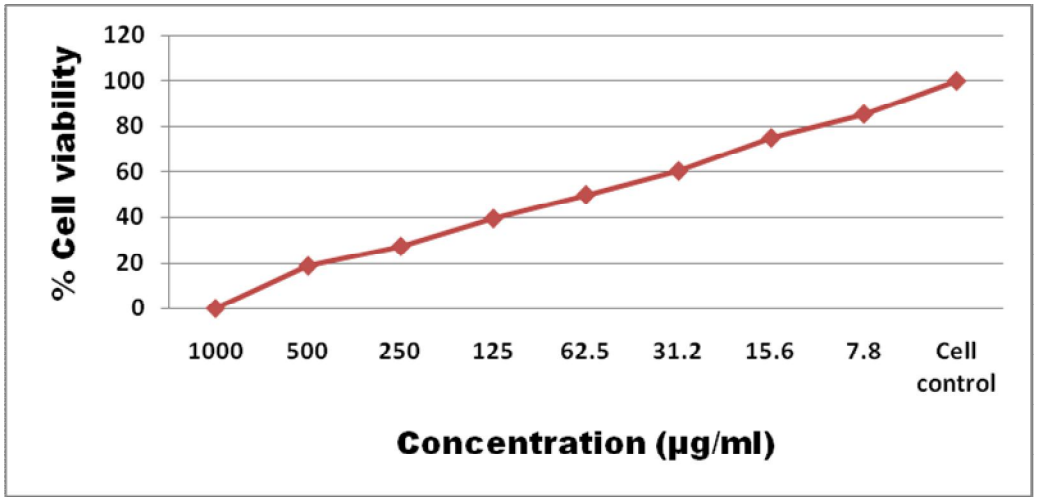


**Fig. 2: Effect of ethanolic extract of *Tecomastans leaf extract* on MCF-7 cell viability**

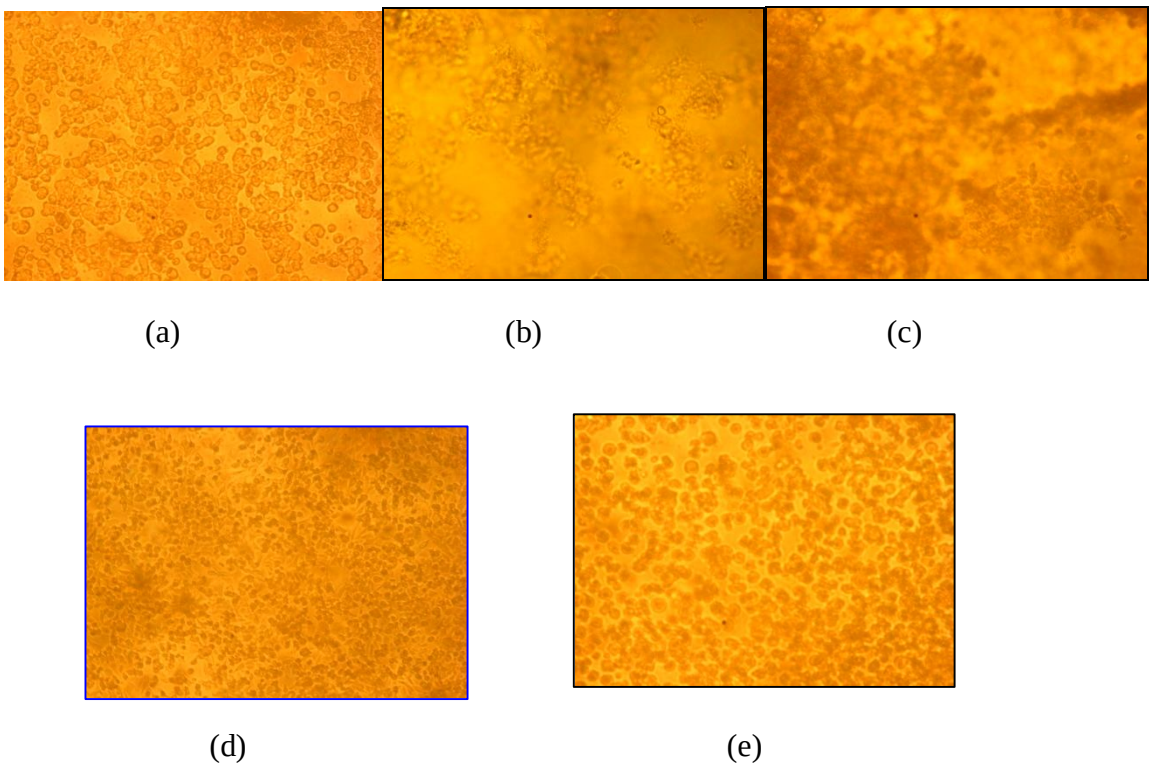


**Fig. 3: Photomicrograph of MCF-7 cell line a) Control, b) Cell Toxicity at 1000µg/ml , c) Cell Toxicity at 250µg/ml, d) Cell Toxicity at 62.5µg/ml, e) Cell Toxicity at 31.2 µg/ml**

## CONCLUSION

The present results showed that *Tecomastans*(L.) ethanolic Leaf extract might be a potential alternativeagent for human breast cancer therapy. Hence, it is anticipated that *T. stans* would be a useful pharmaceutical material to treat breast cancer. Future research should focus on the molecularmechanism of *T. stans*for anticancer action.There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s) to treat breast cancer.

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