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Anti Proliferative Activity of Various Parts of *Tecoma stans* (L.) Against Human Breast Cancer Cells *Invitro*

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ABSTRACT

Tecoma stans (L.), known as Yellow bells, belongs to family Bignoniaceae. It is extensively used in treating of various maladies traditionally. The current study, evaluate *invitro* Anti proliferative activity of ethanolic crude extract of root (ERETS), stem bark(ESETS) and flowers(EFETS) of Tecoma stans against Human Breast Cancer Cell lines (MCF-7) at different concentrations using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. IC50 (Inhibitory Concentration) of root (ERETS), stem bark (ESETS) and flowers (EFETS) extracts were found to be 46.0 µg/ml, 42.0 µg/ml and 70 µg/ml respectively. Root, stem bark and flowers extracts showed significant antiproliferative activity in dose dependable manner on the cell lines (MCF-7) but maximum activity was found to be with ethanolic extract of stem bark of *Tecoma stans* (ESETS).Thus, *T. stans* is a potent plant with anticancer activity and this plant can be taken in to account for further studies.

Keywords: *Tecoma stans*, MCF- 7 cancer cell line, MTT assay, Anti proliferative activity.

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INTRODUCTION

Cancer is a dreadful malady which accounts for more than 100 different types and is characterized by irregular proliferation of the cells which required multidimensional approach for its treatment, control, prevention and is a second leading cause of death worldwide [1-5]. Breast cancer is one of the chronic disease which may experience by women (32.1%) during her life time and is most commonly diagnosed cancer in them [6-9]. There were approximately 1.38 million new cases of Breast Cancer in the year 2008 and by 2020 this number is anticipated to intensify to 1.7 million [10]. Due to unavailability of potent drugs, overpriced chemotherapeutic agents and side effects of antiproliferative drugs, cancer can be a reason for death. Hence, efforts are still in progress for the search of anticancer agents occurring naturally, which are responsible to prevent, ally or reverse cancer development. Plants possess a specific role in this aspect. It is anticipated that compounds which are derived from plants one or another way constitutes more than 50% of antiproliferative agents [2, 3].

Interest in a huge number of folk plants, natural products has increased [11-15]since they won't cause toxicity [16]. Plants are storehouses of lead molecules. Plant derived agent's plays a vital role in treating cancers which includes paclitaxel, vincristine, podophyllotoxin and camptothecin a natural product forerunner from hydrophilic derivatives. Thus natural products are vital medical agents. Even though there are few efforts done for drug discovery which may include CADD, combinatorial chemistry but none could supercede the importance of agents occurring naturally in the field of drug discovery and development [17, 18]. A large number of chemotherapeutic agents derived from plants, such as Vinblastine, Taxol, Camptothecin and Podophyllotoxin are used as anticancer agents [19]. The choice of crude plant extracts for screening plans has the possibility of being more successful in its primary steps than the screening of pure compound(s) separated from natural products [20, 12].

Tecoma stans (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 [21]. Traditional use of leaves of *T.stans* in throughout Mexico and Central America for diabetes and urinary disorder control [22-24]. Roots are used as diuretic and vermifuge [25]. Traditionally flowers and bark are used for treatment of various cancers. Among them stem bark showed better antimicrobial activity [26]. Its Leafs shows Anthelmintic Activity [27], Antispasmodic effect [28], Antibacterial activity [29], Anticancer Activity [30, 31] and Wound Healing property [32]. Studies reveals that Flower possess Antidiabetic Activity [33] and anticancer activity [34] while roots shows Antibacterial activity [35]. Aerial Parts shows Antioxidant Activity [36]whereas Bark shows Wound Healing property [32].

Rationale in selection of this plant for the current study is based on its traditional use to treat various Cancers [26] and based on the results obtained from our previous study [31].

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MATERIALS AND METHODS

Plant Collection and Extraction

The root, stem bark and flowers of *Tecoma stans*were procured 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 [37].

Cell line and Culture

Breast cancer- MCF-7 cell lines were 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 (100 µg/ml) in a humidified atmosphere of 50 µg/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.

*Invitro*Assay for Cytotoxicity Activity (MTT Assay)

The Cytotoxicity of samples on MCF-7 was determined by the MTT assay [38].Cells (1 × 10^5/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 (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 or isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (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

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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, flavonoids, alkaloids, tannins, phenolic compounds. Preliminary reports have attributed the roots, stem bark and flowers of *T. stans* with *invitro*Anti-cancer activity (Table.1, Fig.1, 2). The Photomicrograph of MCF-7 cell lines at various concentrations are shown in Fig.3. The IC50 of root (ERETS), stem bark (ESETS) and flowers (EFETS) was found to be 46.0µg/ml, 42.0µg/ml and 70µg/ml respectively.

Table 1: *Invitro* Anticancer effect of various extracts of *Tecoma stans*on MCF-7 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)			Cell death (%)		
				ERETS	ESETS	EFETS	ERETS	ESETS	EFETS
1	1000	Neat	0.05	6.2	10.4	12.5	93.8	89.6	87.5
2	500	1:1	0.12	16.6	25.0	20.8	83.5	75.0	79.2
3	250	1:2	0.15	22.9	31.2	29.1	77.1	68.8	70.9
4	125	1:4	0.18	35.4	37.5	39.5	64.6	62.5	60.5
5	62.5	1:8	0.22	43.7	45.8	52.0	56.3	54.2	48.0
6	31.2	1:16	0.26	56.2	54.1	62.5	43.8	45.9	37.5
7	15.6	1:32	0.37	66.6	77.0	79.1	33.4	23	20.9
8	7.8	1:64	0.43	81.2	89.5	91.6	18.8	10.5	8.4
9	Cell control	-	0.48	100	100	100	0	0	0

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Fig. 1: Effect of various extract of *Tecoma stans*on MCF-7 cell viability

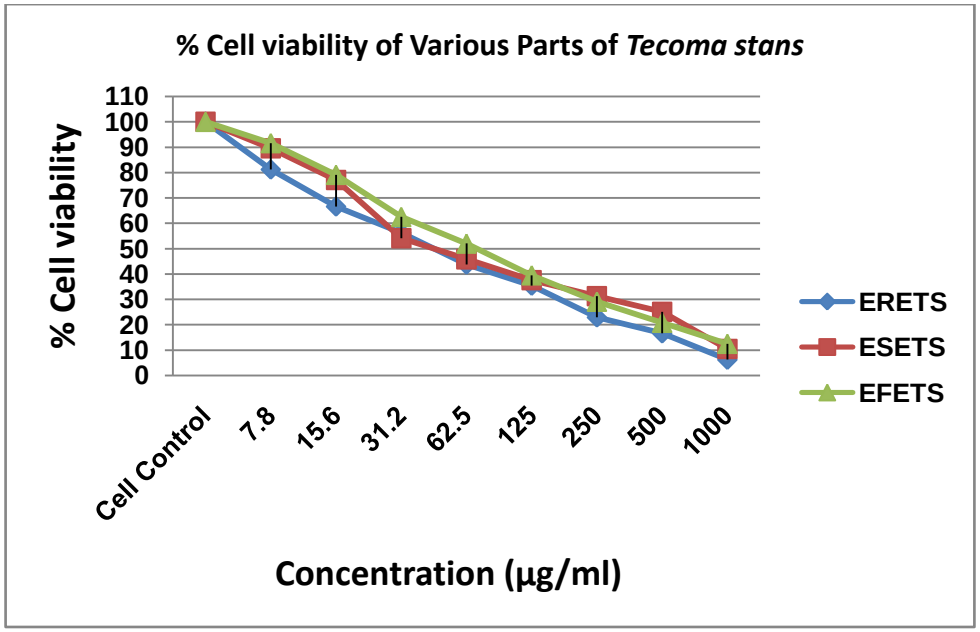

Fig. 2: Effect of various extract of *Tecoma stans*on MCF-7 cell death

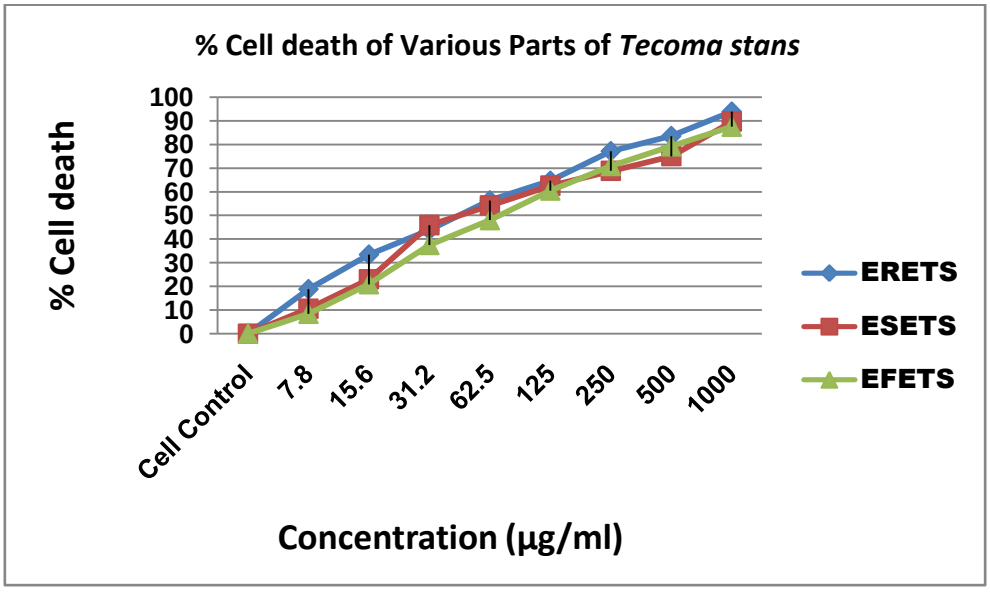

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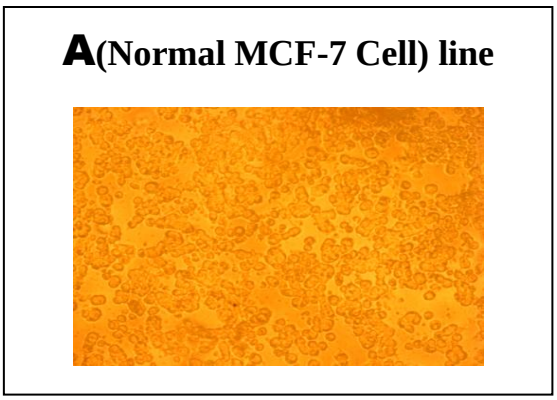
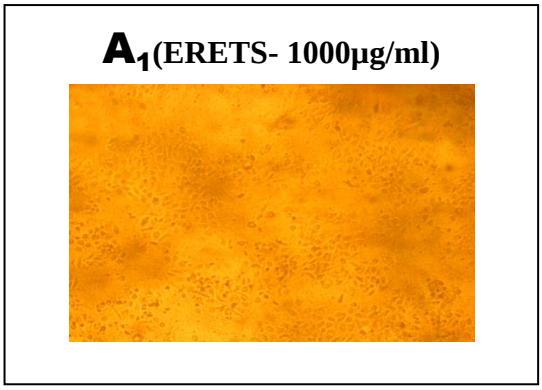
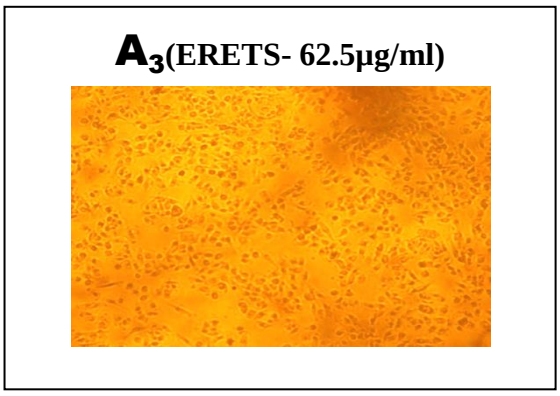
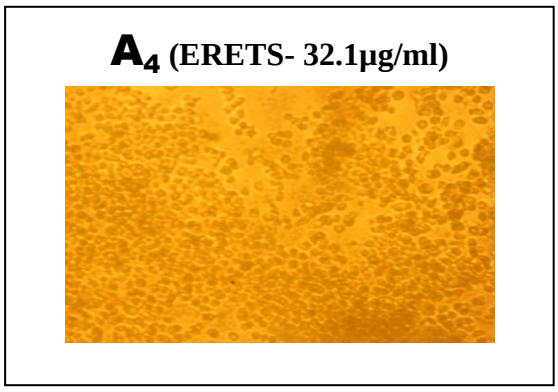
Fig. 3: Photomicrograph of MCF-7 cell line. A- Control; Cell Toxicities of ERETS (A_1-A_4), ESETS (B_1-B_4), And EFETS (C_1-C_4)at 1000µg/ml, 250µg/ml, 62.5µg/ml and 31.2 µg/ml respectively.









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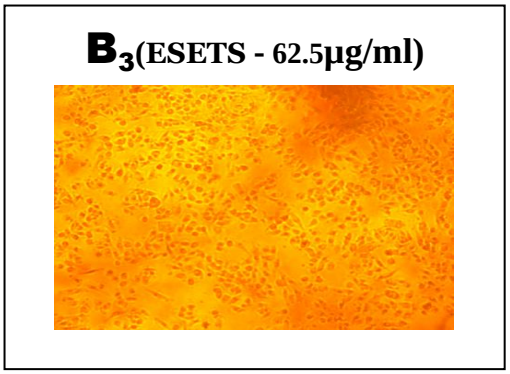

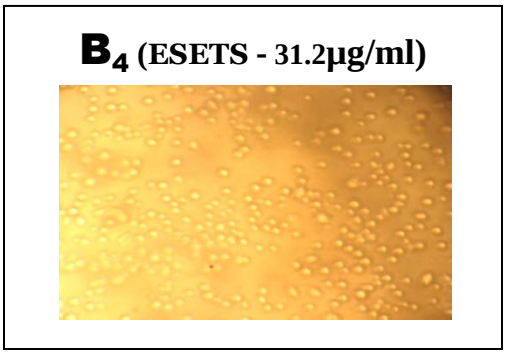

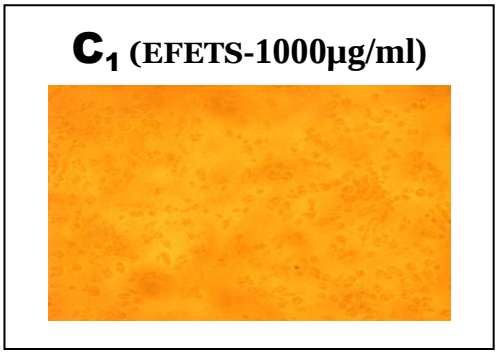

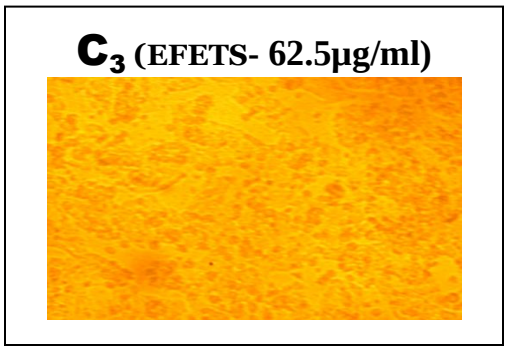

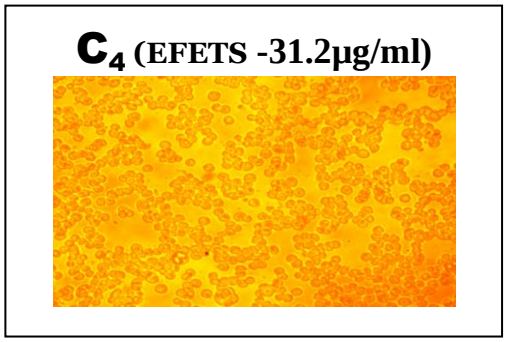

DISCUSSION

Novel scientific strategies for the valuating of natural products with biological activity needed the introduction of wide-scale screening programs. The chief aims of analyzing crude plant extracts is to identify bioactive compounds which can be used as lead substance in the formulation of semi synthetic drugs or isolate bioactive agents for direct use as drugs. In this study, we explored the anticancer potential of *Tecoma stans* in a well-characterized MCF-7 cell line by using MTT assay, which is an automated bioassay screening, currently developed based on colorimetric methods that quantify the proliferation of cell cultures [38,39], these techniques which are considered rapid and cost effective for the assessment of anticancer [40,41]. Literature data confirmed that flavonoids, triterpenes have been shown to possess antimutagenic, antimalignant and antibacterial effects [18, 42-44]. Although all the extracts showed significant anticancer activity, in the present study stem bark (ESETS) showed

asignificant *invitro* anticancer activity against human breast cancer cell line(MCF-7) at increasing concentrations when compared to other extracts. Effective concentration (IC50) of root (ERETS), stem bark (ESETS) and flowers (EFETS) extracts were found to be 46.0 µg/ml, 42.0 µg/ml and 70 µg/ml respectively. Thus, stem bark (ESETS) showed an effective anticancer activity at IC50 42.0 µg/ml.

CONCLUSION

The results of the present study revealed that stem bark (ESETS) showed the best activity compare to Root (ERETS) and Flower (EFETS). Thus stem bark (ESETS) of *T. stans*might be a potential alternative agent for human breast cancer therapy. Hence, it is concluded that*T. stans* would be a useful pharmaceutical material for the treatment of breast cancer. Since the stem extract showed the significant anti-cancer activity, it can be taken as a lead for the future study. There is a need for further investigation of this plant in order to identify, characterize and isolate its active anticancer principle(s) to treat breast cancer. Future research should focus on the Toxicity and *invivo* studies and also on molecular mechanism which is responsible for anticancer activity.

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