

Cytotoxicity Analysis of Crude Leaf Extracts from *Tecoma castanifolia* (D. Don) Melch on Brine Shrimp and MCF-7 Cell Line

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Abstract: To assess the possibility of cytotoxic potential of *T.castanifolia* leaf extract. Since this family of plants have effective cytotoxic potential against cancer, analysis of this particular species was carried out. The acetone, ethylacetate and ethanol extracts were subjected to brine shrimp lethality assay and cytotoxicity analysis against MCF-7 cell line. MTT assay of these three extracts showed the ethyl acetate extract to be most effectively cytotoxic. Further studies can be carried out in this aspect to bring out the complete effectiveness of the extract and compounds therein.

Keywords: Plant extract, cytotoxicity, Brine shrimp, MCF-7, MTT assay.

1. Introduction

Cancer is one of the leading causes of mortality worldwide and it has been that way since a long time. Worldwide, one in seven deaths is due to cancer; cancer causes more deaths than AIDS, tuberculosis, and malaria combined (American Cancer Society. *Global Cancer Facts & Figures* 3rd Edition). Current estimates from the American Cancer Society and from the International Union Against Cancer indicate that 12 million cases of cancer were diagnosed last year, with 7 million deaths worldwide; these numbers are expected to double by 2030 (27 million cases with 17 million deaths) [Aggarwal et al, 2009]. Breast cancer is the most commonly diagnosed cancer among women in the vast majority (140 of 184) of countries worldwide, making it the only cancer that is common among women in all regions of the world (IARC CancerBase). There are different types of breast cancer based on the origin of cells from which the tumor has arisen and their point of occurrence. The two major types include ductal and lobular carcinoma and based on their infiltration they can be divided as *in situ* or invasive. The treatments that are available slow down tumor progression and/or eradicate cancer cells but do not always promise a complete cure with zero relapse and a greater survival period. There has been an increase in research to discover better alternative drugs. The concentration has been on the ethno medicinal plants and their products.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. India is usually referred as "Botanical garden of the world" due to its large diversity in flora. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter [2]. Botanicals are less expensive and easily available because of their natural occurrence in abundance (Opara and Wokocho, 2008). The Bignoniaceae, the bignonias, are a family of flowering plants in the order Lamiales (Vernon H. Heywood et al., (2007)). Bignoniaceae family having 100 genera and more than 800 plant species found in various tropical regions of India. (Kirtikar K.R, 1999). Many species

of Bignoniaceae have some use, either commercially or ethno botanically. Several species are known to be used as a folk medicine for diabetes, skin disorders, gastro and urinary disorders etc., (Mohammed Rahmatullah et al, 2010). *Tecoma* is a genus of 14 species of shrubs or small trees in the trumpet vine family. *Tecoma sambucifolia* H.B.K. alcoholic extract of flowers also demonstrated cytotoxicity against human hepatoma cell line (Alguacil et al., 2000) [3]. One species, *T.stans* have been known to possess anti diabetic activity and the compounds like tecomine and tecostanin have been isolated from it [4-8]. Based on such literatures, the cytotoxic potential of one of the members of Bignoniaceae, *Tecoma castanifolia* (D. Don) Melch was analysed in this study.

2. Materials and Methods

Materials

The solvents viz., acetone, ethylacetate and ethanol were of analytical grade and purchased from HiMedia Laboratories Ltd., Mumbai. 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Foetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Modified Eagle's Medium (MEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Collection and Extraction of Plant Material

The leaves of the plant were collected in Chennai and the plant was authenticated from Botanical Survey of India, Coimbatore. The voucher number for the same is BSI/SRC/5/23/2014-15/Tech./842. The leaves were cleaned thoroughly and healthy leaves were shade dried. The dried leaves were then coarsely ground and cold extracted with various solvents for a period of 7 days with continuous shaking. The solvents were changed thrice during the period and filtered through Whatmann No.1 filter paper. It was then rotary evaporated to obtain a gummy extract which were stored in a glass vial.

Brine Shrimp Lethality Assay

The eggs of Brine shrimp (*Artemia salina*) were collected from an aquarium shop and hatched in a tank with constant oxygen supply and artificial sea water (38g sea salt in 1 litre of distilled water). 10 mg of each sample was dissolved in 150µl of DMSO and 1850 µl of deionised water. The extracts were tested at various concentrations from 1.25mg, 1mg, 0.5mg up to 0.025mg by adding respective volume from the stock to a pre marked microtitre plate containing 5 ml of sea water. All concentrations were tested in triplicates. With the help of a Pasteur pipette 10 living nauplii were transferred to each well. For the next 24 h the vials were observed and the number of nauplii survived in each vial was counted with the help of magnifying glass [9,10]. Potassium dichromate was used as standard cytotoxic agent. (Modified method from Meyer et al., 1982; Zhao et al., 1992). From this, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extract. The LC50 value was calculated using probit analysis.

Cell lines and Culture medium

MCF-7 (Human breast carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Foetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO₂ atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm [11,12]. The percentage growth inhibition was calculated using the following formula

$$\% \text{ Growth Inhibition} = \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

3. Results

Brine Shrimp Lethality Assay

The LC50 values calculated for each extract is tabulated in Table 1. Of the three extracts tested the ethyl acetate extract showed very potent activity as compared to the control, Potassium dichromate. The LC50 i.e., 50% mortality of the test organisms were obtained around 370µg/ml concentration from ethyl acetate extract.

Table 1: LC50 Values of Brine Shrimp Lethality Assay

S. No	Name of Extract	LC50
1.	Acetone	906µg/ml
2.	EthylAcetate	370µg/ml
3.	Ethanol	1233µg/ml
4.	Potassium Dichromate	495 µg/ml

MTT Assay

The three extracts were tested against MCF-7, a breast cancer cell line. The effectiveness of inhibition of the three extracts is graphically represented in Figure1. The GI50 values of acetone, ethyl acetate and ethanol extracts are >1000µg/ml, 335µg/ml and 926.67µg/ml respectively.

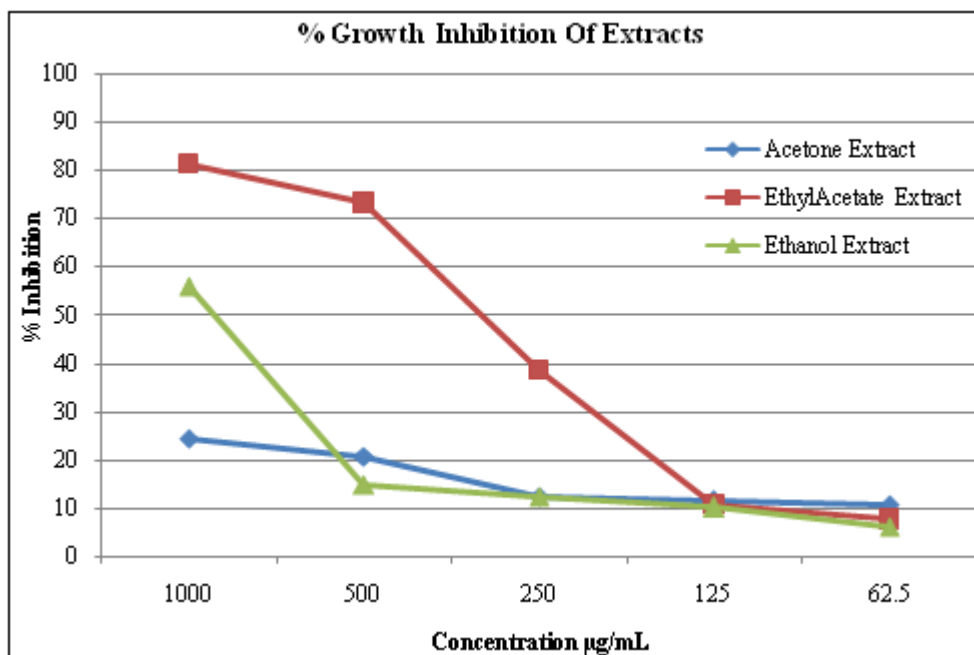


Figure 1: Graphical representation of Cell Growth Inhibition of the Extracts on MCF-7

4. Discussion

The genus of *Tecoma* has been traditionally used in various parts of the world to treat a variety of conditions and cancer is recorded to be one of it. The leaves of the plant is known to contain phenolic acid, beta-sitosterol, triterpenoids etc., both phenol and flavonoids possessing antioxidant activity. *Tecoma fabrisi* and *Tecoma impetiginosa* are used to treat some forms of cancer [13].

The three solvent extracts showed potent cell growth inhibitory activity. This may be due to their action on any one of the following- mitosis, biochemical processes etc. The biological activity shown by plants is mainly due to their secondary metabolites like alkaloids, phenolics, flavonoids, terpenoids, saponins etc. These are referred to as bioactive phyto compounds. Several phytochemical studies revealed that the extracts from many species of Bignoniaceae contained secondary metabolites such as saponins, tannins, flavonoids, quinones, alkaloids, anthralene derivatives, reducing sugars, glycosides, carbohydrates, querletin, kaempferol, β -sitosterol, terpenes, steroids, coumarins etc., and their derivatives [14].

From the above results it is clear that the LC50 values of brine shrimp lethality assay correlates with the GI50 values of the MTT assay. Brine shrimp Lethality assay is becoming a preliminary standard assay to assess the cytotoxic potential of plant extracts. Since many plants both land and marine are being tested for their various potentials, this preliminary assay is best suited to eliminate and/or select plant extracts for further study as a cytotoxic agent.

Out of the three crude plant leaf extracts tested here the ethyl acetate extract showed the effectiveness for further studies as an anti cancer agent. Its efficacy can be due to the fact of synergistic effects of various compounds present in the crude extract. The plants belonging to the family Bignoniaceae are one of the important sources of new bioactive compounds and as such several new chemical

entities have been isolated and tested for their efficacy as drugs [14]. The compounds present in the extract must be studied and the reason behind the activity (cell cycle arrest or biochemical interference etc.) should be assessed.

5. Conclusion

The study was conducted to assess the possibility of this plant extract to have a potent cytotoxic potential. From the preliminary assay it is clear that this plant can be further studied for its activity as a cytotoxic agent. Further studies into their way of action, phytochemicals involved etc., need to be explored.

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