

EVALUATION OF *IN VITRO* ANTICANCER ACTIVITY OF ETHANOL AND ETHYL ACETATE EXTRACTS OF *ALBIZIA SAMAN* FLOWERS

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ABSTRACT

To evaluate *in vitro* anticancer activity on the MCF-7 cell line (Human breast cancer cell line) of *Albizia saman* (Leguminosae: family) flower extract. The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer. The cell suspension was diluted with medium containing 5% FBS (Fetal Blood Serum) to obtain final density of 1×10^5 cells/ml. 100 μ l per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. The results obtained from the *in vitro* studies performed using the human breast cancer cell line (MCF-7) reveals that the ethanolic extract of *Albizia saman* flower has a moderate anticancer activity with 94.72% growth inhibition at 200 μ g/ml. The IC₅₀ value was 120.1 μ g/ml and the regression value was 0.999.

Keywords: *Albizia saman*, Anticancer activity, MCF-7 cell line and MTT Assay.

INTRODUCTION

Albizia saman formerly called *Samanea saman* (Leguminosae; family), a fast budding tree normally used as pasture for ornamental purposes. The flowers, bark, leaves, roots, seeds and pods of the tree are so far used as medicine from the traditional system¹. The alcoholic extract of selected leaves inhibit *Mycobacterium tuberculosis* the alkaloid portion of leaves is active on CNS and as laxative. Seeds are masticated for stinging throat. A decoction from the fresh leaves and inner bark are used for colds, diarrhea, and intestinal problem². The literature reveals that *Albizia saman* contains alkaloids, glycosides, terpenoids etc., the extracts of flowers possess antioxidant activity³. The tiny flowers (12–25 per head) are massed in pinkish heads 5-6 cm (2-2.4 inch) across and about 4 cm (1.6 inch) in height⁴. The mature pods of *Albizia saman* are black brown, almost oblong, clumpy, 10 to 20 cm long, 15 to 19 mm wide, 6 mm thick, slightly curved, eventually cracking irregularly, and filled with brownish pulp which is sticky, sweet and edible. The

bottom most line is that pods of *Albizia saman* tree which are rarely used as a plant source for herbs. Knowledge on phytochemical constituents of plant parts is mandatory in understanding the basis for any therapeutic effect. Recently, isolated flavonoids were reported to exhibit anti-carcinogenic activity. In addition to that the flavonoids through their free-radical scavenging activity have multiple biological functions including vasodilatory, anti-microbial, anti-inflammatory, anti-bacterial, immune stimulatory, anti-allergic and anti-viral functions².

Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells⁵. There are 200 different types of cancer that of list humans (Cancer Research UK). Cancer is commonly defined as the uncontrolled growth of cells, with loss of differentiation and commonly with metastasis, spread of the cancer to other tissues and organs. Cancers are malignant growths, where as in contrast, benign growths remain encapsulated and grow within a well defined

area. The causes of cancer are diverse, complex, and only partially understood. Cancer is classified by the type of cells that the tumor cells resemble and are therefore presumed to be the origin of the tumor. Though chemotherapy is now being used as a standard treatment method, search for anti-cancer agents from natural products has increased.

The prevalence of breast cancer in Indian women is more at the age of forty⁶. The incidence of breast cancer has been increasing worldwide for many decades⁷ with Asian countries attaining highest incidence rate⁸. Some breast tumors stay resistant to conventional treatment^{9,10} and may have many side effects which affect the quality of the treatment¹¹. According to the data of World Health organization, chemotherapy is needed for more than 90% of people affected with breast cancer. Hence we selected the flower of *Albizia saman* to treat the cancer cells which may not have significant side effects.

MATERIALS AND METHODS

Plant collection and identification

The fresh *Albizia saman* flowers were collected from Annamalai Nagar, Chidambaram during the month of January to March. Selected samples were taxonomically identified and authenticated by Botanical Survey of India (BSI), Palayamkottai, Tirunelveli District.

Processing of plant materials

The flowers of *Albizia saman* were cleaned, shade dried, segregated, pulverized by a mechanical grinder and passed through a Sieve #40. The powdered plant materials were stored in a clean air tight container until needed for analysis with proper labelling.

Preparation of plant extracts

Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method. The flowers were shade dried at room temperature for 10 days. The dried flowers were stored in an air-tight container for future use. About 175g of powdered plant material was uniformly packed into a thimble and extracted with different solvents separately subsequently. Solvent used were petroleum ether, ethyl acetate and ethanol as per increased polarity. The process of extraction continues for 48 hours. The petroleum ether, ethyl acetate and ethanol extracts were separately concentrated using rotary evaporator and then preserved individually at 5°C in air tight containers until used for further use.

Evaluation of *in vitro* anticancer activity

Cell line

The human breast cancer cell line (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 at 37°C, 5% CO₂, 95% air and 100% relative humidity and the culture medium was changed twice a week⁵.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspension and viable cells were counted using a hemocytometer. The cell suspension was diluted with medium containing 5% FBS to obtain final density of 1x10⁵ cells/ml. 100µl per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples⁵.

They were initially dissolved in dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours at 37°C, 5% CO₂, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations⁵.

MTT Assay

After the extraction of the sample, the viability of the cell was determined through MTT assay⁵. The MTT assay is based on the conversion of the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells, provides a quantitative determination of viable cells¹². 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT reagent (5mg/ml) in phosphate buffered saline

(PBS) was added to each well and plates were incubated at 37°C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl/well of DMSO and then the absorbance was measured at 570 nm using Micro plate reader. The effect of the samples on the proliferation of MCF-7 cell lines can be expressed as % cell viability or % cell growth. The percentage cell growth was then calculated with respect to control as follows

$$\% \text{ Cell Growth} = ([A] \text{ Test} / [A] \text{ control}) \times 100$$

The % Cell inhibition was determined using the following formula

$$\% \text{ Cell Inhibition} = ((100 - [A] \text{ Test}) / [A] \text{ control}) \times 100$$

Where, [A] - Absorbance at 570 nm
Non-linear regression graph was plotted between % Cell inhibition and Log concentration (Figure 3). IC₅₀ was determined using graph Pad Prism software.

RESULTS AND DISCUSSION

The results of cell growth inhibition by the plant extract against human breast cancer cell line

(MCF-7) for various concentrations are shown in Table 1. As the concentration increases there is an increase in the cell growth inhibition, but it is found to be very moderate with only 94.72% growth inhibition at 200µg/ml. The IC₅₀ value was 120.1µg/ml and the regression value was 0.999.

Effect of ethyl acetate and ethanolic extracts of *Albizia saman* flower in various concentrations for anticancer activities were presented in Figure 1 and 2. The percentage of cell inhibition of the different extracts was presented in Figure 3. The results obtained were showed that the ethanolic extract of *Albizia saman* flower has a moderate anticancer activity.

CONCLUSION

The results obtained from the *in vitro* studies, which performed using the human breast cancer cell line (MCF-7) reveals that the ethanolic flower extract of *Albizia saman* has moderate anticancer activity. Even though there was an increase in the cell growth inhibition, when concentration of sample was increased, the IC₅₀ value was more than 100µg/ml for cell line studies as shown by the MTT assay method. Hence the level of anticancer activity of the ethanolic extract of *Albizia saman* flowers can be concluded to be moderate effective.

Table 1: Percentage of Cell Inhibition

Test Concentration (µg/ml)	% Cell Inhibition
12.5	0.9083
25	0.4955
50	0.9083
100	26.259
200	94.715
IC ₅₀	120.1
R ₂	0.9998

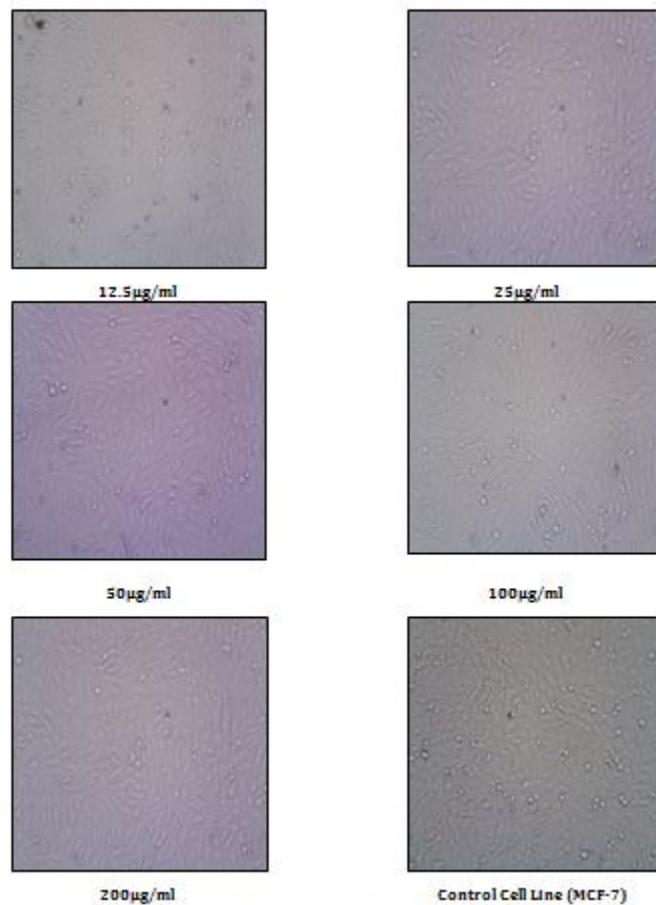


Fig. 1: Anticancer Activity shown by Ethyl acetate extract of *Albizia saman* flower in various concentrations

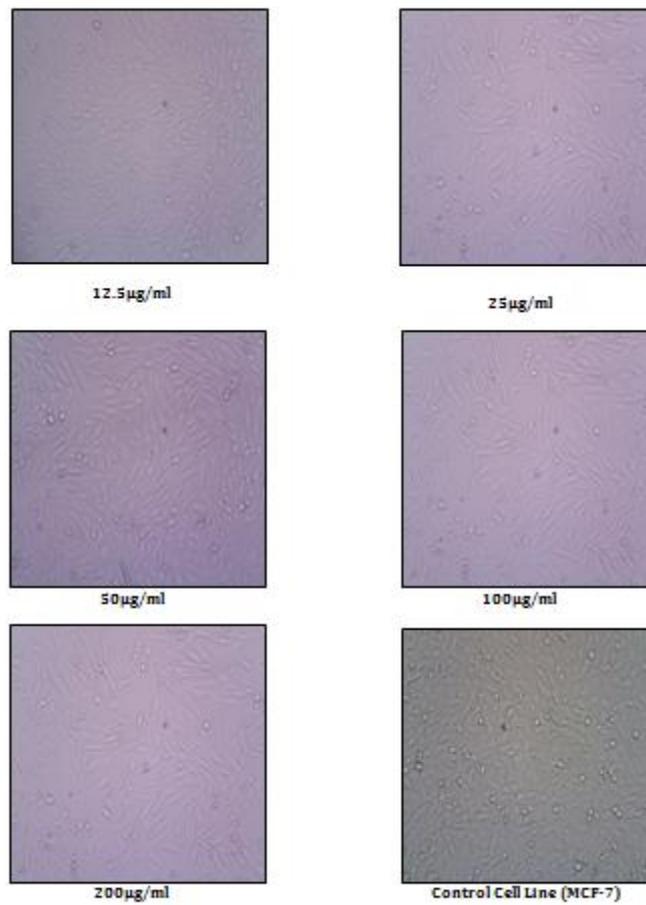


Fig. 2: Anticancer Activity shown by Ethanolic extract of *Albizia saman* flower in various concentrations

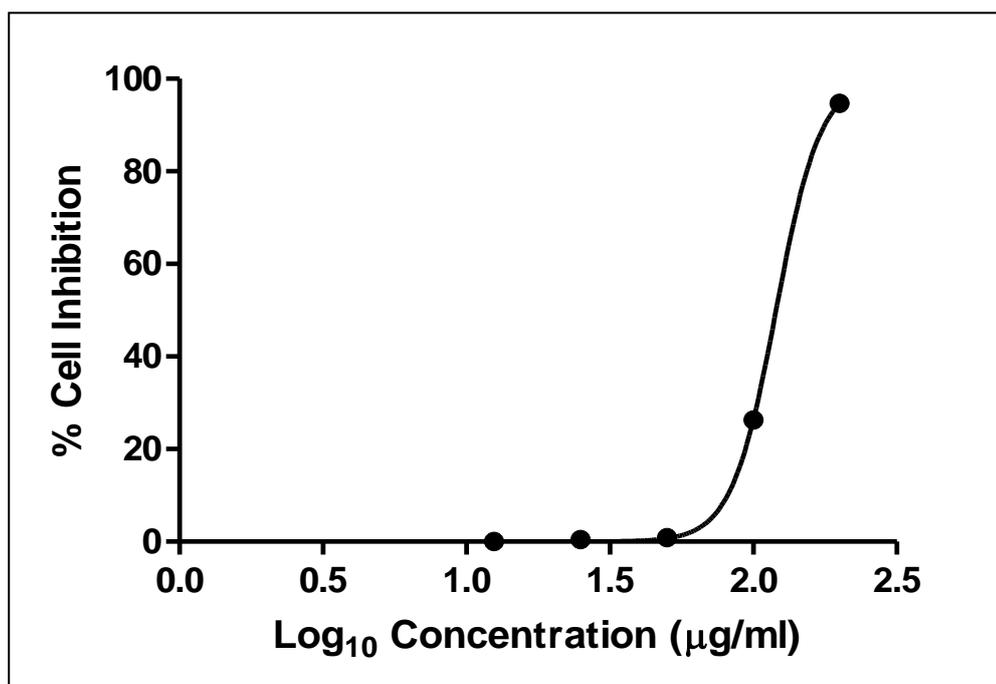


Fig. 3: Percentage of Cell Inhibition Vs Log Concentration

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