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EVALUATION OF IN VITRO ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF ALBIZIA SAMAN FLOWERS

Narra venkatesh *[1], Kopuri Gayathri Devi[1], Gunji Venkateswarlu [2], Akula Murali Sri Sudhakar[3].

[1]B.Pharm Research Scholars, A M Reddy Memorial College of Pharmacy, Petlurivaripalem Narasaraopeta, Guntur Dist, Andhra pradesh.

[2]Assoc. Prof, Department of Pharmacognosy, A M Reddy Memorial College of Pharmacy, Petlurivaripalem Narasaraopeta, Guntur Dist, Andhra pradesh.

[3]Prof.Department of Pharmaceutics, A M Reddy Memorial College of Pharmacy, Petlurivaripalem Narasaraopeta, Guntur Dist, Andhra pradesh.

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 1x105cells/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 370C, 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 200µg/ml. The IC50 value was 120.1µg/ml and the regression value was 0.999.

***Key Words*:** Albizia saman, anticancer activity, MCF-7 cell line and MTT Assay.

***Corresponding Author:** Narra venkatesh, B.Pharm Research Scholar, A M Reddy Memorial College of Pharmacy, Petlurivaripalem Narasaraopeta, Guntur Dist, Andhra Pradesh.

Email Id: narraamrcp@gmail.com

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INTRODUCTION

The word albizzia has come from Albizzia an Italian naturalist of the eighteenth century[1]. AL is an exotic species whose invasion is from Australia to India. Its vernacular name is Shirish. There are some common names of AL given below Hindi-Garso, Siris, Sanskrit-Barhapuspha,Bhandi, Kalinga , Urdu-Darash, West Indies-Woman'Tongue, Brazil-Heart-to-black, Ceylon-Kona, English-Parrottree French-Acacia lebbeck, Bois noir AL is found throughout India, ascending to 13000 m. in the Himalayas[2]. It is widely available plant in the tropical and subtropical Asia and Africa with economic importance for industrial medicinal uses. AL is a leguminous plant belonging to the family Fabaceae (Formerly Leguminosae), member of the subfamily Mimosae. AL is large deciduous tree with grayish bark; young shoots glabrous. Leaves are evenly 2- pinnate and the leaflets are in 5-9 pairs, 2.5-5.0 cm long, broadly oblong and pale green , unequal sided, very obtuse glabrous above and reticulately veined beneath. Flowers are stalked, white fragarant in globose umbellate heads 2-3.8cm diameter. Peduncles 3.8-7.5cm long solitary or 2-4 together from the axils of the upper leaves. Calyx 4 mm. long teeth short, Corolla 1 cm long ; tube glabrous ;lobes 2.5 mm long. Stamens much longer than the corolla . Pod is 10-30 cm long and 2.5-5.0 cm broad, flat straw coloured and contains 4-12 pale brown seeds. Flowering & fruiting periods are April to June.

PHYTOCHEMICALS

Two new tri-O-glycoside flavonols: kaempferol and quercetin 3-O-á-rhamnopyranosyl(1,6)-aglucopyranosyl(1,6)-agalactopyranosides, were identified from the leaves of Albizia lebbeck[3]. Pod of the A. l. contains 3',5 dihydroxy4',7 dimethoxy flavone and N-benzoyl L Phenyl alaninol[4]. The beans of the plants contain albiginc acid- a new triterpenoid sapogenin[5]. (Barua & Raman1959). The plant also contains saponins[6], Macrocyclic alkaloids,[7] Phenolic glycosides,[8]and Flavonols[9]. Low moisture content makes the shelf life for the seed long. Low lipid content is a favorable factor in preventing in rancidity of seeds stored for long periods .The ash contents (7.84%)of this seed is higher than that of other legumes which has been reported to range between3.0-4.8%(Elegbede1998), an indication that it may possess a higher mineral content[10,11].

Saponins are glycosides components often referred to as 'natural detergent' because of their foamy nature[12]. It has been established that saponins have anti-carcinogenic activity, immune modulation activities and regulation of cell proliferation as well as health benefits such as cholesterol lowering capacity. The toxic effect of cyanogenic glycoside decreases heart rate, decreases sympathetic activity & decreases systemic vascular resistance (Seiglar 1998).However for the AL seeds it is low. Tannin reduces protein solubility by forming a complex with protein, thereby causing a reduction I digestibility & causing

depressed growth (Siglar 1998). The level of Tannin in the seed is negligible (Ahn et.al.1989). All these things mentioned above are the favorable condition for animal supplement diet. Therefore AL has a potential to be utilized as a cheap source ofprotein, energy, & mineral supplement for animals. The alkaloids from the seeds of AL are fungicidal and cytotoxic to selected lines of cancer cells growing in vitro (Rahman et.al. 1986).Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells[13]. 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 forty[14]. The incidence of breast cancer has been increasing worldwide for many decades [15] with Asian countries attaining highest incidence rate[16]. Some breast tumors stay resistant to conventional treatment [17, 18] 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 a to treat the cancer cells which may not have significant side effects.

MATERIALS AND METHODS

Plant collection and identification: The fresh Albizia saman flowers collected from local area of Narasaraopet and the taxonomical identification of the leaves was confirmed by Dr. MadhavaSetty, a botanist from the Department of Botany, S. V. University, Tirupati Specimen Voucher no:3148, Preserved for further reference at our laboratory Processing of plant materialsThe 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 370C, 5% CO2, 95% air and 100% relative humidity and the culture medium was changed twice a week[19].

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 370C, 5% CO2, 95% air and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples.[19]

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 37oC, 5% CO2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

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 370C 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

% Cell Growth = ([A] Test / [A]control) x 100

The % Cell inhibition was determined using the following formula

% Cell Inhibition = ((100- [A] Test) /[A] control) x 100

Where, [A] - Absorbance at 570 nm

Non-linear regression graph was plotted between % Cell inhibition and Log concentration (Figure 2). 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 ethanolic extracts of Albizia saman flower in various concentrations for anticancer activities was presented in Figure 1. The percentage of cell inhibition of the different extracts was presented in Figure.2.The results obtained were showed that the ethanol extract of *Albizia saman* flower has a moderate anticancer activity.

Table 1: Percentage of Cell Inhibition

TestConcentration (µg/ml)	% Cell Inhibition
12.5	0.9083
25	0.4955
50	0.9083
100	26.259
200	94.715
IC50	120.1
R2	0.9998

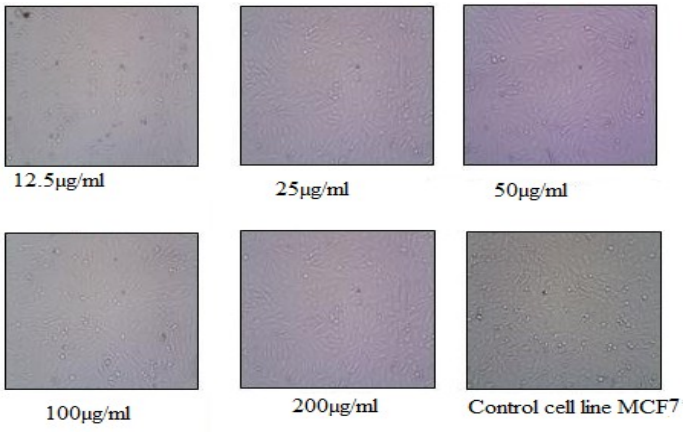

Fig no 1 Anti cancer activity of ethanol extract of Albizia flowers

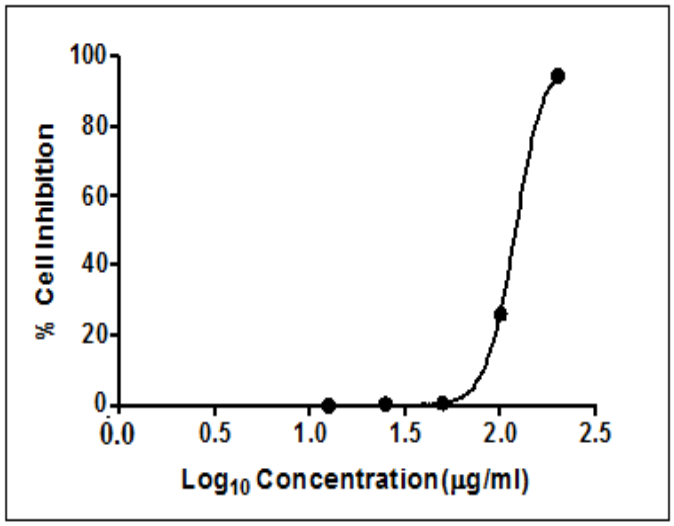

Fig no 2 percentage of Cell inhibation Vs Log Conc

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.

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