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Anti-cancer activity of Bauhinia tomentosa (Flowers) against human liver cancer

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Abstract
The present study has been performed experimentally by in vitro method to examine the anti-cancer activity of flowers of Bauhinia tomentosa. The report on to the research reveals a significant anti-cancer activity at different concentrations of the sample solution. The flowers of Bauhinia tomentosa was tested for its anti-cancer activity against liver cancer HePG2 cell line by MTT assay. The CTC_{50} value of the sample was 426.18µg/ml against liver cancer HePG2 cell lines. Significant results were observed there by explaining the use of this plant in the traditional system of medicine.

Keywords: MTT assay, anticancer activity, Bauhinia tomentosa, Liver cancer HePG2, pharmacological actions etc.

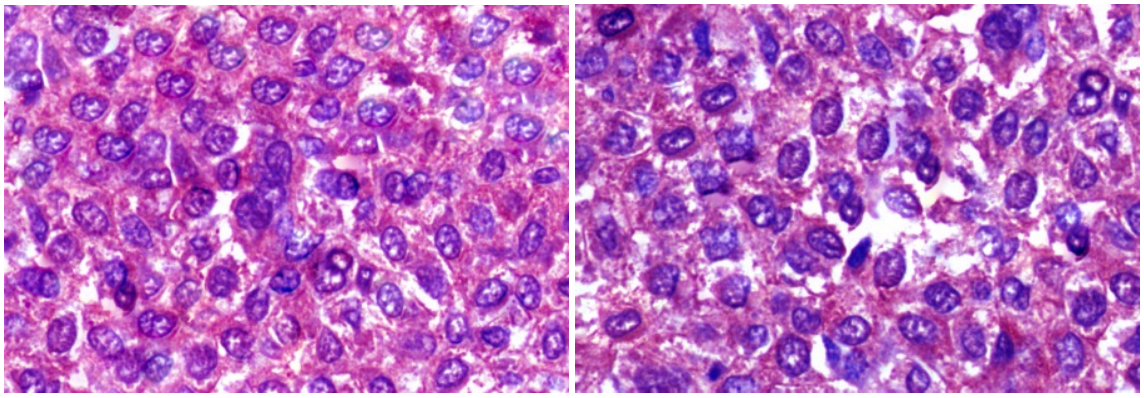
Introduction
Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. The screening of natural products has been the source of innumerable therapeutic agents [1]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [2]. Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell [3]. Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumor, they are not totally free from side effects [4]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [5]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders [6].
Bauhinia (Fabaceae) comprises ca. 300–350 species of trees, shrubs and vines distributed mostly in tropical region [7, 8, 9]. Bauhinia tomentosa called as variegated bauhinia, St. Thomas tree, bell bauhinia, orchid tree, hairy bauhinia, mountain ebony, yellow tree bauhinia in English; commonly known as "Kanjana" in Tamil, "Phalgu" in Sanskrit [10] and as adavimandaramu in Telugu [11]. Flowers bell-shaped, up to 7 cm long, beautiful and distinctive, pendulous, solitary, with large, lemon-yellow petals, 1-3 of which have dark maroon patch at the base and turning to a veined reddish brown with age. The flowers appears usually in pairs (rarely 1 or 3), on short axillary or leaf-opposed peduncles; bracts linear, 6-13 mm long; pedicels 5 mm long, 2-bracteolate [12].

Materials and Methods
Collection of Flowers
Fresh flowers of Bauhinia tomentosa were collected from Jail Corner, Trichy district, Tamilnadu, India, during the month of January and identified by Dr. S. John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS003 dated: 08/01/2016). St. Joseph's College (Campus), Trichy. Tamilnadu, India.

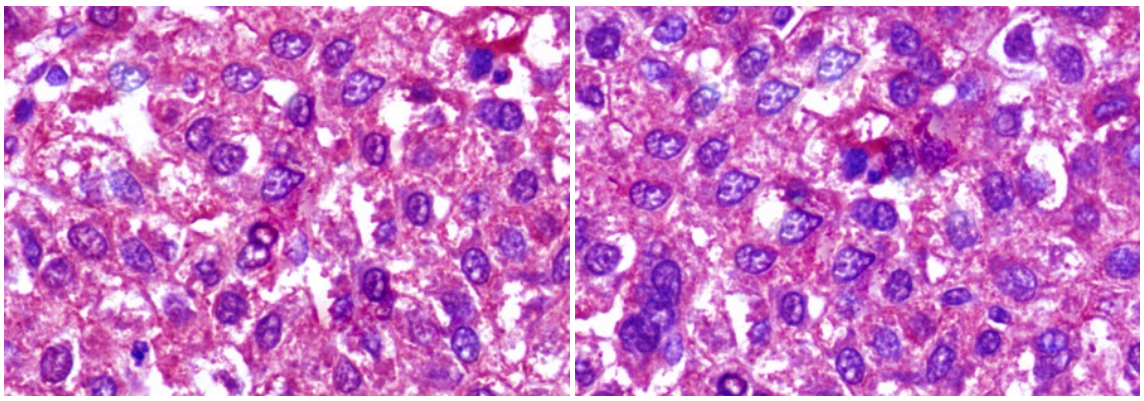
Extraction and fractionation
Fresh flower (1kg) of Bauhinia tomentosa collected at Jail corner, Trichy district, Tamilnadu, India were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in *vacuo* and the aqueous extract was successively fractionated with petroleum ether (60-80 ^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250 ml).

Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

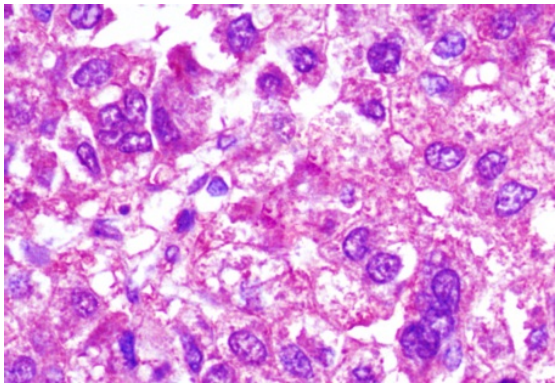
MTT Assay method
HePG2 cell line figures



1. 62.5 µg/ml 2. 125 µg/ml



3. 250 µg/ml 4. 500 µg/ml

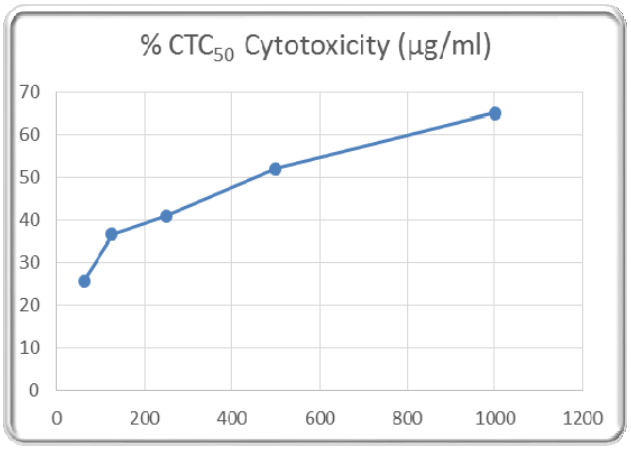


5. 1000 µg/ml

Fig (1-5): Effect of the compound isolated from the ethyl acetate fraction of *Bauhinia tomentosa* flowers against human Liver cancer HePG2 Cell line in different concentrations.

Table 1: The CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Bauhinia tomentosa* flowers against human Liver cancer HePG2 Cell line

S. No	Concentration of test sample (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50} (µg/ml)
1	1000	65.14	426.18
2	500	52.03	
3	250	40.87	
4	125	36.54	
5	62.5	25.63	



Graphical representation of the CTC_{50} values of the compound isolated from the ethyl acetate fraction of Bauhinia tomentosa flowers against human Liver cancer HePG2 Cell line.

Mtt Assay

MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) 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.

Cell Lines and Culture Medium

HePG-2 (Liver cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 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 cm^2 culture flasks and all experiments were carried out in 96 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 DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

% Growth inhibition =

$$100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Result and Discussion

The MTT assay is based on the reduction of MTT (3-(4,5-dimethyl thiazolyl)- 2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different concentration of the compound isolated from the ethyl acetate fraction of Bauhinia tomentosa flowers were subjected for MTT assay and results are presented in table.1. The photographs (Fig. 1 to Fig. 5) show the effect of the compound on the human liver cancer HePG2 cell line.

Conclusion

The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of Bauhinia tomentosa shows that all concentrations are having anticancer activity. The sample concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml show 65.14 µg/ml, 52.03 µg/ml, 40.87 µg/ml, 36.54 µg/ml, 25.63 µg/ml CTC_{50} value against the human liver cancer HePG2 cell line respectively. Thus B. tomentosa flowers to have the potential to act as a source of useful anticancer drugs and also to improve the health status due to the presence of compound that is vital for good health. Further work is required in order to establish the identity of the chemical component responsible for anticancer activity.

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