



## ANTI-CANCER ACTIVITY OF *TABEBUIA ROSEA* (FLOWERS) AGAINST HUMAN LIVER CANCER

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### ABSTRACT

Nature has been a powerful source of enormous medicines for thousands of years and number of modern drugs has been extracted and exploited from natural sources, for its use in traditional medicine. The present study has been performed experimentally by in vitro method to examine the anti cancer activity of flowers of *Tabebuia rosea*. The report on to the research reveals a significant anti cancer activity at different concentrations of the sample solution. The flowers of *Tabebuia rosea* was tested for its anti cancer activity against liver cancer HePG2 cell line by MTT assay. The  $CTC_{50}$  value of the sample was 205.3 $\mu$ g/ml against liver cancer HePG2 cell lines. Significant results were observed thereby explaining the use of this plant in the traditional system of medicine. Traditional herbal medicines have a long history of use and are generally considered to be safer than artificial drugs. Over 50% of all modern scientific drugs are natural products that play an important role in drug development in pharmaceutical industries.

### KEY WORDS

MTT assay, anticancer activity, *Tabebuia rosea*, Liver cancer HePG2, pharmacological actions etc.,

### INTRODUCTION

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<sup>1</sup>. 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<sup>2</sup>. 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<sup>3</sup>. 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<sup>4</sup>.

*Tabebuia rosea* (Bertol.) DC. is belonging to the family Bignoniaceae, It's commonly known as "Pink Trumpet Tree" often grown as an ornamental tree for its grand and majestic pink or purple flowers which offer different shades of colours. The graceful beauty is a treat for the eyes, but the tree has medical uses as well<sup>5</sup>. Tea made from the leaves and bark is known to have a fever reducing effect. The herbal products

obtained from the bark of *Tabebuia* trees are called Tahebo, lapacho, pandarco and iperoxo. Traditionally, it has been used for treating ulcers, syphilis, gastrointestnal problems, candidiasis, cancer, diabetics, prostatitis, constipation and allergies<sup>6</sup>. Recently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from plant products and such investigations about plant products have recently regained prominence with the increasing understanding of their biological significance and increasing recognition of the origin and function of their structural diversity<sup>(7,8)</sup>. Recently several herbals have been screened for anticancer activity and many patients with cancer take plant extracts in addition to chemotherapy<sup>9</sup>.

### MATERIALS AND METHODS

#### Collection of Flowers

Fresh flowers of *Tabebuia rosea* were collected from Jail Corner, Trichy district, Tamil Nadu, India, during the month of May and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS 002

dated: 06/11/2015). St. Joseph's College (Campus), Trichy, Tamil Nadu, India.

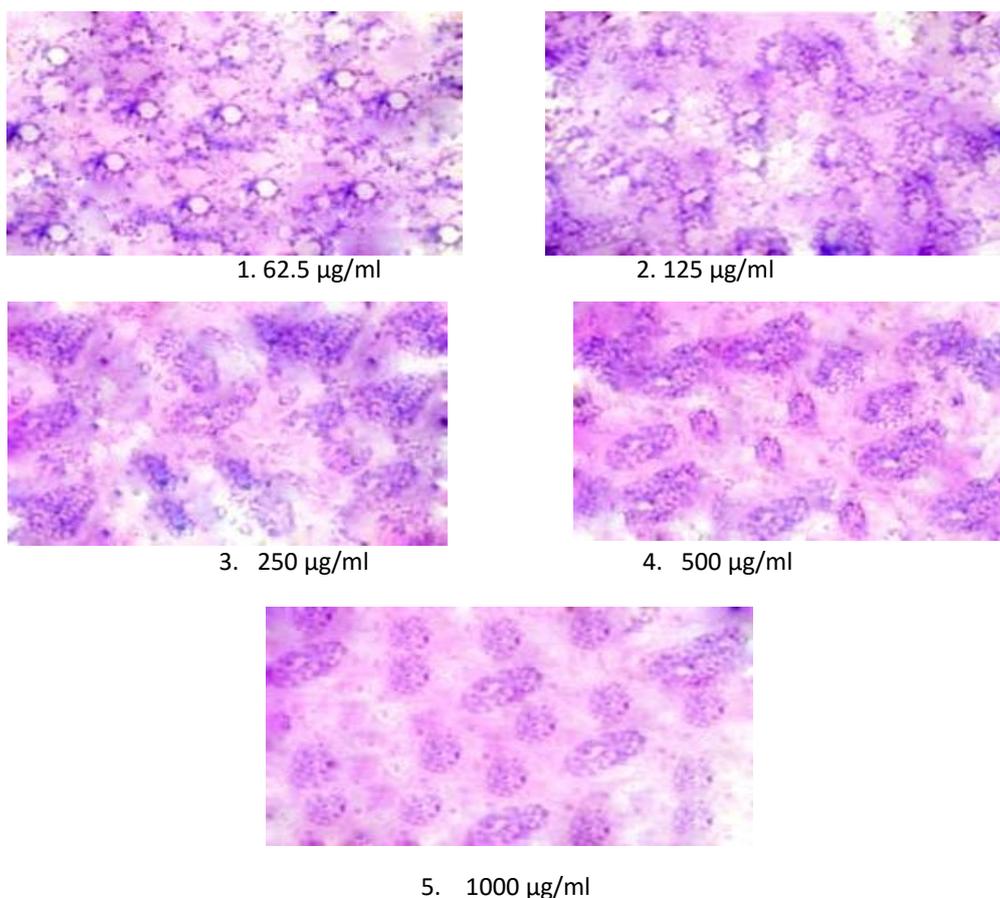
#### Extraction and fractionation

Fresh flower (1kg) of *Tabebuia rosea* collected at Jail corner, Trichy district, Tamil Nadu, 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°C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). 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

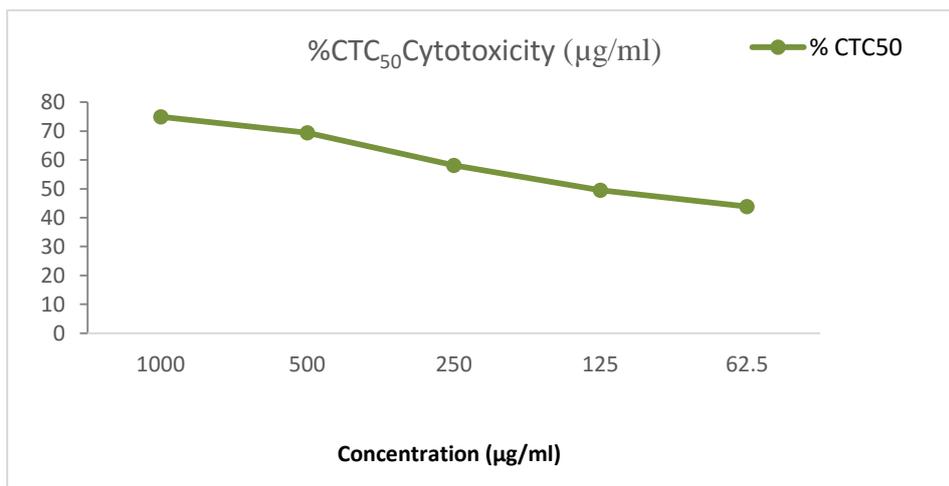
#### HePG2 cell line figures:



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

S. No	Concentration of test sample (µg/ml)	% CTC <sub>50</sub> Cytotoxicity (µg/ml)	CTC <sub>50</sub> (µg/ml)
1	1000	74.95	
2	500	69.43	
3	250	58.12	205.3
4	125	49.52	
5	62.5	43.86	

**Table.1:** The CTC<sub>50</sub> values of the compound isolated from the ethyl acetate fraction of *Tabebuia rosea* flowers against human Liver cancer HePG2 Cell line



**Graphical representation of the CTC<sub>50</sub> values of the compound isolated from the ethyl acetate fraction of *Tabebuia rosea* 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<sub>2</sub> 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<sup>2</sup> 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<sup>5</sup> 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<sub>50</sub>) values is generated from the dose-response curves for each cell line.

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

## 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 *Tabebuia rosea* 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 *Tabebuia rosea*

shows that all concentrations are having anticancer activity. The sample concentrations of 1000 $\mu$ g/ml, 500  $\mu$ g/ml, 250 $\mu$ g/ml, 125 $\mu$ g/ml and 62.5 $\mu$ g/ml show 74.95 $\mu$ g/ml, 69.43 $\mu$ g/ml, 58.12 $\mu$ g/ml, 49.52 $\mu$ g/ml, 43.86 $\mu$ g/ml  $CTC_{50}$  value against the human liver cancer HePG2 cell line respectively. These concentrations were able to induce apoptosis on human cancer cell lines and its anticancer activity was found to be precise. Further work is required in order to establish the identity of the chemical component responsible for anticancer activity. Studies are in improvement in our laboratory to explain the molecular structure. This contributes towards the development of potent anticancer drug.

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