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Original Research Paper

ANTI CANCER ACTIVITY OF *CASSIA AURICULATA* (FLOWERS) AGAINST HUMAN LIVER CANCER

N. Muruganantham[1]*, S. Solomon[2] and M. M. Senthamilselvi[3]

[1]Department of Chemistry, Roever Engineering College, Perambalur,Tamil Nadu, India

[2]Department of Chemistry, Periyar E.V.R. College (Autonomous), Trichy, Tamil Nadu, India

[3]Government Arts College, Kulithalai, Tamil Nadu, India

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. 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 and they play an important role in drug development in pharmaceutical industries. The present communication constitutes a review on medicinal properties and pharmacological actions of *Cassia auriculata*. This plant is known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases. The present study has been performed experimentally by in vitro method to examine the anti cancer activity of a variety of concentrations of ethanolic extract of flowers of *Cassia auriculata*. The report on to the research reveals a significant anti cancer activity at different concentrations of the extract. The ethanolic extract of flowers of *Cassia auriculata* was tested for its anti cancer activity against liver cancer HePG2 cell line by MTT assay. The CTC_{50} value of sample was 352.4µg/ml against liver cancer HePG2 cell lines. Significant results were observed thereby proving the use of this plant in the traditional system of medicine.

Keywords: MTT assay, Anticancer activity, *Cassia auriculata*, Liver cancer HePG2, Pharmacological action.

INTRODUCTION

The *Cassia auriculata* belongs to family Cesalpinaceae which has been claimed to posses the wound healing and antioxidant activities. According to the literature survey a detailed chemical investigation on the flowers of *Cassia auriculata* has not been carried out yet, hence a thorough phytochemical and pharmacological study can be done. Plants and plant products both as extracts and derived compounds are known to be effective and versatile chemo-preventive agents against a variety of types of cancers (Aruna and Sivarama Krishnan, 1990; Graham *et al*., 2000; Moongkarndi *et al*., 2004). Traditional background of Indian medicine shows widespread use of plant products in cancer (Cha, 1977; Gupta, 1979; Rabi and Gupta, 1995; Hussian *et al*., 1993). A remarkable surge of interest in chemoprevention research has thus led to the classification of many phytochemicals as effective chemo-preventive agents (Cordell *et al*., 1999). Today there are at least 120 distinct chemical substances derived from plants that are considered as important drugs and active ingredients. Natural compounds are perfectly suited to the current molecular-target approach of drug development, as well as the use of combinations. They produce few adverse effects; many act as tonics and stimulators that slow down

multiple aspects of disease progression. Fresh flower of *Cassia auriculata* is widely used in Indian traditional medicine (Shawney *et al*., 1978). The flower extracts of the plant have anti-diabetic activity (Jain and Sharma, 1967) and they have an emollient effect (Dhar *et al.*, 1968). The alcoholic leaf extract of *Cassia auriculata* is effective in alcoholic liver injury (Rajagopala *et al*., 2003). There are a few experimental studies to show the anti-viral activity of the plant (Dhar *et al*., 1968). Although this plant has been widely studied, biochemical studies on the anti-carcinogenic effects of *Cassia auriculata* has not been reported. Many chemotherapeutic drugs eliminate cancer cells by inducing, a genetically programmed form of cell death (Nanba *et al*., 1994). It is therefore important to establish the chemopreventive efficacy of the plant by evaluating cytotoxicity and apoptosis induction in cancer cell lines before whole animal studies or clinical trials begin. We therefore decided to screen the flower extract for its anti-cancer activity against liver cancer HePG2 cell lines. *Cassia auriculata* is one of the most important species of the genus Cassia which is rich in anthraquinones and polyphenols. Activity of the plants is associated with the presence of chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates. Traditionally this plant is effective in treating skin infections in man and animals. All parts of this plant have one or more medicinal action especially antimicrobial activity. *Cassia auriculata* commonly known as Tanner‟s Cassia is an important medicinal shrub used in Asia. *Cassia auriculata* profoundly used in tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia. The flowers are widely used in Ayurveda tradition system. It is used as Avaraipanchanga chooranum and the main constituents of Kalpa herbal tea. The flowers are used to treat urinary discharges, nocturnal emissions and throat irritation. It gives relief against skin ailments. *Cassia auriculata* plant contains preliminary phytochemical constituents such as alkaloids, phenols, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates and anthraquinone derivatives are responsible for the pharmacological activities. The plant has been widely used in traditional system of medicine as a cure for rheumatism.The plant has been reported to possess antipyretic hepatoprotective, antidiabetic, antiperoxidative and antihyperglyceamic and microbicidal activity. The present study has been undertaken to investigate the anticancer potential of ethanolic extract of *Cassia auriculata* flowers.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh flowers of *Cassia auriculata* were collected from O. Koothur Village, Ariyalur district, Tamil Nadu, India, during the month of November and Identified by Head, PG & Research Department of Botany, Periyar E.V.R. College, Trichy, Tamil Nadu.

Flower Extraction

2 kg fresh flowers of *Cassia auriculata* were soaked with 90% ethanol at room temperature (25°C-30°C). After 72 hrs the ethanolic extract was filtered. This extract was concentrated in vacuum and the dry powder obtained was dissolved in DMSO to get required concentrations and were used for screening anti cancer activities.

MTT Assay Method

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

HePG2 (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 a 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 made 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.

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

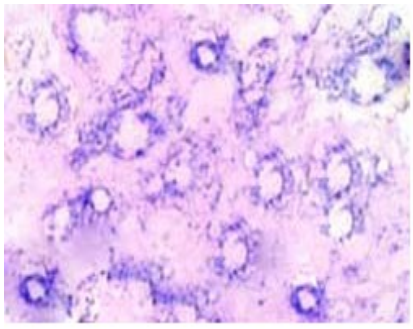
RESULT AND DISCUSSION

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 ethanolic extract *Cassia auriculata* flowers were subjected for MTT assay and results are presented in table.1. The photographs (Figure 1 to Figure 5) show the effect of *Cassia auriculata* flower extracts on human Liver cancer HePG2 cell line.

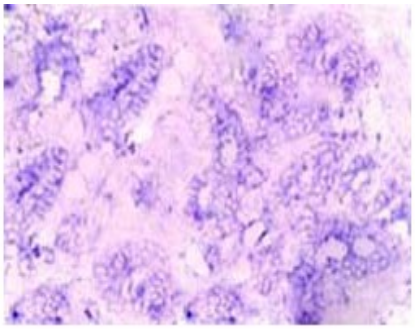
CONCLUSION

The MTT assay of ethanolic extract of flowers of *Cassia auriculata* shows that all concentrations are having anticancer activity. The sample concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml show 73.14 µg/ml, 57.86 µg/ml, 41.07 µg/ml, 35.43 µg/ml, 24.57 µg/ml IC50 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 progress in our laboratory to elucidate the molecular structure of that component. This contributes towards the development of valuable anticancer drug.

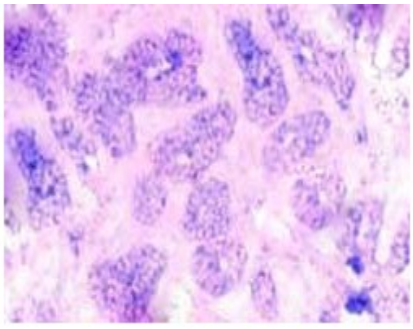
HePG2 Cell Line Figures



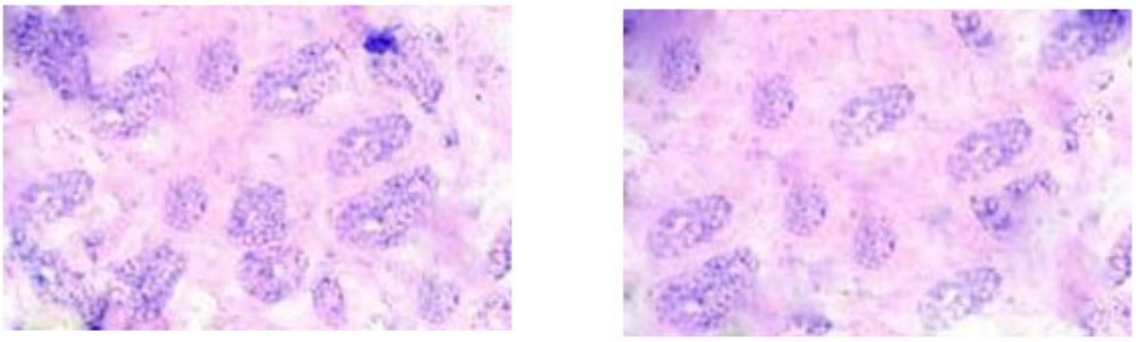
62.5 µg/ml



125µg/ml



250µg/ml

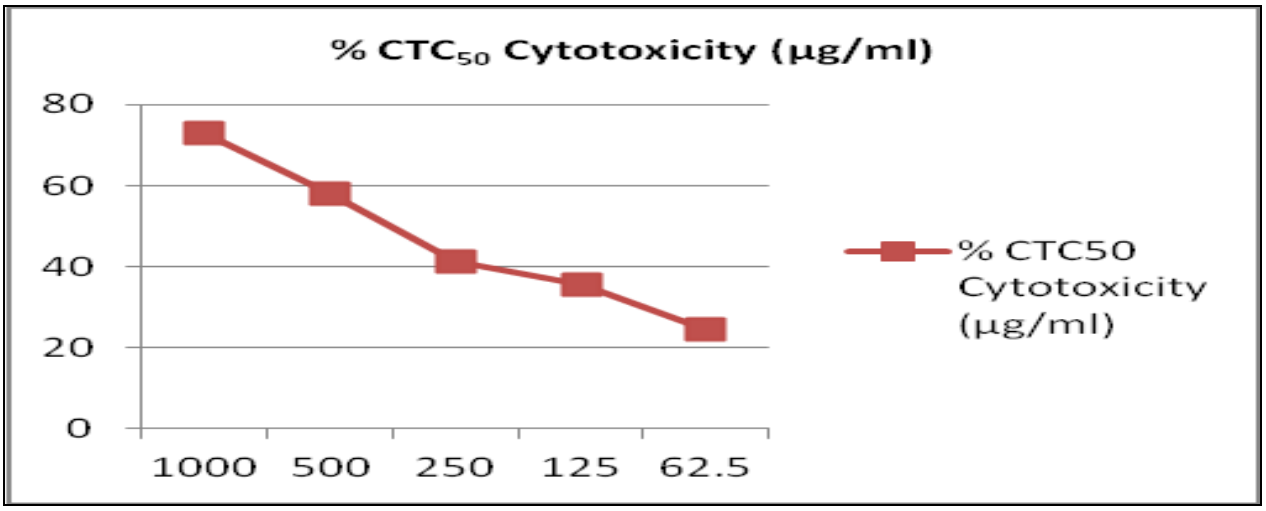


500 µg/ml 1000 µg/ml

Figures 1: *Cassia auriculata* f lower extract against human Liver cancer HePG2 Cell line in different concentrations

Table.1: The CTC_{50} values of *Cassia auriculata* flower extract against human Liver cancer HePG2 Cell line

S. No.	Concentration of extracts (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	CTC_{50}
1	1000	73.14	352.4µg/ml
2	500	57.86	
3	250	41.07	
4	125	35.43	
5	62.5	24.57	



Figures 2: Graphical representation of the CTC_{50} values of *Cassia auriculata* flower extract against human Liver cancer HePG2 Cell line.

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Correspondence Author:
N. Muruganantham
Department of Chemistry, Roever Engineering College, Perambalur,Tamil Nadu, India

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