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## ANTI CANCER ACTIVITY OF *CASSIA AURICULATA* (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. 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<sub>50</sub> 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.

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### INTRODUCTION

The *Cassia auriculata* belongs to family Cesalpinaceae which has been claimed to possess 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 ophthalmia. The flowers are

### 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

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 antihyperglycemic 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.

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<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 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<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

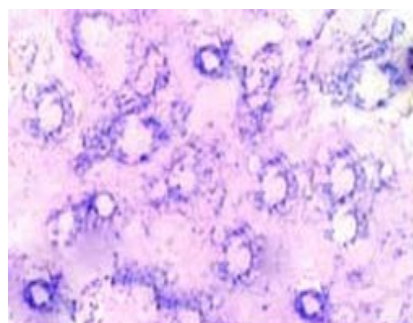
The MTT assay is based on the reduction of MTT (3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different concentration of ethanolic extract *Cassia auriculata* flowers were subjected for MTT assay and results are presented in 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

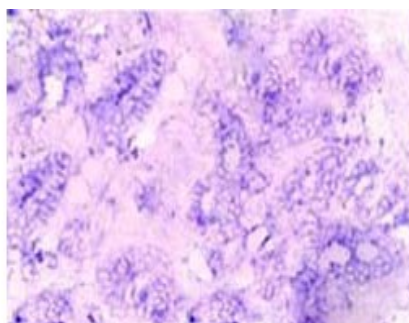
The MTT assay of ethanolic extract of flowers of *Cassia auriculata* shows that all concentrations are having anticancer activity. The sample concentrations of 1000 µg/ml, 500 µg/ml, 250

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 IC<sub>50</sub> 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.

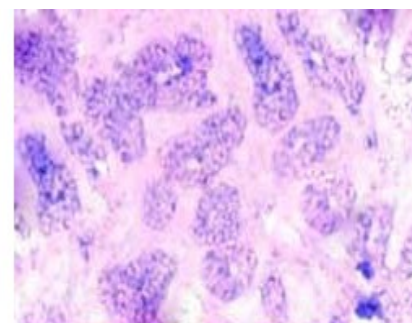
#### HePG2 Cell Line Figures



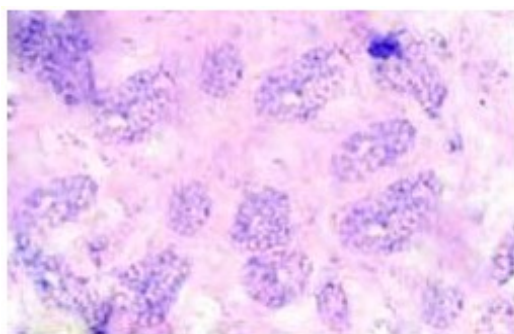
62.5 µg/ml



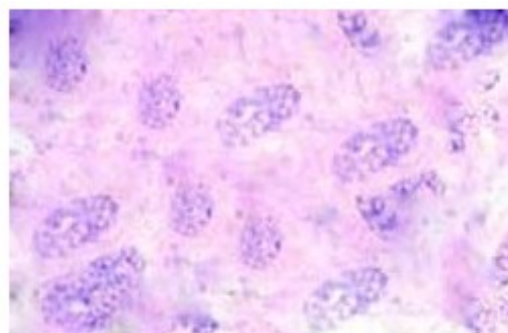
125µg/ml



250µg/ml



500 µg/ml

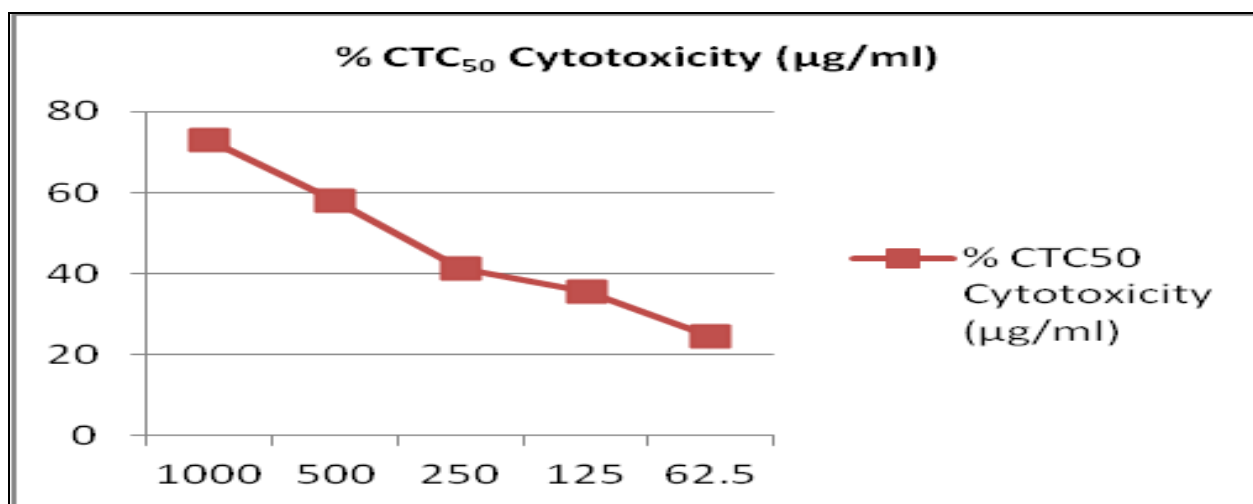


1000 µg/ml

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

**Table.1:** The CTC<sub>50</sub> values of *Cassia auriculata* flower extract against human Liver cancer HePG2 Cell line

S. No.	Concentration of extracts (µg/ml)	% CTC <sub>50</sub> Cytotoxicity (µg/ml)	CTC <sub>50</sub>
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<sub>50</sub> values of *Cassia auriculata* flower extract against human Liver cancer HePG2 Cell line.

## REFERENCES

1. Aruna, K and Sivarama, Krishnan VM (1990), "Plant products as protective agents against cancer", *Indian Journal of Experimental Biology*, 28, 1008.
2. Cha, S (1977), "Potential anticancer medicinal plants. A statistical evaluation of their frequencies of appearance in oriental medicine formularies", *Korean Journal of Pharmacognosy* 8, 14.
3. Cordell, GA; Farnworth, NR and King, Horn CWW (1999), "Plant derived anti cancer agents for therapy (Abstr)", *4<sup>th</sup> International*



- Congress on Phytotherapy*, PLI, Munich, Germany.
4. Darzynkiewicz, Z; Bruno, S; Del, Bino; G, Gorczyca; W, Hots MA and Lassota, P *et. al.* (1992), Features of apoptotic cells measured by flow cytometry”, *Cytometry*,14.
  5. Dhar, ML; Dhawan, BN; Mehrotra, BN and Ray, C (1968), “Screening of Indian plants for biological activity”, *Indian Journal of Experimental Biology*”, 6, 232e47.
  6. Gao, J; Huang, F; Zhang, J; Zhu, G; Yang, M and Xiao, P (2006), “Cytotoxic cycloartane triterpene saponins from *Actaea asiatica*”, *Journal of Natural Products*”,69 (10),1500e2.
  7. Graham, JG; Quinn, ML; Fabrican,t DS and Fransworth, NR(2000), “Plants used against cancer-an extension of the work of Jonathan Hartwell”, *Journal of Ethnopharmacology*, 73, 347e77.
  8. Gupta, SK (1978), “Apocynaceous plants of Varanasi with notes on their medicinal importance”, *Journal of Research Indian Medicine Yoga and Homeopathy*, 14,140e2.
  9. Hong, C; Firestone, GL; Boeldanes, LF (2002), “Bcl-2 family-mediated apoptotic effects of 3,3<sup>0</sup>-diionoilmethane (DIM) in human breast cancer cells”, *Biochemical Pharmacology*, 63,1085e97.
  10. Hsu, YL; Kuo, PL; Zeng, WS and Lin, CC (2006), “Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis”, *Food and Chemical Toxicology*, 44,704e13.
  11. Hussian, SJ; Alvi, AB and Jahan, MA (1993), “Study on Unani medicinal plants, *Aster-atiquus*”, *Journal of Research and Education in Indian Medicine*, 2, 35e9.
  12. Jain, SR and Sharma, SN (1967), “Hypoglycaemic drugs of Indian indigenous origin”, *Planta Medica*, 15,439e42.
  13. Kaur, G; Stetler, Stevenson M; Sebers, S; Worland, P; Sedlacek, H and Myers, C *et. al.* (1992), “Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275”, *Journal of the National Cancer Institute*,84 (22),1736e40.
  14. Keum, YS; Kim, J; Lee, KH; Park, KK; Surh, YJ and Lee, JM (2002), “Induction of apoptosis and caspase-3 activation by chemopreventive [6]-paradol and structurally related compounds in KB cells”, *Cancer Letters*,177, 41e7.
  15. Kumaran, A and Joel, Karunakaran R (2007), “Antioxidant activity of *Cassia auriculata* flowers”, *Fitoterapia*, 78 (1), 46e7.
  16. Lohmann, CM; League, AA; Clark, WS; Lawson, D; De Rose, PB and Cohen, C (2000), “Bcl-2:Bax and Bcl-2:Bcl-x ratios by image cytometric quantitation of immunohistochemical expression on ovarian carcinoma: correlation with prog-nosis”, *Cytometry*,42,61e6.
  17. Mackey, TJ; Borkowski, A; Amin, P; Jacobs, SC and Kyprianou, N (1998), “Bcl-2/Bax ratio as a preventive marker for therapeutic responses to radiotherapy in patients with prostate cancer”, *Urology*, 52,85e90.
  18. Moongkarndi, P; Kosem, N; Luanratana, O; Jongsomboonkusol, S and Pongpan, N (2004), “Antiproliferative activity of Thai medicinal plant extracts on human breast adenocarcinoma cell line”, *Fitoterapia*, 75,375e7.
  19. Mosmann, T (1983), “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays”, *Journal of Immuno-Logical Methods*”, 65,55e63.
  20. Ayyanar, M and Ignacimuthu, S (2008), “Pharmacological Actions of *Cassiaauriculata* L. and *Cissusquadrangularis Wall-* A short review”, *Journal of Pharmacology and Toxicology*, 3 (3), 213-32.
  21. Baansiddhi, J and Pechaaply, D (1988), “Botanical report of some Thai medicinal plants part I”, *Department of Medical Sciences*, Bangkok, 8-9.
  22. Barnali, Paul *et. al.*(2013), “Isolation and Structural Determination of an Antibacterial Constituent from the Leaves of *Cassia alata* Linn.”, *Journal of Pharmacognosy and Phytochemistry*, 2,326-32.

23. Ibrahim, D and Osman, H (1995), "Antimicrobial Activity of *Cassia alata* from Malaysia", *J. Ethnopharmacol*, 45 (3), 151-6.
24. Irene, M Villaseñor *et al.* (2002), "Bioactivity studies on *Cassia alata* Linn. leaf extracts", *Phyto therapy*, 16(S1),93-6.
25. Latha, M and Pari, L (2003), "Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism", *Clin. Exp Pharmacol Physiol*, 30,38-43.
26. Palanichamy, S and Nagarajan, S (1990), "Antifungal activity of *Cassia alata* leaf extract", *J Ethnopharmacol*, 29,(3), 337- 40.
27. Srivastava, J *et al.*, "Medicinal plants: an expanding role in development", *Word Bank Agriculture and Forestry Systems*, Washington, 320.
28. Sharanaiahumesha, *et al.*(2013), "Antioxidant and antidiabetic activities of medicinal plants: A short review", *Int j Res Phytochempharmacol*, 3(1),40-53.
29. Shi, J *et al.* (2004), "Saponins from edible Legumes: Chemistry, processing and health benefits", *J. Med. Food*, 7,67-78.
30. Sukumaran *et al.* (2011), "Phytochemical constituents and antibacterial efficacy of the flowers of *Peltophorumpterocarpum* (DC.) Baker ex Heyne", *Asian Pacific Journal of Tropical Medicine*, 4(9),735-8.
31. Nakamura, C; Yasumoto, E; Nakano, K; Nakayachi, T; Hashimoto, K and Kusama, K *et al.* (2003), "Changes in intracellular concentrations of polyamines during apoptosis of HL-60 cells", *Anticancer Research*, 23 (6C) 4797e803.
32. Zhang, M; Peter, C; Cheng, K; Lawrence, C; Chium, M; Elaine, Y *et al.* (2006), "Cell cycle arrest and apoptosis induction in human breast carcinoma MCF-7 cells by carboxymethylated b-glucan from the mushroom *Scleretia* of *Pleurotus tuber-regium*", *Carbohydrate Polymers*, 66, 455e62.
33. Ye, X; Krohn, RL; Liu, W; Joshi, SS; Kuszynski, CA; McGinn, TR *et al.* (1999), "The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells", *Molecular and Cellular Biochemistry*, 196(1-2), 99e108.
34. Towbin, H; Staehelin, T and Gordon, J (1997), "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications", *Proceedings of the National Academy of Sciences*, USA, 76, 4350e5354.
35. Wyllie, AH (1980), "Glucocorticoid-induced apoptosis is associated with endogenous, endonuclease activation", *Nature*, 284, 555e6.

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