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ANTICANCER ACTIVITY OF *ABELMOSCHUS ESCULENTUS* (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 Abelmoschus esculentus. The report on to the research reveals a significant anti-cancer activity at different concentrations of the sample solution. The flowers of Abelmoschus esculentus was tested for its anti-cancer activity against liver cancer HePG2 cell line by MTT assay. The CTC_{50} value of the sample was 444.22µg/ml against liver cancer HePG2 cell lines. Significant results were observed thereby explaining the use of this plant in the traditional system of medicine.

KEY WORDS

MTT assay, anticancer activity, Abelmoschus esculentus, Liver cancer HePG2, pharmacological actions etc.,

INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [1]. 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 [2]. 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 [3]. 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 [4]. 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 [5]. *Abelmoschus esculentus* (Family:Malvaceae) is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits containing round, white seeds. *Abelmoschus* suggesting the musky odour produced by the seeds. This plant is commonly known as okra, gumbo, or lady's finger, and in Southern Asia, usually a variant of "bhindi" or "vendi.". It is a perennial originated in the Ethiopian Hilltops. It grows largely in India, Africa, America, and Brazil. Its fruits are harvested when immature and eaten as a vegetable. Traditionally parts of the plants are assumed to have medicinal properties like antioxidant antispasmodic, demulcent, diaphoretic, diuretic, emollient, and stimulant [6,7]. Abelmoschus esculentus is a widely cultivated and consumed vegetable in tropical and subtropical countries. It is a source of protein, vitamins C and A, iron, and calcium and dietary fiber [8-10]. It contains large quantities of glycans, which are responsible for the viscosity of aqueous suspension and the stringy, gum like consistency that is particularly desirable in soups. Nowadays, the most important producing countries are India, Nigeria, Pakistan, Ghana, and Egypt [11].

MATERIALS AND METHODS

Collection of Flowers

Fresh flowers of *Abelmoschus esculentus* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of February and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS005 dated: 03/06/2016). St.Joseph's College (Campus), Trichirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (3 kg) of *Abelmoschus esculentus* collected at S. Pudur, Sivagangai (Dt), 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^{0}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:

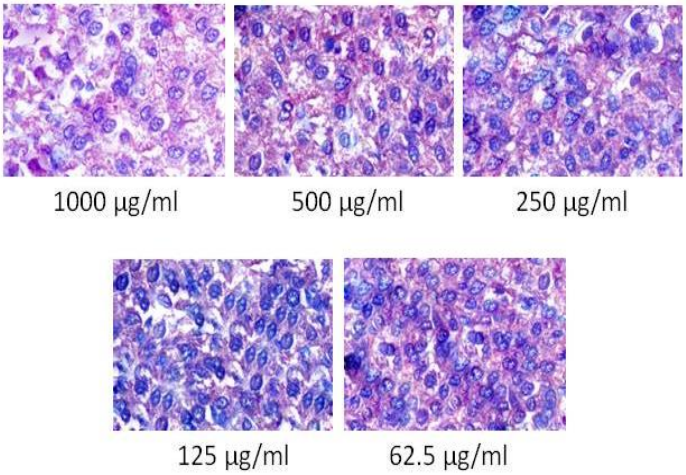
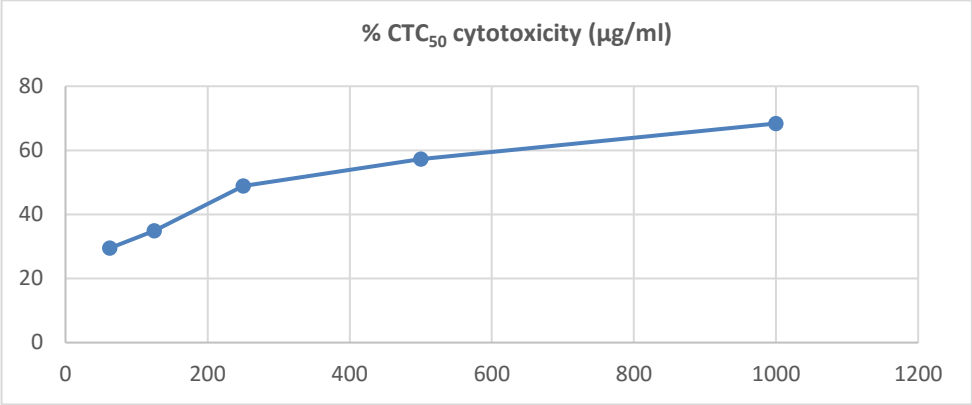


Fig. (1-5): Effect of the compound isolated from the ethyl acetate fraction of *Abelmoschus esculentus* flowers against human Liver cancer HePG2 Cell line in different concentrations.

S. No	Concentration of test sample (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	IC_{50} (µg/ml)
1	1000	68.37	
2	500	57.28	
3	250	48.91	444.22
4	125	34.86	
5	62.5	29.49	

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



Graphical representation of the CTC_{50} values of the compound isolated from the ethyl acetate fraction of *Abelmoschus esculentus* 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 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 FBS). 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.

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

RESULTS AND DISCUSSION

The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus* 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 68.37 µg/ml, 57.28 µg/ml, 48.91 µg/ml, 34.86 µg/ml, 29.49 µg/ml CTC_{50} value against the human liver cancer HePG2 cell line respectively.

CONCLUSION

The MTT assay of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus* shows that all concentrations are having anticancer activity. So, it could be concluded that this compound has the potential to act as a source of useful

anticancer drugs and could be used to improve the health status.

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