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Anti-cancer activity of *Cucurbita maxima* flowers (Pumpkin) against human liver cancer

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ABSTRACT

In the earlier times, many people used plants to cure diseases just by experimental data without knowing anything about its compounds. Now because of industrialism and lifestyle changes the presence of pumpkin in the diet of families really has decreased, and in parallel the prevalence of variety of cancers, especially liver cancers, has increased. A number of evidence has been documented to demonstrate the potential of these plants in pharma industry. In recent years Cucurbita maxima an important medicinal plant that has been extensively used in pharmacological analysis and toxicological studies was found and these phytochemicals were considered to be greater importance in pharma industry. The present study was carried out to evaluate the anticancer activity of the compound isolated from ethyl acetate fractions of Cucurbita maxima. This study was explains a very good anticancer action of against liver cancer. The compound isolated from ethyl acetate fraction of Cucurbita maxima flowers was tested for anti cancer activity against human liver cancer HePG2 cell line by MTT assay. The CTC_{50} value of sample was 212.7 µg/ml against liver cancer HePG2 cell lines. Significant results were observed there by proving the use of this plant in the traditional system of medicine.

Keywords: MTT assay, anticancer activity, *Cucurbita maxima,* Liver cancer HePG2, pharmacological actions.

1. INTRODUCTION

The plant *Cucurbita maxima* (commonly known as pumpkin) belongs to Cucurbitaceae. The family is widely cultivated throughout the world for use as vegetable as well as medicine. Both of its fruits and the aerial parts are commonly consumed as vegetable. It is a large climbing herb, annual or perennial. Its aerial part consists of flexible succulent stem with trifoliate leaves [1]. The plant has been used traditionally as medicine in many countries such as China, India, Yugoslavia, Brazil and America [2,3]. Traditionally it is used in most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immune modulator and antibacterial agents [4]. Several in vitro and in vivo studies with crude pumpkin fruits extract as well as various purified fractions, including proteins and poly saccharide, However, Pharmacology of its aerial parts has not yet been explored scientifically. As such, the present investigation was carried out to evaluate the anticancer activity of the methanol extract of *Cucurbita maxima* Duchesne aerial parts (MECM) against in vivo and in vitro Ehrlich Ascites Carcinoma (EAC) tumor model.

Cancer is a multi factorial diseases and economical burden worldwide. There are numerous chemo preventive agents used to cure various types of diseases including cancer. These drugs show an adverse side effect through alteration in gene normal action. The current treatments based on radiotherapy and chemotherapy which are effective but also show adverse consequences. Constituents of medicinal plants such as flavonoids and phenols play a significant role in cancer control through the regulation of genetic pathways without any side effect [5, 6].

Over the past few decades, cancer has remained as the largest cause of mortality worldwide and the number of

individuals living with cancer is steadily expanding. Hence, a major portion of the current pharmacological research is involved with the anticancer drug design customized to fit new molecular targets [7]. Due to the enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities. Traditionally various plants have long been used in the treatment of cancer [8- 11].

Plant shave proven to be the most useful in curing diseases and provide an important source of pharma and medicine. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiologicalaction on the body of human [12]. Prostate cancer is the most common type of cancer occurred in men. Hormones, in particularly the androgens, are essential for the development, growth and maintenance of the prostate. Certain pathological assaults may trigger the hyper stimulation of androgen and/or growth factors leads to prostate cancer. Androgen ablation therapy is the first step in the prostate cancer treatment [13,14]. Other than that various treatment strategies like chemotheraphy, radiation theraphy, hormones and surgery [15]. The present study has been undertaken to investigate the anticancer potential of the compound isolated from ethyl acetate fraction of *Cucurbita maxima* flowers.

2. MATERIALS AND METHODS

2.1 Extraction and fractionation

Fresh flowers (1kg) of *Cucurbita maxima* were collected at O.Koothur village, Ariyalur district, during the month of August and identified by Dr.JohnBritto, Director, Rabinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Trichirappalli-2, Tamilnadu, India. The flowers 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 was taken for screening of anti cancer activity against human liver cancer HePG2 cell line.

2.2 MTT Assay method

2.2.1 MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium 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.

2.2.2 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).

2.2.3 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.

2.2.4 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.

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

3. RESULTS AND DISCUSSION

The MTT assay is based on the reduction of MTT (3-(4, 5- dimethyl thiazolyl)-2,5diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The different concentration of the compound isolated from ethyl acetate fraction of *Cucurbita maxima* flowers were subjected for MTT assay and results are presented in Table 1 and Fig 1. The photographs (Fig. 2 to Fig. 6) show the effect of *Cucurbita maxima* flowers of the compound (test drug) isolated from ethyl acetate fraction on human Liver cancer HePG2 cell line.

Table 1: The CTC_{50} values of the compound isolated from ethyl acetate fractions of *Cucurbita maxima* flowers against human Liver cancer HePG2 cell line.

S.No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity	CTC_{50}
1	1000	72.05	
2	500	68.94	
3	250	54.22	212.7 µg/ml
4	125	43.19	
5	62.5	37.80	

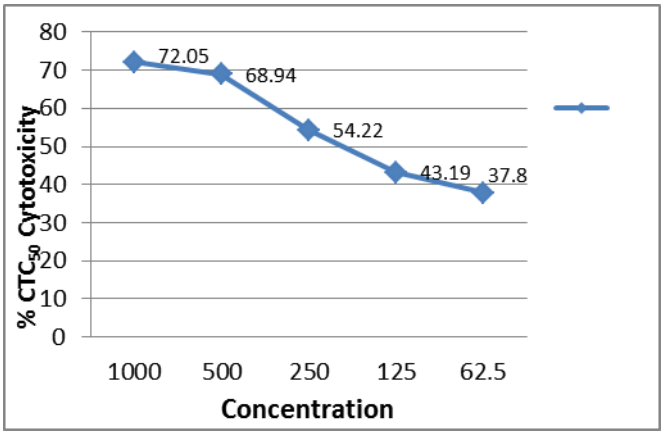
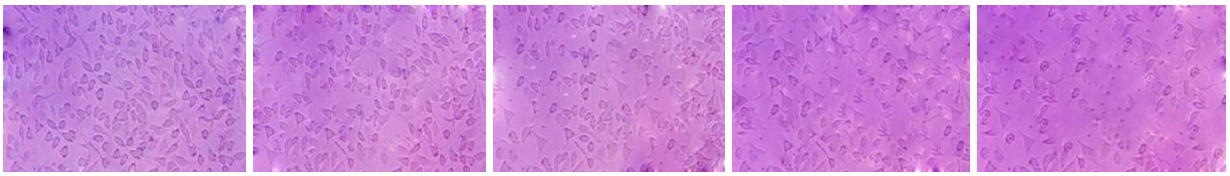


Figure 1: Graphical representation of the CTC_{50} values of the compound isolated from ethyl acetate fractions of *Cucurbita maxima* flowers against human Liver cancer HePG2 cell line.



Figures (2-6): Compound isolated from ethyl acetate fractions of *Cucurbita maxima* flowers against human Liver cancer HePG2 Cell line in different concentrations.

4. CONCLUSION

The MTT assay of the compound isolated from isolated ethyl acetate fractions of *Cucurbita maxima* 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 showed 72.05µg/ml, 68.94µg/ml, 54.22µg/ml, 43.19µg/ml and 37.80µg/ml of CTC_{50} (212 µg/ml) 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.

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