

## Anti-cancer Activity of *Cucumis sativus* (Cucumber) Flowers Against Human Liver Cancer

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### ABSTRACT

Cucumbers are scientifically known as *Cucumis sativus* and belong to the same botanical family as melons (including watermelon and cantaloupe) and squashes (including summer squash, winter squash, zucchini and pumpkin). Commercial production of cucumbers is usually divided into two types. "Slicing cucumbers" are produced for fresh consumption. "Pickling cucumbers" are produced for eventual processing into pickles. Slicing cucumbers are usually larger and have thicker skins, while pickling cucumbers are usually smaller and have thinner skins. Cucumbers have not received as much press as other vegetables in terms of health benefits, but this widely cultivated food provides us with a unique combination of nutrients. At the top of the phytonutrient list for cucumbers are its cucurbitacins, lignans, and flavonoids. This Present study explains a very superior anticancer action against liver cancer. The compound isolated from ethyl acetate fraction of *Cucumis sativus* flowers 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 103.7µg/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, *Cucumis sativus*, Liver cancer HePG2, pharmacological actions etc.,

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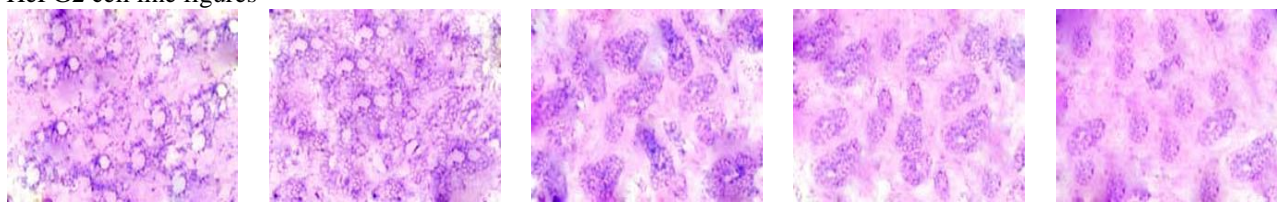
### INTRODUCTION

Cancer is a general term applied for series of hateful diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. The main forms of treatment for cancer in humans are surgery; radiation and cancer chemotherapeutic agents<sup>1</sup>. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicine, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance<sup>2</sup>. Due to the prevalence, morbidity, and mortality of the malignant diseases, they represent significant medical, social and financial burden on the society. At present the pharmacological therapy of cancer is limited to symptomatic treatments that do not alter the course of the underlying disease. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the treatment of cancer diseases due to their potent pharmacological activity, Low toxicity and less time, economic viability and renewable sources, long history of use, better patient tolerance, public

acceptance, cultivation and processing conditions environmental friendly, a voiding environmental pollution by the chemical industry<sup>3-11</sup>.

Research on the anti-cancer benefits of cucumber is still in its preliminary stage and has been restricted thus far to lab and animal studies. Interestingly, however, many pharmaceutical companies are actively studying one group of compounds found in cucumber—called cucurbitacins—in the hope that their research may lead to development of new anti-cancer drugs. Cucurbitacins belong to a large family of phytonutrients called triterpenes. Cucurbitacins A, B, C, D and E have all been identified within fresh cucumber. Eventually, we expect to see human studies that confirm the anti-cancer benefits of cucumbers when consumed in a normal, everyday meal plan<sup>12</sup>. A second group of cucumber phytonutrients known to provide anti-cancer benefits are its lignans. The lignans pinoresinol, lariciresinol, and secoisolariciresinol have all been identified within cucumber. Interestingly, the role of these plant lignans in cancer protection involves the role of bacteria in our digestive tract. When we consume plant lignans like those found in cucumber, bacteria in our digestive tract take hold of these lignans and convert them into enterolignans like enterodiols and enterolactone. Enterolignans have the ability to bind onto estrogen receptors and can have both pro-estrogenic and anti-estrogenic effects. Reduced risk of estrogen-related cancers, including cancers of the breast, ovary, uterus, and

MTT Assay method  
HePG2 cell line figures



1.62.5 µg/ml

2.125µg/ml

3. 250µg/ml

4.500 µg/ml

5. 1000 µg/ml

Figures(1-5) of the compound isolated from ethyl acetate fractions of Cucumis sativus flowers against human Liver cancer HePG2 Cell line in different concentrations.

Table.1: The CTC<sub>50</sub> of the compound isolated from ethyl acetate fractions of Cucumis sativus flowers 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> (µg/ml)
1	1000	82.15	
2	500	73.06	
3	250	69.74	103.7
4	125	56.21	µg/ml
5	62.5	49.83	

prostate has been associated with intake of dietary lignans from plant foods like cucumber<sup>13,14</sup>. The present study has been undertaken to investigate the anticancer potential of ethyl acetate fraction of Cucumis sativus flowers.

## MATERIALS AND METHOD

### Extraction and fractionation

Fresh flowers (1kg) of Cucumis sativus were collected at O.Koothur village, Ariyalur district, during the month of August and identified by Dr.John Britto, 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°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 anti-cancer activity against human liver cancer.

### MTT Assay method

#### 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<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 the compound from ethyl acetate fractions of Cucumis sativus flowers were subjected for MTT assay and results are presented in table.1 and Fig-6. The photographs (Fig. 1 to Fig. 5) shows the effect of Cucumis sativus flowers extracts on human Liver cancer HePG2 cell line.

## CONCLUSION

The MTT assay of the compound isolated from ethyl acetate fractions of Cucumis sativus flowers shows that all concentrations are having anticancer activity. The sample

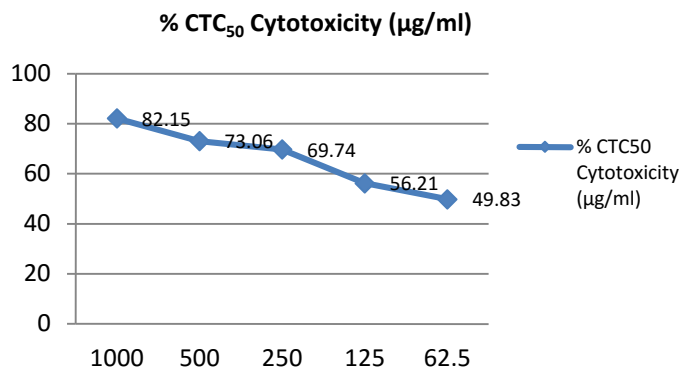


Figure 6: Graphical representation of the CTC<sub>50</sub> values of the compound isolated from ethyl acetate fractions of *Cucumis sativus* flowers against human Liver cancer HePG2 Cell line.

concentrations of 1000µg/ml, 500 µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml shows 82.15 µg/ml, 73.06 µg/ml, 69.74 µg/ml, 56.21 µg/ml and 49.83 µg/ml of CTC<sub>50</sub> values 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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