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Research Article

# *IN - VITRO* ANTI - CANCER STUDIES OF CHEBULINIC ACID ON COLON ADENOCARCINOMA HT-29 CELL LINES

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**ABSTRACT**

Objective: This paper aims that ***in-vitro*** anti-cancer activity of Chebulinic acid on Colon adenocarcinoma HT-29 cancer cell lines.

Methods: Cell lines were examined by using MTT cell growth inhibition assay.

Results: The maximum percentage inhibition of cancer cell lines for Chebulinic acid was found to be 41.2% at a dose of 200µg/ml.

Conclusion: within the work, It has been shown that the inhibition of cancer cell growth, contractile responses of cardiovascular muscles, anti-fungal, anti-bacterial activities etc. Hence Chebulinic acid can be used as a potent anti-cancer agent.

**Keywords:** *Terminalia chebula*, Chebulinic acid, Anti-cancer activity, HT-29 cancer cells, Inhibition.

## INTRODUCTION

Colon adenocarcinoma[1] is the most common type of gastrointestinal cancer. This type of cancer begins in the cells of glandular structures in the inner layer of the colon and spreads first into the wall of the colon and potentially into the lymphatic system and other organs. Colon adenocarcinoma progresses slowly and may not present symptoms for up to five years. As the cancer grows, symptoms become more likely and can include rectal bleeding, fatigue, shortness of breath, angina, and changes in bowel habits, abdominal discomfort, anemia, or bowel obstruction. About 5 to 10 percent of colon[2] cancers are initially discovered during a digital rectal exam (DRE), in which a primary care physician inserts a lubricated, gloved finger into the patient's rectum. A blood test also can show the possibility of colon cancer, as can various tests that examine the colon. These tests include colonoscopy, flexible sigmoidoscopy, or double-contrast barium enema. Colon adenocarcinoma is treated with surgery, chemotherapy, or radiation therapy or with a combination of two or three of these treatments. The most common treatment for colon adenocarcinoma is surgery, which can remove the cancerous tumor from the body. Surgery is generally recommended for 90 percent of colon cancer patients. A radical bowel resection–also known as a partial colectomy or hemicolectomy is the type of surgery performed on most patients. During this procedure, a surgeon removes the section of the colon containing the tumor, as well as nearby lymph nodes[3]. Chebulinic acid[4, 5] was an ellagitannin widely present in plants such as *Terminalia chebula*[6] fruit up to 30%. It is easily dissolved in methanol, ethanol, and ethyl acetate but sparingly soluble in water. The extraction of Chebulinic acid is done by Soxhlet[7] extractor and it also be extracted by HPLC[8]. Chebulinic acid showed many bioactivities including inhibition of cancer cell growth[9] like human leukemia K562 cells[10], inhibiting the contractile responses of cardiovascular muscles[11] and anti-fungal, anti-bacterial activities etc. It helps to remove toxins, unwanted fat from the body, improves skin glow and complexion also. In the present study, the effect of Chebulinic acid on colon adenocarcinoma HT-29 cell lines by using MTT cell growth inhibition assay was studied.

## MATERIALS AND METHODS

### Materials

The dry fruits of *Terminalia chebula* were collected from the local market Visakhapatnam. Clean the fruits and dried under sunlight for 1 day. The dried fruits were powdered and used as a raw material and stored in the air tight container. It is finely grounded to 120 mesh size.

### Chemicals

Cancer cell lines (HT-29), 4.5 g/l glucose, 2 mm l-glutamine, 5% foetal bovine serum (fibs) Standard drug (5 fluoro uracil), MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), 1x phosphate buffer saline (1x PBS), Dimethyl sulfoxide (DMSO).

### Preparation of Extract

Chebulinic acid from 50% (v/v) ethanol was extracted from the Colum chromatography [12] which was followed by the optimized conditions by Soxhlet extractor. The extract from Column Chromatography was used for anti-cancer [13] studies on HT-29 cell lines.

### Maintenance of HT-29 (Colon adenocarcinoma) Cell culture

HT-29 cell lines were obtained from the National Centre for Cell Science, Pune. Cell lines were grown in Minimal essential medium (MEM) supplemented with 4.5 g/L glucose, 2 m ML-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37 $^0$C in 5% $CO_2$ incubator.

### MTT assay

The MTT assay used to determine the inhibitory effects of test compounds on cell growth in vitro. The trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of 5x10$^3$ cells/well in growth medium and cultured at 37 $^0$C in 5% $CO_2$ to adhere. After 48hr incubation, the supernatant was discarded and the cells were pre-treated with growth medium and were subsequently mixed with different concentrations of test samples(12.5, 25, 50, 100 and 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as control and blank. Each well then received 5 µl of fresh MTT (5mg/ml in PBS) followed by incubation for 2hr at 37 $^0$C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilise the colour Formosan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 492 nm on an ELISA reader.

The percentage inhibition of cancer cell lines can be calculated as

**100-(At-Ab) / (Ac-Ab) × 100**

At: Absorbance of test,

Ab: Absorbance of blank,

Ac: Absorbance of control.

## RESULTS AND DISCUSSION

The Chebulinic acid of *Terminalia chebula* ha*s* showed significant activity at various concentrations and its effect was compared with the standard drug 5-fluorouracil. The maximum percentage inhibition of cancer cell lines was observed as 41.2% at 200 µg/ml. From the Figure, it was found that the Chebulinic acid inhibits the HT-29 cancer cell lines.

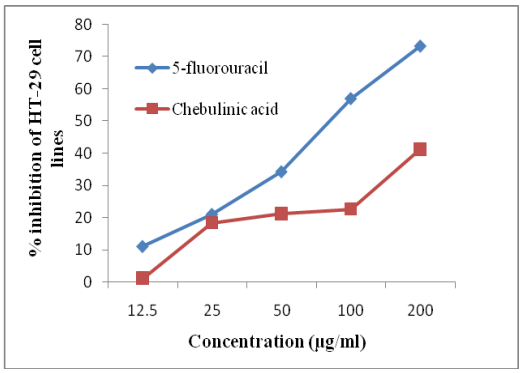


**Fig: Effect of Chebulinic acid and standard on % Inhibition of HT-29 cancer cell lines**

## CONCLUSION

Chebulinic acid was a main compound in *Terminalia chebula* species. It shows the inhibition of cancer cell growth, contractile responses of cardiovascular muscles, anti-fungal, anti-bacterial activities etc. The anti-cancer studies of Chebulinic acid on Colon adenocarcinoma HT-29 cancer cell lines was carried out by using MTT cell growth inhibition assay. The results showed that the maximum percentage inhibition of cancer cell lines for Chebulinic acid was found to be 41.2% at a dose of 200µg/ml. Hence Chebulinic acid can be used as a potent anti-cancer agent.

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