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In vitro anticancer activity of ethanolic extract of pericarp and seed parts of *Momordica charantia* on colon cancer cell lines

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Abstract

Objective: To investigate *In vitro* anticancer activity of ethanolic extract of pericarp and seed parts of *Momordica charantia* on colon cancer cell line.

Materials and Methods: *In vitro* anticancer activity was carried out by 3 (4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide assay. Different concentrations of the ethanolic extracts were tested on cell lines HT29.

Results and Discussion: IC 50 value were determined by measuring absorbance at 570nm. The IC50 value of seed and Pericarp was found to be 62.84 µg/ml and 143.75µg/ml respectively. The *Momordica charantia* showed moderate to potent anticancer activity. The above results showed *Momordica charantia* seed was better therapeutic activity than pericarp in the treatment of colon cancer. The Therapeutic activity may be due to the phytoconstituents present in the seed extract and further the seed have to be evaluated for GCMS studies.

Keywords: *Momordica charantia*, anti-cancer, MTT, HT29, GCMS

Introduction

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative system of health namely Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. These systems are rightfully existed side by side with allopathy are not in domain of obscurity^[1-3]. The reason behind exponential growth and commercialization in the field of herbal medicines in both developing and developed countries is mainly due to their natural origin, low cost, easy availability and lesser side effects. But the pharmacopoeia standards on raw materials or finished products are not available^[4, 5]. World Health Organization has set specific guidelines for the assessment of safety, efficacy and quality of herbal medicines^[6]. *Momordica charantia* is rich in nutrients like thiamine, beta carotene, foliate, riboflavin and minerals like calcium, iron, phosphorous, manganese, potassium, magnesium, zinc and dietary fibres. Regular use of bitter gourd juice boosts body stamina and prevents chronic fatigue and effective in early stage of cholera and other types of diarrhoea. It is used in the treatment of various diseases such as hypertension, diabetes mellitus and it is also a good digestive agent and helps in stimulating the secretion of gastric juice^[7-9].

Materials and methods

The fruit of *Momordica charantia* were collected from Thyagaraja Nagar, Chennai, Tamil Nadu, India during November 2019. The plant material was identified by professor Dr. J. Jayaraman, Ph. D. Director, plant Anatomy Research centre, west Tambaram, Chennai. A voucher specimen (PARC/2019/4177) was submitted at C. L. Metha College of Pharmacy, Chennai- 97. The Pericarp and seeds were separated, shade dried, Pulverized and extracted by hot percolation (Soxhlet) method by using ethanol as solvent. The extract obtained was tested for preliminary phytochemical screening and tested for *in vitro* anti-cancer studies by MTT assay.

A) Anticancer Activity studies using MTT

For Anticancer studies, serial two-fold dilutions (3.125-100µg/ml) were prepared from this for carrying out anticancer studies. Human colorectal adenocarcinoma cell line (HT29) were procured from NCCS, stock cells was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS).

The viability of the cells is checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5% CO₂ incubator.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line.

IC₅₀ Value

The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC₅₀ of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC₅₀ values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC₅₀ values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve.

Table 1: Qualitative Analysis

Sample	Seed	Pericarp
Alkaloids	+	+
Saponins	-	+
Tannins	-	+
Cardiac Glycosides	+	-
Flavonoids	-	+
Phenols	+	+
Steroids	+	+
Terpenoids	+	+
Quinones	-	+
Proteins	+	+

MTT ASSAY

Table 2: MTT assay of Seed

Mcse Concentration µg	Mean	Mean-Od	Standard Deviation	Viability
3.12	1.236	1.223	0.0122	97.57
6.25	1.195	1.192	0.0155	94.30
12.5	1.103	1.100	0.0240	87.07
25	0.922	0.919	0.0230	72.75
50	0.825	0.822	0.0384	65.04
100	0.340	0.337	0.0188	26.64

IC 50 Value= 62.84

Table 3: MTT Assay of Pericarp

MCPC Concentration µG	Mean	Mean-OD	Standard Deviation	Viability
3.12	1.251	1.249	0.0171	98.78
6.25	1.205	1.202	0.0136	95.14
12.5	1.162	1.159	0.0200	91.77
25	1.017	1.014	0.0487	80.27
50	0.871	0.868	0.0407	68.74
100	0.595	0.592	0.0318	46.87

IC 50 VALUE=143.75

Table 4: 5-Fluorouracil

5-FU Concentration µG	Mean	Mean-OD	Standard Deviation	Viability
3.12	0.829	0.826	0.0116	65.41
6.25	0.552	0.549	0.0291	43.41
12.5	0.361	0.358	0.0226	28.33
25	0.240	0.237	0.0220	18.78
50	0.136	0.133	0.0177	10.57
100	0.053	0.050	0.007	3.96

IC 50 VALUE = 5.01

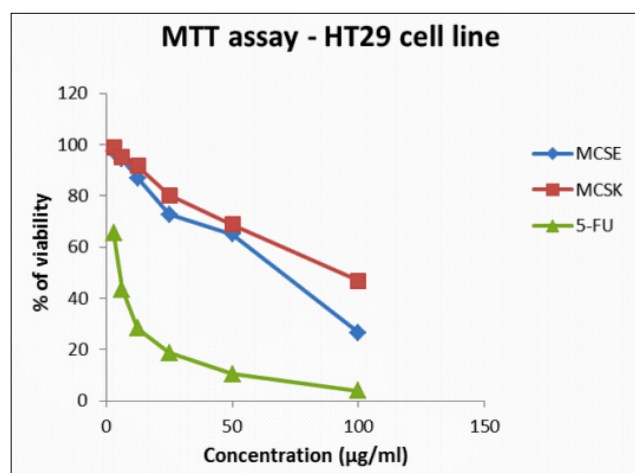


Fig 1: GRAPH

Cytotoxicity Assay - Ht29 Cell Line A. Momordica Charantia Seed

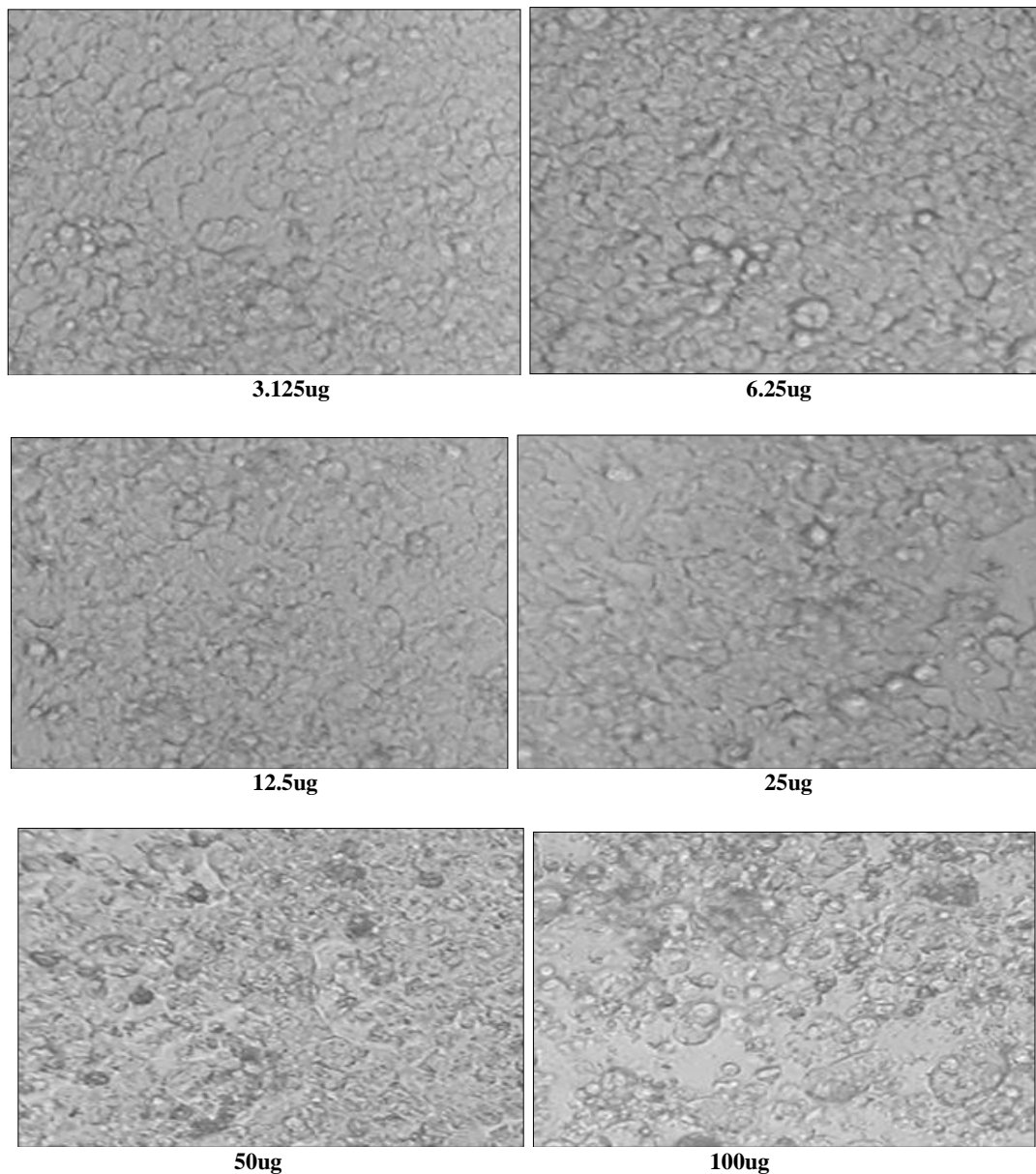
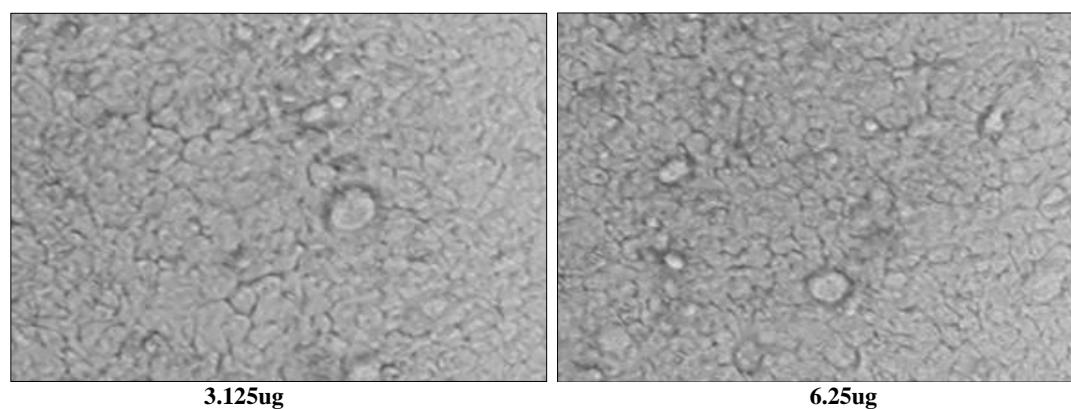
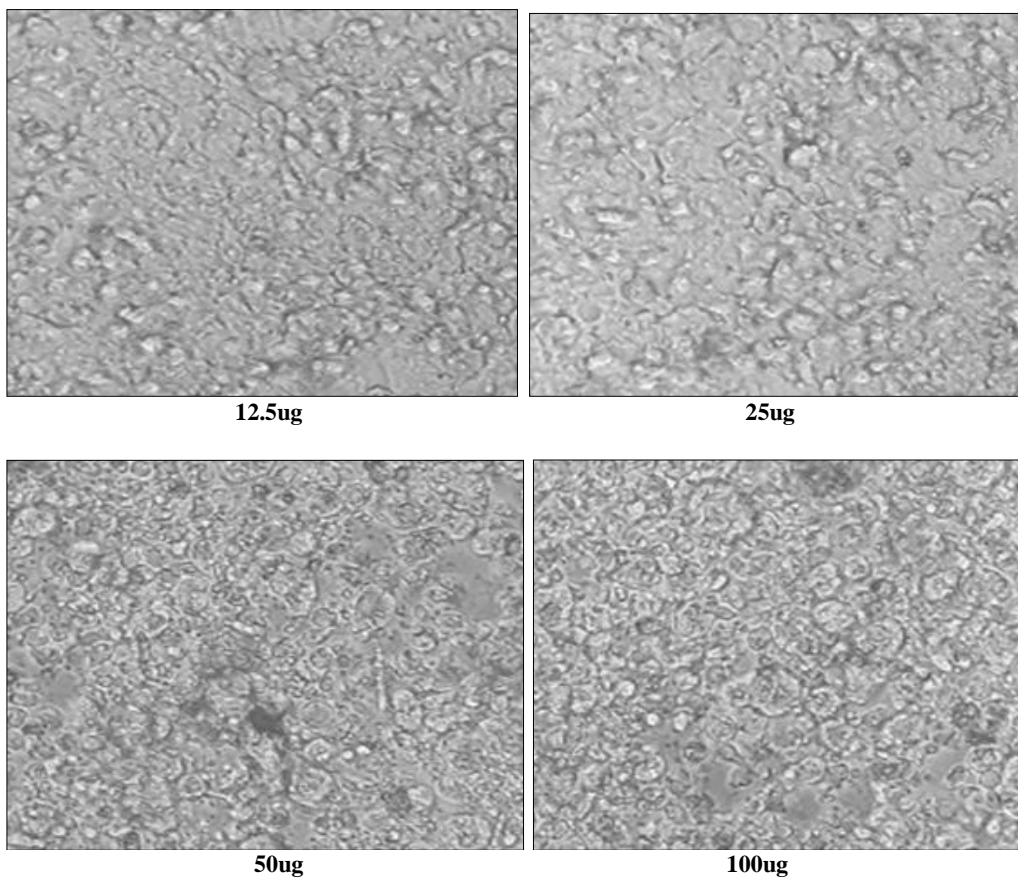


Fig 2: Momordica charantia Seed

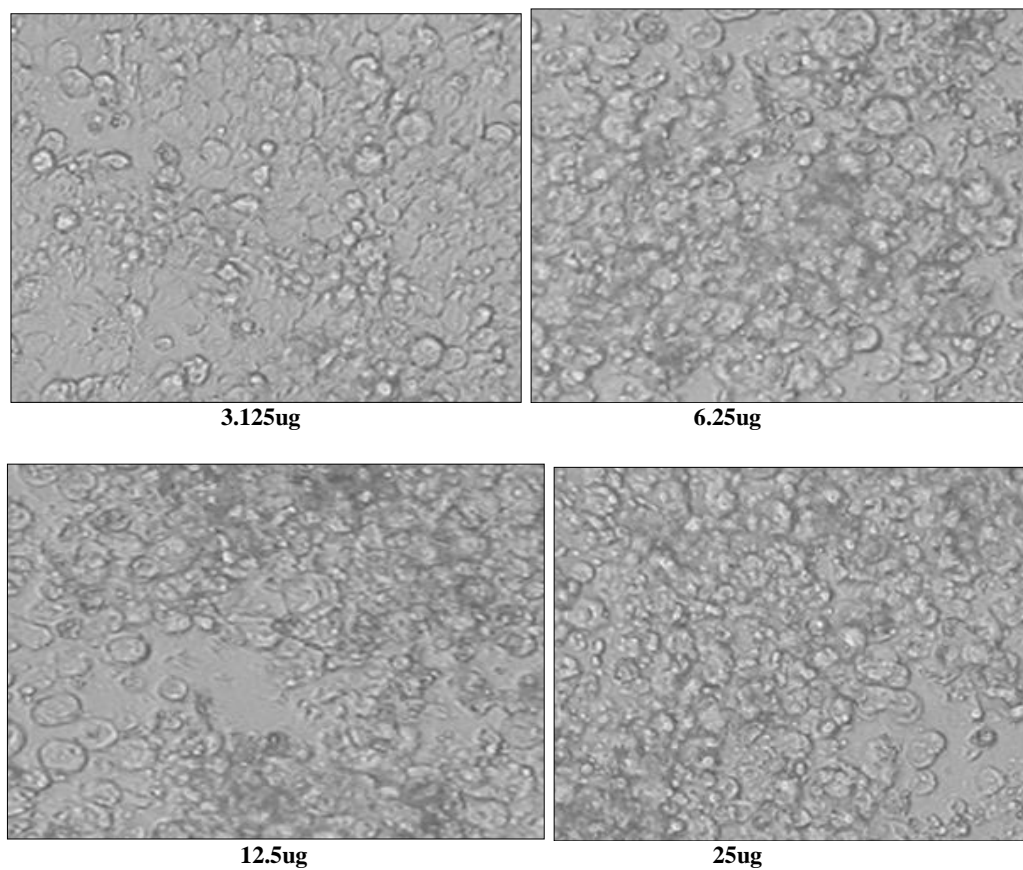




B. Momordica Charantia Pericarp

Fig 3: Momordica charantia Pericarp

C.5- Fluorouracil



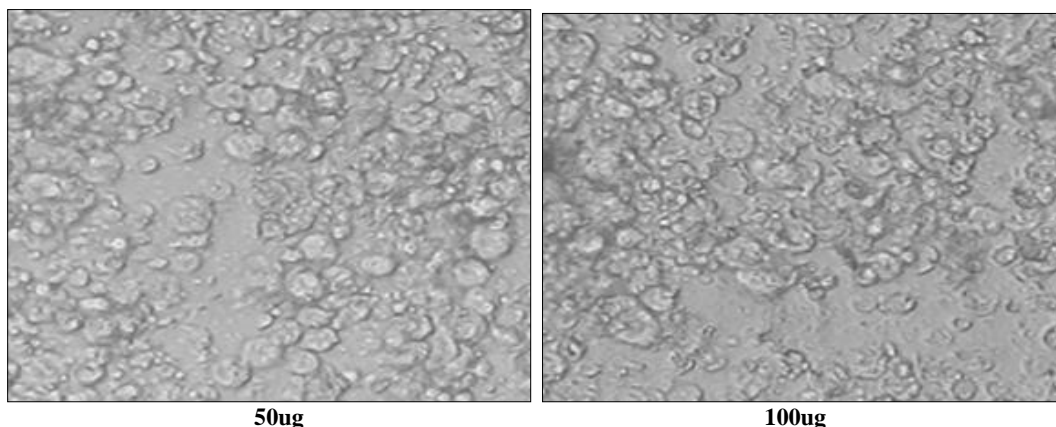


Fig 4: 5-Fluorouracil

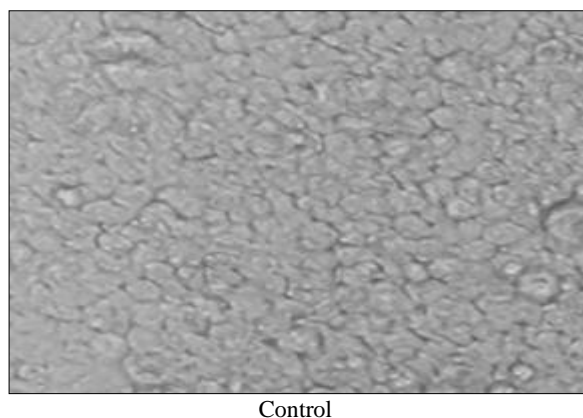


Fig 5: Control

Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past twenty years. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells. The *Momordica charantia* plant parts pericarp and seed were selected for study. The solvent used for extraction is ethanol. The ethanolic extract of *momordica charantia* pericarp and seed were measured. The qualitative phytochemical analysis of *Momordica charantia* ethanolic extract of pericarp and seed were performed. The pericarp showed the presence of alkaloids, saponins, tannins, flavonoids, phenols, steroids, terpenoids, quinones and proteins. The seed showed the presence of alkaloids, cardiac glycosides, phenols, steroids, terpenoids and proteins. The *in vitro* anticancer activity was studied using cell lines. HT29 cell lines were used for the study. MTT assay was performed. IC 50 value were determined by measuring absorbance at 570nm. The IC50 value of seed and Pericarp was found to be 62.84 $\mu\text{g/ml}$ and 143.75 $\mu\text{g/ml}$ respectively. Thus, *momordica charantia* showed moderate to potent anticancer activity.

Discussion

The well-known common medicinal *Momordica charantia* plant parts pericarp and seed were selected for study. The plant has vast review in research and proven multiple therapeutic activities. The universal solvent ethanol was used for extraction. The yield of ethanolic extract of *momordica charantia* pericarp and seed were quantified. The qualitative phytochemical analysis of *Momordica charantia* ethanolic extract of pericarp and seed showed the presence of alkaloids, phenols, steroids and terpenoids

The *in vitro* anticancer activity was studied using HT29 cell lines and the IC 50 value of MC seed was found to be 62.84 $\mu\text{g/ml}$ which was better than pericarp of MC was 143.75 $\mu\text{g/ml}$. This showed plant part seed of MC was having potent anticancer activity which was comparable with standard. The above results showed *momordica charantia* seed was better therapeutic activity than pericarp in the treatment of colon cancer.

Conclusion

Natural Product research was giving enormous opportunities in Drug Discovery which help in better compatibility with the human body, lesser side effects. The medicinal plant constituents do wonders in the new invention of Drug molecules, the plant *momordica charantia* also such a common plant which was used traditionally for longer period and had proven diabetes and cancer activity. This above phytochemical, *in vitro* and GCMS studies of plant part seed and pericarp of MC will give new incentive to the natural system of medicine in the treatment of colon cancer. The Plant can be further tested for preclinical and clinical studies in the treatment of colon cancer.

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Conflict of Interest

No conflict of Interest

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