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Research Article

"NEW ERA ABOUT TREATMENT" - EVOLUATION OF ANTICANCER PROPERTIES OF EVERGREEN MEDICINAL PLANT *ANNONA MURICATA* (GRAVIOLA)

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ABSTRACT

The development in the field of modern medicine temporarily subdued the traditional herbal medicine. But it has now staged a comeback and a "herbal renaissance" is blooming across the world. One such plant is graviola, is an indigoes medicinal plant belonging to the family Annonaceae. The objective of the study is the *invitro* anticancer activity of the ever green medicinal plant graviola. The hydro alcoholic extract of graviola proved to be effective against Breast cancer cell line (MCF-7). Annonaceous acetogenins is "not only are effective in killing tumors that have proven resistant of anticancer agents, but also seem to have a special affinity for such resistant cells". The findings of present study suggests that a promising new anticancer agent. The hydro alcoholic extract of graviola is posses most cytotoxicity and anticancer properties.

INTRODUCTION

The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood. Considerable works have been done on these plants to treat cancer, and some plant products have been marketed as anticancer drugs, based on the traditional uses and scientific reports. These plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues [5]. Several reports describe that the anticancer activity of medicinal plants is due to the presence of antioxidants in them. In fact, the medicinal plants are easily available, cheaper and possess no toxicity as compared to the modern (allopathic) drugs [2]. Cancer is a disorder developed due to some molecular changes within the cell. It becomes the second major cause of death in the human after cardiovascular disease. Every year, millions of people are diagnosed with cancer, leading to death. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation. Cancer is the second largest cause of death which killed 7.6 million people worldwide in 2005. The number is believed to become 9 million in 2015 and 11.5 million in 2030 (World Health Organization, 2007). The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management [3, 4].The fruit *Annona Muricata* highly effective against liver cancer cells and breast cancer cells. The fruit from the *Annona Muricata* is a miraculous natural cancer cells. These were found to selectively kill colon cancer cells at 10000 times more effective of chemotherapy.

MATERIALS AND METHOD

Plant Collection and Identification

Annona Muricata used in the study was identified in the botanical survey of VICAS botany department, the reference material has been kept under reference VICAS/SC/05/15-16. Fresh whole fruit was collected randomly from the region of in around Kolli Hills, Tamilnadu. Fresh fruit was air dried and then homogenized to fine powder and stored in air tight bottle.

Anticancer Activity

The *in vitro* anticancer potential of fruit of *Annona Muricata* was followed in different cancer cell line by MTT (3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) colorimetric assay.

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Cell lines and Culture Medium

MCF-7 (Human Breast Carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM and DMEM respectively supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an 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).

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. Serial two fold dilutions were prepared from this for carrying out cytotoxicity studies [8].

Determination of Cell Viability by MTT Assay (Francis and Rita, 1986)

Principle

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubulize the formed formazan. 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.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

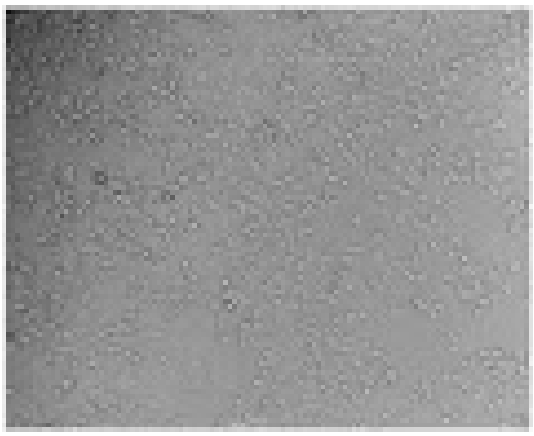
RESULT AND DISCUSSION

Anticancer and Cytotoxic Activity

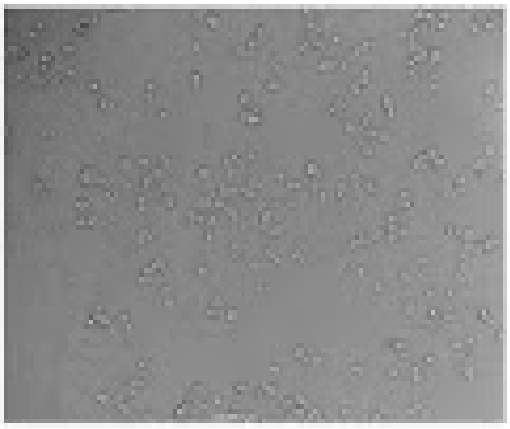
The cytotoxic effect of hydro alcoholic fruit extract of *Annona Muricata* is shown in Table and Figure. *Annona Muricata* exhibited potent cytotoxicity against the cancerous MCF-7 cell lines with average CTC_{50} values of 493.87 ± 8.7 µg/ ml respectively. However, against the normal Vero cell lines, the average CTC_{50} value was found to be 493.87 ± 8.7 µg/ml. This indicated that hydro alcoholic fruit extract of *Annona Muricata* possesses strong cytotoxicity against the cancer cell line, but is safe towards the normal cells.

Table - Anticancer Activity of Hydro Alcoholic Fruit Extract of *annona Muricata*

S. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	Hydro alcoholic extract of *Annona Muricata*	1000	78.50±1.0	493.23±8.7
		500	51.37±0.5	
		250	46.50±0.3	
		125	38.91±2.3	
		62.5	33.85±1.0	



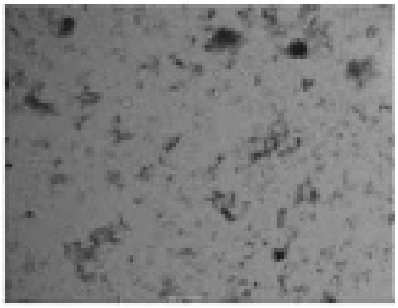
MCF-7 Control



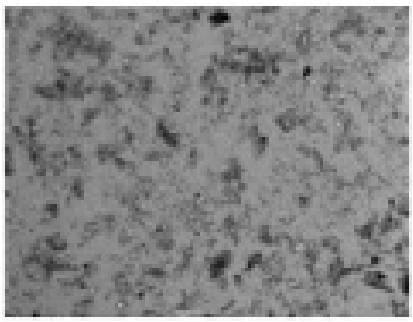
MCF-7 62.5 µg/ml

Figure-Anticancer Activity of Hydro Alcoholic Fruit Extract Of *Annona Muricata*

MCF-7 125 µg/ml

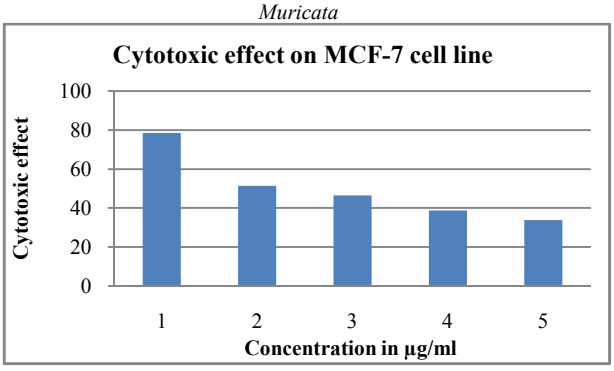


MCF-7 250 µg/ml



MCF-7 1000 µg/ml

Figure - Anticancer Activity of Hydro Alcoholic Fruit Extract Of*annona Muricata*



The biological selective activity of any compound might depend on the type of chemical composition and the concentration of active constituents as well as the types and developmental stages of the cancer. The screening of plants for their anticancer properties use cell-based assays and established cell lines, in which the cytotoxic effects of plants extracts or isolated compounds could be measured [1].

The result of our study revealed that hydro-alcohol extract of leaves of *Annona Muricata* has a cytotoxicity effect onMCF-7 (Human breast adenocarcinoma *cell line*) in a concentration dependent manner. The extract showed good therapeutic values against MCF-7 cell line with CTC_{50} values 493.87± 8.7 respectively. Morphological studies also confirmed that the hydro alcoholic extract of fruit of *Annona Muricata* has got potential cytotoxicity effect.

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