ANTICANCER POTENTIALS OF *CIRULLUS COLOCYNTHIS*(L.) *SCHRAD* FRUIT

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ABSTRACT

*Citrullus colocynthis* (L) *Schrad* fruit is traditionally used as medicine for treating skin, intestinal and inflammatory infections. Aqueous and ethanol extracts of this plant fruit were evaluated for their *in-vitro* anticancer activities by making use of cancer cell line cytotoxicity by MTT assay (MCF-7 cell line) and trypan blue dye exclusion assay (EAC Cell line). The results obtained indicated that the plant extracts has potent cytotoxic activity on MCF-7 as well as EAC cell line. The results of the present findings strengthen the potential of the selected plants as a resource for the discovery of novel anticancer agents.

Keywords: *Citrullus colocynthis*, fruit, Anticancer, MTT assay, tryphan blue dye exclusion

Introduction

Medicinal plants and its products have been the backbone of traditional system of medicine throughout the world for over thousands of year. It is continued to provide new remedies to human being without any side effects. Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases. It is estimated that more than 1300 Indians die due to cancer. Mortality rate due to cancer is was increased up to 6%. The disease is widely prevalent and in the West, almost a third of the population develops cancer at some point of time during their life. Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at the molecular level. Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would prevent, slow, or reverse cancer development. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents. Cancer chemoprevention with natural phytochemical compounds is an emerging strategy to prevent the cancer. The MTT assay is a colorimetric assay for assessing cell metabolic activity. The MTT in vitro cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of synthetic derivatives, natural products and natural product extracts. Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions that damage living cells, spoil foods. Having known the importance of *Citrullus colocynthis* (L) *Schrad* fruit, TSM, this study was undertaken to screen *in vitro* anticancer by MTT and dye exclusion methods.

Materials and Methods

Collection, identification, and authentication of the selected medicinal plants

*Citrullus colocynthis* (L) *Schrad* fruit was collected from the nearby regions of Mannargudi, Thiruvarur district (Tamil nadu). The plants were identified and authenticated by Dr. John Britto, Director, Rabinath Herbarium, St. Josephs College, Tiruchirappalli. India. Voucher specimens of the collected plants were deposited in the herbarium center of the host institute. The plant materials were dried under shade at room temperature pulverized by a mechanical
blender and sieved through 40 meshes then stored in airtight closed bottle until required. All the plant materials were powdered individually and mixed in equal proportions.

**Extraction**

The shade-dried, powdered fruit samples (100 g) were extracted in water and ethanol by using Soxhlet apparatus. The resultant extracts were filtered by using Whatman No 1 filter paper and then concentrated in a rotary evaporator and were stored in a refrigerator at 4°C in small sterile glass bottles for further analysis.

**In Vitro Anti-Cancer Activity by MTT assay**

The human breast cancer cell line (Michigan Cancer Foundation-7 (MCF 7)) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained in Minimal Essential Media (MEM) supplemented with 10% Fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100µg/mL) in a humidified atmosphere of 5% CO₂ at 37°C. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. Cytotoxic assay was evaluated by the method of Mosmann and Tim. Viable cells were determined by the absorbance at 570nm. The standard as Paclitaxel used to compare the test sample. Measurements were performed and the concentration required for inhibition Concentration (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer. The medium without samples served as control and triplicates were maintained for all concentrations. The effect of the samples on the antiproliferation of MCF-7 was expressed as the % Cytotoxicity using the following formulas:

\[
\% \text{ Cytotoxicity} = 100 - \left(\frac{\text{Abs (sample)}}{\text{Abs (control)}}\right) \times 100.
\]

**Short-term in vitro cytotoxicity assay (trypan Blue dye exclusion method)**

Cell lines were obtained from National Centre for Cell Sciences,Pune (NCCS). The medium and Trypsin Phosphate Versene Glucose (TPVG) was brought to room temperature by thawing. The assay was performed using the trypan blue exclusion method of Talwar and Halder with few modifications.

**Results and Discussions**

Cancer chemoprevention with natural compounds from medicinal plants is an emerging strategy to prevent the cancer. The MTT *in vitro* cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of synthetic derivatives, natural products and natural product extracts. Medicinal plants continue to play an important role in the healthcare system of a majority of the world's population. Traditional medicine is widely used in India. The herbal products have been classified under “dietary supplements” and are included with vitamins, minerals, amino acids and other products intended to supplement the diet. In fact, there are several medicinal plants all over the world, including India, which are being used traditionally for the prevention and treatment of cancer. Various phytochemicals seems to be associated with these activities. In particular, the phytochemicals such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes, and minerals have been found to elicit antioxidant and anticancer activities. These chemicals block various hormonal actions and metabolic pathways that are associated with the development of cancer. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extracts.

The lowest *in vitro* animal cell growth inhibition by the test aqueous and ethanol extracts was determined to be 18.12 and 21.40 % at 25µg/mL for sample and standard respectively; while the highest growth inhibition was 71.19 and 82.54% at 200 µg/mL for aqueous and ethanol extracts.
extract respectively (Table 1 and Figure 1). The IC$_{50}$ values of aqueous and ethanol extracts were 126.50 and 88.27µg/mL respectively.

Table. 1- Effect of varying concentrations of extract of Aqueous and Ethanol extract on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentrations (µG/mL)</th>
<th>CCAE</th>
<th>CCEE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absorbance (Optical density)</td>
<td>Cytotoxicity (%)</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>0.311</td>
<td>18.126</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.268</td>
<td>29.509</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.217</td>
<td>42.907</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>0.109</td>
<td>71.190</td>
</tr>
<tr>
<td>5</td>
<td>Cell Control</td>
<td>0.380</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Half inhibition concentration (IC$_{50}$) (µG/mL)</td>
<td>126.50</td>
<td>-</td>
</tr>
</tbody>
</table>

Cytotoxicity of CCF extracts on MCF-7 cell line at 100 and 200µg/mL concentrations were depicted in Plate I (1-6). The most identifiable morphological features of apoptosis (dark coloured cells) were observed by inverted light microscopy with the aqueous extract (CCAE) treated cell lines. The CCAE treated cell lines appeared as cells undergoing apoptosis with prominent features such as detachment from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighbouring cells. However, the untreated cell lines appeared normal (control) and were confluent. The CCEE effectively induced apoptosis. The cell lines treated with various concentrations of the test extract and standard were incubated for 24hr and were compared with the untreated cells to assess any morphological changes. It was noted that the morphology of
sample and standard treated cancer cells significantly changed compared to control cells after the incubation period. The test sample and standard treated cell lines appeared less uniform with a loss of membrane integrity, although remained intact at lower concentrations. Whereas at higher concentrations, the sample and standard treated cells showed remarkable difference with the control group. The significant changes such as loss of intact membrane, karyopyknosis, cell detachment from the plate and change of morphological features were evident when compared to untreated cells.

![Figure 2](effect_of_ccf_extracts_on_eac_cell_line_cytotoxicity.png)

The test plant extract at 100 μG/mL concentration had 55% cell. An increasing concentration from 100 to 200 μG/mL increased the effect of CCEE drastically, it produced 81% cytotoxicity on EAC cell lines. At similar concentration, CCAE produced only 61% cell cytotoxicity (Figure 2).
The decrease in the viable cell count and increase in the rate of non-viable cell indicated the extracts stimulate the growth and activity of the immune cells and lead to the production of interleukins. As the EAC was injected into mice first result in increased survival time indicate the delay in vascular permeability of the cells. The standard drug 5-fluorouracil would arrest the cell cycle and inhibit the nucleic acid synthesis and enrol about indirect cytotoxicity. The most identifiable morphological features of apoptosis were observed by inverted light microscopy in the sample and standard treated cells. The treated cells appeared like cells undergoing apoptosis with prominent features such as detaching from the culture plate, cytoplasmic condensation, cell shrinkage and condensation and aggregation of the nuclear chromatin, and loss of contact with neighbouring cells. However, the untreated cells appeared normal and were confluent. Chemoprevention and dietary modification studies are underway to identify promising candidates for reduced cancer risk. It is concluded that the aqueous and ethanol extract had anticancer properties against breast carcinoma MCF-7 cell line. Among this, ethanol extract possessed potential anticancer activity than aqueous extract. They act as chemopreventive agents, which act on tumour via modulations of numerous cell signaling pathways.

Gupta et al., mentioned the effects of secondary metabolites like saponins, tannins, cardiac glycosides, alkaloid, flavonoids, steroids and terpenoids. These phytochemicals disturb cell membrane, which is responsible for major functions such as osmoregulation, transport, lipid synthesis, and peptidoglycan cross-linking. The integrity of the plasma membrane is necessary for survival of organism in a non specific way, leading to cell death. Bansal et al., and Upadhyay et al., stated the effectiveness of phytochemicals on cancer cell lines. Tannins cause inhibition in the cell membrane synthesis by forming irreversible complexes with prolene rich protein. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell. Primay and secondary metabolites of plants interferes with uncontrollable growth of cell lines.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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