IN-VITRO CYTOTOXICITY ACTIVITY OF SOLANUM XANTHOCARPUM AGAINST MCF7, HEla, A549 AND CACO2 CELL LINES BY MTT ASSAY

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Abstract

The present investigation aims to evaluate anticancer activity of methanolic extract of Solanum xanthocarpum fruits. The extract is investigated for its inhibitory effect on MCF7, HeLa, A549, CaCo2 cell lines. Percentage viabilities of cell lines are assessed by adopting the MTT method. The extract has significant cytotoxicity on MCF7, HeLa, A549, CaCo2 cell lines in the concentration range between 10 to 100 µg/ml as per MTT assay. IC50 values of Solanum xanthocarpum on MCF7, HeLa, A549, CaCo2 cell lines are 27.99, 75.55, 54.66 and 156.36 respectively. From the performed assay, methanolic extract has more cytotoxic effect on MCF7 and least activity on CaCo2 cell line. Thus the extract of Solanum xanthocarpum fruits has identified to have anticancer activity.

Keywords: Cytotoxicity, Solanum xanthocarpum, methanol extract, cell lines, MTT

Introduction

Cells viability and proliferation rates are good indicators of cells health. Cell health and metabolism are affected by physical and chemical agent’s viability of Cells and proliferation rates reflect health of the cells. Cell health and metabolism are affected by physical and chemical agents these reagents hinder the growth of cancer cells by various mechanisms viz., obliteration of cell-membranes, retarding the protein synthesis by binding over to receptors. Further, inhibition of the growth of cancer cells may also be caused due to elongation of oligodeoxynucleotide and prevention of enzymatic reactions.

The reagents employed for this purpose must be bio-compactable and do not because side reactions. Many of the synthetic drugs have ill-effects and cussing other disorders. In this context, the chemicals derived from the plant materials are interesting researcher in the recent
Plants constitute one of the major sources of drugs in modern as well as traditional medicine throughout the world. The bioactive substances in plants are mainly produced as secondary metabolites. **Methods have been developed** to cure various diseases like gonorrhea, rheumatism, cough, asthma, catarrhal fever and sore throat by using extracts from the bio-materials. In fact our research group has investigated Cytogenetic properties of some mangroves belong to Krishna-Godavari estuary.

Solanum xanthocarpum (SX) plant is medicinal herb and is known for its medicinal values. The plant belongs to the Solanaceae family. It is commonly known as Indian night shade or Yellow berried nightshade. Alcoholic or aqueous extracts of Solanum xanthocarpum (SX) plant have shown hypotensive effect, antiviral activity (against Ranikhet disease virus) and against sarcoma-180 in mice. In the present study, the cells viability and proliferation in the alcoholic extract of Solanum xanthocarpum furt has been investigated.

**MATERIALS AND METHODS**

**Plant Collection**

Fruits of the ‘*Solanum xanthocarpum*’ were collected from the Kuragallu rural area of district Guntur, Andhra Pradesh, India. The voucher -specimen was preserved in the chemistry lab of KL university.

**Preparation of Extract**

The collected fruits of the *Solanum xanthocarpum* were cleaned and dried under shade for a period of one month. The dried fruits were ground to fine powder. Powders of the fruits were successively extracted with Methyl alcohol (60- 80 C) using a Sock let extractor. Extractions of
the solvent were collected, filtered and vacuum dried in a rotary evaporator at 40 ± 5°C. The dried product obtained is analyzed for its cytotoxic activity.

**Cytotoxicity bioassays by MTT method**

The various Cell lines tested by MTT methods were presented in Table 1. The chemical used in this investigation were of Analytical Grade quality. DMEM (Dulbecco's modified Eagle's medium), MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl, tetrazolium bromide, EDTA Phosphate Buffered Saline (PBS) and trypsin, were procured from Sigma Chemicals. Fetal Bovine Serum (FBS) was purchased from Gibco. 25 cm² and 75 cm² flask and 96 well plated were purchased from Eppendorf India.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound Name</th>
<th>Cell Line tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solanumxanthocarpum (SX) extract</td>
<td>MCF7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HeLa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A549</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CaCo2</td>
</tr>
</tbody>
</table>

The Cancer cell lines were purchased from NCCS, Pune. Cells were maintained in MEM supplemented with 10% FBS and antibiotics penicillin/streptomycin (0.5 mL⁻¹), in an atmosphere of 5% CO₂/95% air at 37°C.

**Preparation of Testing Extract**

The required quantity of the extract was correctly weighed and dissolved in DMSO so as to obtain the concentration of 1 mg/ml. This solution was subsequently diluted to a series of concentrations ranging from 10 to 100 µg/ml.

**MTT ASSAY**

This method is based on colorimetric estimation. In this method, the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) caused by ‘mitochondrial succinate dehydrogenase’ present in live cells, was used as a basis. In live cells, the presence of enzyme causes the reduction while in dead cell (or their compounds), the reduction is not
affected. Thus, the reduction is based on number of live cells and not on dead cells. In live cells the MTT penetrates into ‘mitochondria’ and get reduced to form insoluble deep purple coloured formazan crystals. On treating the crystals with DMSO, the cell were dissolved in the solvent and thereby releasing the Formosan. The Formosan is assayed spectrophotometrically as max at 570 nm.

The IC50 values obtained for the extract wires pet to different cell lines were presented in Table 2. Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates.

Cells were trypsinized and performed the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells well in 100 μl media in 96 well plate culture medium and incubated overnight at 37 °C. After incubation, take off the old media and add fresh media 100 μl with different concentrations of test compound in represented wells in 96 plates. After 48 hrs Discarded the drug solution and added the fresh medic with MTT solution (0.5 mg / mL^-1) was added to each and the plates were incubated at 37 °C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

\[
\% \text{ Inhibition} = \frac{100 (\text{Control} - \text{Treatment})}{\text{Control}}
\]

The Ic50 value was determined by using linear regression equation i.e. \( Y = Mx + C \). Here, \( Y = 50 \), M and C values were derived from the viability graph.

**Results and Conclusion**

The IC50 values are tabulated in table2. The present study describes *in vitro* anti cancer activity of Solanum xanthocarpum alcoholic extract. From the result of the cytotoxicity evolution of the extract, it displayed a potent activity against MCF7 and A549 cell lines with the IC50 values 27.99 and 54.66. The cytotoxic effects also plotted in graph concentration versus viability percentage.
Table 2: Cytotoxicity by MTT assay of Solanum Xanthocarpum Methanolic extract

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Sample Name</th>
<th>Cell Line</th>
<th>IC₅₀ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SX methanolic extract</td>
<td>MCF 7</td>
<td>27.99</td>
</tr>
<tr>
<td>2</td>
<td>SX methanolic extract</td>
<td>HeLa</td>
<td>75.55</td>
</tr>
<tr>
<td>3</td>
<td>SX methanolic extract</td>
<td>A549</td>
<td>54.66</td>
</tr>
<tr>
<td>4</td>
<td>SX methanolic extract</td>
<td>CaCo2</td>
<td>156.37</td>
</tr>
</tbody>
</table>

![Graphs](image1.png)

**Figure 1:** Results of cell viability assay of different concentrations of Solanum xanthocarpum extracts on MCF7, HeLa, A549 AND CaCo2 are shown in the graphs.

**REFERENCES**


