Evaluation of Invitro Anticancer Activity of Hydroalcoholic Extract of Justicia tranquibariensis

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ABSTRACT
Justicia tranquibariensis a native of India and many other tropical regions is a common garden plant that has been used traditionally for treatment of a number of diseases. In the current study the hydro-alcoholic extract of the roots of the plant have been tested for anticancer activity. The extract was prepared by cold maceration method, hydroalcohol was the solvent used. The in-vitro anticancer studies were performed against human cancer cell line (HeLa) and MTT assay was used to analyze the cell growth inhibition. The results showed that the hydroalcoholic extract of roots of Justicia tranquibariensis possessed a moderate amount of anticancer activity and the IC50 value was greater than 200 μg/ml.

Keywords: HeLa, MTT assay, IC50.

INTRODUCTION
The use of plant extracts and photochemical are of great significance in therapeutic treatments. However, medicinal plants, and indeed plants in general, synthesize toxic substances which in nature act as a defense against infections and herbivores but which often affect the organisms that feed on them. Thus, an assessment of their cytotoxic potential is necessary to ensure relatively safe use of medicinal plants. Cancer is a dangerous disease and controlling this disease is of great importance to public health. There is a necessity for search of new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem, cytotoxicity to the normal cells. Photocatalytic examination has been making rapid progress and herbal products are becoming popular as sources of plausible anticancer compounds. It claims more than six million people lives a year. Cervical cancer is one of the leading causes of death from gynaecologic cancers. Therefore, this study is crucial as a stepping stone to explore the activity of Justicia tranquibariensis extract in the inhibition of human cervical carcinoma cells before proceed to animal toxicology study.

MATERIALS AND METHODS
Plant Collection and Identification
Roots of Justicia tranquibariensis was collected in the month of January and February months of 2015. The plant material was authenticated by National institute of Herbal Science (PARC/2015/463), West Tambaram, Chennai. Later the plant material was size reduced, dried in sunlight, pulverized, passed through sieve no. 40, stored in air tight container and used for further extraction.

Preparation of Extract
Using a soxhlet extraction method, the powder of dried flowers were processed with petroleum ether (40-50ºC) for 18 hrs in order to remove fat and unwanted components. The treated powder was further extracted using hydroalcoholic solution (25:75) by cold maceration process for about 48 hours. The extract was later concentrated by evaporating the solvent using a water bath maintaining at 60 - 80O C at ambient conditions to get a crude hydroalcoholic extract devoid of solvents.

IN-VITRO EVALUATION OF ANTICANCER ACTIVITY BY MTT ASSAY
Cell Culture
The HeLa cell lines (human cervical adenocarcinoma cell line) was provided by National Centre for Cell Science, Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained
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10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO₂, 95% air and the culture medium was changed twice a week.

Cell Treatment

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylene diamine tetra acetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS, to obtain final density of 1x105 cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity was maintained. The cells were treated with different concentrations of the test samples after 24 hours prepared by serial dilution method. Cells were initially dissolved in dimethylsulfoxide (DMSO), further diluted with serum free medium to achieve twice the desired final maximum test concentration. The required final drug concentrations of 25, 50, 75, 100, 200, 300 µg/ml were obtained by adding aliquots of 100 µl of the different drug dilutions to the appropriate wells already containing 100 µl of medium. After addition of the drug the plates were incubated for an additional 48 hours at 37°C. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT Assay

After 48 hours of incubation, to each well added 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) and incubated at 37°C for 4 hours. The medium with MTT was removed and the formed formazan crystals were solubilized in 100µl of DMSO solution. Using a micro plate reader the absorbance was measured at 570 nm. The percentage cell inhibition was determined using the formula.

\[
\text{Percentage Cell Inhibition} = \left[ 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \right] \times 100.
\]

Table 1. Percentage cell growth inhibition of hydro-alcoholic extract of Justicia tranquibariensis on HeLa cell lines by MTT assay

<table>
<thead>
<tr>
<th>Si NO</th>
<th>CONCENTRATION OF EXTRACT (µg/ml)</th>
<th>ABSORBANCE</th>
<th>PERCENTAGE INHIBITION OF CELL GROWTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.60698±0.01184</td>
<td>-3.26077</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.49257±0.021085</td>
<td>1.03429</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.36369±0.005142</td>
<td>25.54823</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.30834±0.004967</td>
<td>24.65825</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>0.239645±0.00532</td>
<td>36.65734</td>
</tr>
<tr>
<td>6</td>
<td>CONTROL</td>
<td>0.5976±0.00579</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig1. Anticancer activity of Justicia tranquibariensis extract against HeLa cells

Statistical Analysis

The absorbance values were denoted as mean ± SEM. IC₅₀ was determined using Graph Pad Prism software. The IC₅₀ is half the maximal inhibitory concentration of the toxic compound which results in the reduction of biological activity by 50%.
RESULTS AND DISCUSSION

In Vitro Anticancer Activity

The results for cell growth inhibition by the extract against HeLa cell lines for various concentrations are shown in Table 1. As the concentration increases there is an increase in the cell growth inhibition but is found to be very less with only 36.65734% growth inhibition at 200 µg. The regression value was difficult to analyze. The results obtained showed that hydro-alcoholic extract of Justicia tranquibariensis had a very moderate anticancer activity.

CONCLUSION

The results obtained from the in-vitro cancer studies performed using the HeLa cell lines reveals that the hydro-alcoholic root extract of Justicia tranquibariensis has a moderate anticancer activity. Even though there was increase in the cell growth inhibition when concentration of sample was increased, the IC_{50} value was more than 100 µg/ml for the cell line studies as shown by the MTT assay method. Hence the level of cytotoxicity of the hydroalcoholic extract of Justicia tranquibariensis roots can be concluded to be less effective.

REFERENCES

