

EVALUATION OF *IN VITRO* ANTICANCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *TABERNAEMONTANA DIVARICATA*

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ABSTRACT

Tabernaemontana divaricata 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 hydroalcoholic extract of the flowers of the plant have been tested for anticancer activity. The extract was prepared by soxhlet extraction method, petroleum ether and hydroalcohol were the solvents 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 flowers of *T.divaricata* possessed a moderate amount of anticancer activity and the IC₅₀ value was greater than 100 µg/ml.

Keywords: HeLa, MTT assay, IC₅₀, medicinal flower.

INTRODUCTION

Medicinal plants have been in use from time immemorial and their utility has been increasing day by day in the present world. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced.¹ Plants represent a source of leads for many pharmaceutical compounds and the phytochemical compounds and secondary metabolites present in plants have been used in treating a number of human ailments. Drugs obtained from medicinal plants comprise 25% of total drugs in developed countries and about 80% in developing countries.²

Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells. Though Chemotherapy is now being used as a standard treatment method,³ search for anticancer agents from natural products has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activities a number of plants have been explored. *Tabernaemontana divaricata* under the family Apocynaceae is an ornamental, flowering, evergreen shrub that generally grows to a height of 6ft and comes under the genus *Tabernaemontana* which consists of 100-110 species of flowering plants.⁴ It is a common garden plant found in tropical countries including Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand.⁵ The flowers are white and sweetly fragrant,⁶ the leaves, flowers, roots and stem of the plant have medicinal value and have been used traditionally for the treatment of ulcers and rheumatism,⁷ other medicinal properties of the plant include Anxiolytic,⁶ Antidiabetic⁸ and Anticonvulsant⁹ activities. The main aim of the present work was to evaluate the anticancer activity of dried flowers of *T.divaricata*.

MATERIALS AND METHODS

Plant collection and identification

Flowers of *T.divaricata* were collected in the month of January and February at mornings and evenings and authenticated by Dr. N. Ravichandran, CARISM, SASTRA University, Thanjavur- 613 401. The herbarium is kept in the department.

Preparation of Extract

The fresh flowers of the plant were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. Using a soxhlet extraction method, the powder of dried flowers were processed with petroleum ether (40-50°C) for 18 hrs in order to remove fat and unwanted components. The treated powder was further processed with hydroalcoholic solution (25:75) by using same extraction process for 18 hrs. The extract was concentrated by evaporating the

solvent using a water bath maintaining at 60-80° 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 human cervical adenocarcinoma cell line (HeLa) was provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 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-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order 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. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. The required final drug concentrations of 18.75, 37.5, 75, 150, 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 hr at 37° C, 5% CO₂, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay

After 48h of incubation, to each well 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4h. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbance was measured at 570 nm. The % cell inhibition was determined using the following formula. % Cell Inhibition = $[100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}}] \times 100$.

TABLE 1: Percentage cell growth inhibition of hydroalcoholic extract of *T.divaricata* on HeLa cell lines by MTT assay

S. No.	Concentration of the extract (µg/ml)	Absorbance	Inhibition of Cell growth (%)
1	18.75	0.406333±0.012284	-2.35097
2	37.5	0.392667±0.011086	1.09152
3	75	0.378667±0.005312	4.617968
4	150	0.308±0.004967	22.41814
5	300	0.260667±0.004989	34.34089
6	Control	0.397±0.004243	0

n=3

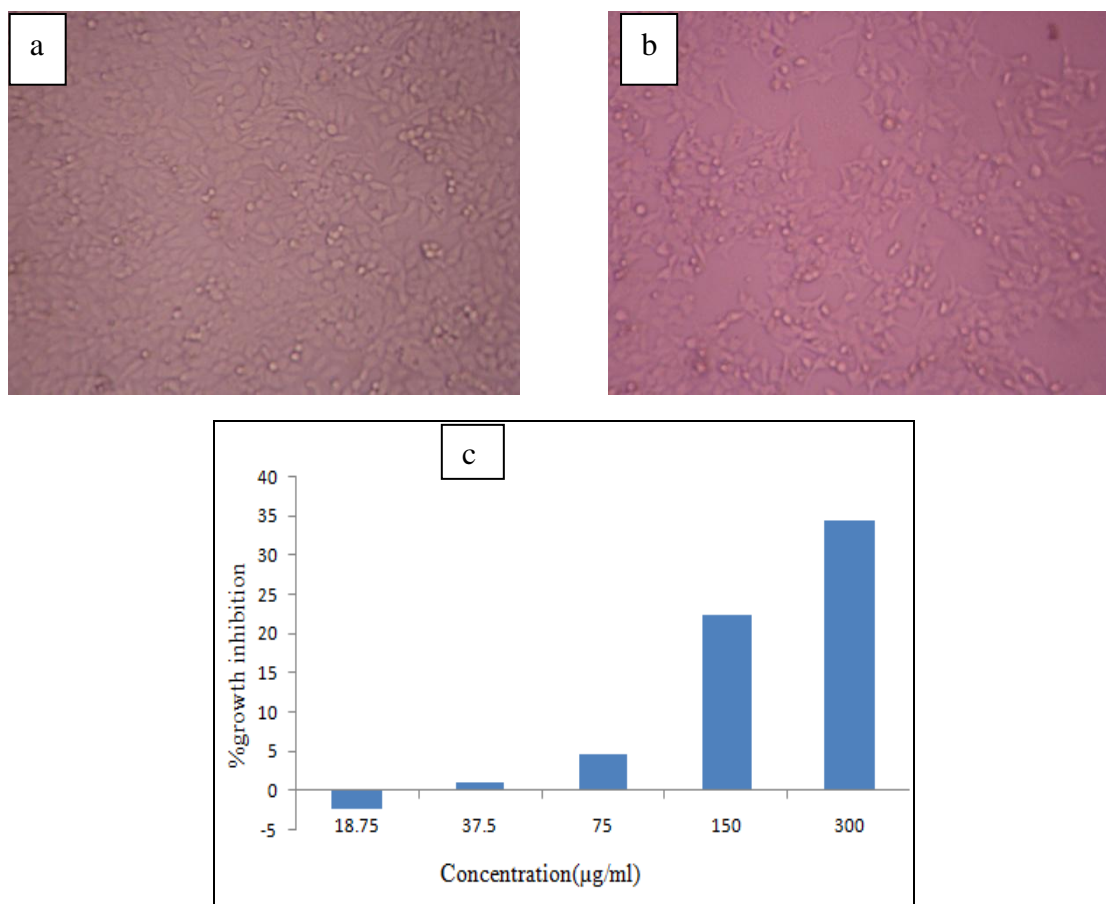


FIG 1. Anticancer activity of *T.divaricata* extract against HeLa cells; a) control cells, b) 300 µg extract treated cells, c) Concentration Vs % growth inhibition

Statistical analysis

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

RESULTS AND DISCUSSION

In vitro anticancer activity

The results for cell growth inhibition by the extract against HeLa cell lines for various concentrations is 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 34.34089 % growth inhibition at 300 µg. The IC₅₀ value was more than 100 µg/ml and the regression value was difficult to analyse.

The results obtained showed that hydroalcoholic extract of *T.divaricata* had a very moderate anticancer activity which was

supported by a number of studies as follows: *Clerodendrum phlomidis* crude extracts of petroleum ether, ethyl acetate, chloroform and ethanol obtained from the root of the plant were tested for cytotoxic activity on Mouse embryonic fibroblasts cell line (NIH 3T3) and HeLa cell lines using MTT assay where ethanol extract had no cytotoxic activity and the other extracts had moderate to weak cytotoxic activity on both the cell lines.¹⁰ In another study four trifoliolate plant extracts in different solvents were tested for cytotoxic activity against HeLa cell lines and MCF7 cell lines and extract showed less significant activity against HeLa cell lines but showed good activity against MCF7.¹¹ In a research the methanolic extracts of *Artocarpus heterophyllus* was tested for anticancer activity by MTT assay on different cell lines like HEK293, A549, HeLa and MCF-7. The IC₅₀ value was found to be 35.26 µg/ml by MTT assay against A549 but the extract had no activity against HeLa and MCF-7 cell lines.¹²

CONCLUSION

The results obtained from the *in-vitro* studies performed using the HeLa cell lines reveals that the hydroalcoholic flower extract of *T.divaricata* has a moderate anticancer activity. Even though there was increase in the cell growth inhibition when concentration of sample was increased, the IC₅₀ 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 *T.divaricata* flowers can be concluded to be less effective.

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