Research Article

# *In vitro* Cytotoxicity Studies on *Tabernaemontana divaricata* leaves Extracts by Sulforhodamine B Assay Method

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**ABSTRACT**

Indian herbal medicines are gaining more importance and popularity across the globe because of their remarkable therapeutic values, low side effects, low cost and ease of availability. *Tabernaemontana divaricata* (TD), commonly called as Tagar/Crape Jasmine/Chandni, is a traditional medicine known to have antioxidant, anti-infective, anti-diarrhoeal, anti-tumour, analgesic, anthelmintic, antipyretic and reversible acetyl cholinesterase inhibitory activities**.** In the present study, crude petroleum ether and ethanolic leaf extracts of TD were screened for *in vitro* cytotoxicity activity against different cell lines. Petroleum ether and ethanolic crude leaf extracts of TD (PETD and ETD) were prepared by ultrasonication. The crude extracts were studied on human colon cancer HCT15, human breast cancer MCF7 and human leukemia MOLT4 cell lines using Sulforhodamine B (SRB) assay method. Growth inhibition of 50 % ($GI_{50}$) was analyzed by comparing it with standard drug Adriamycin (Doxorubicin). Both the extracts showed mild results on human breast cancer cell line MCF7 and human leukemia cell line MOLT4 and negative results on human colon cancer cell line HCT15. The cell growth inhibition of leukemia and breast cancer cells correlates to the ethnomedical use of extracts. The present study gives the overall perception about importance of *Tabernaemontana divaricata* in medicine and nutraceuticals purposes.

**Keywords:** *Tabernaemontana divaricata,* human colon cancer cell line HCT15, human breast cancer cell line MCF7, human leukemia cell line MOLT4, Sulforhodamine B assay method.

## INTRODUCTION

Cancer has become a big health hazard to humans globally. In India, 1.1 million new cancer cases were estimated, indicating the contribution to 7.8% of the global cancer burden.[1] Cancer is the second most common disease in India responsible for maximum mortality due to the poor availability of facilities of prevention, diagnosis and treatment of the disease.[2]

From ancient times, plants are well known as a major source of modern medicines. According to World Health Organization (WHO), 80% of the world population relies on herbal medicines as a primary health care need. In developed countries, the contribution of herbal medicines is 25% while in developing countries the contribution is as much as 75%. Herbal medicines have much more economic importance in India since ancient days. Treatment with herbal medicines is considered to be very safe as they have low side effects, are in sync with nature and the treatment with herbal medicine is independent of any age or sex.[3]

*Tabernaemontana divaricata* (TD) commonly known as Tagar/Chandni/Crape jasmine belongs to the family Apocynaceae. It is an evergreen shrub; leaves contain alkaloids and non-alkaloid constituents such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes. Traditionally *Tabernaemontana divaricata* is used to treat various diseases like diarrhea, abdominal tumors, asthma, epilepsy, eye infections, fever, fractures, inflammation, leprosy, paralysis, piles, rabies, rheumatic pain, skin diseases and urinary disorders. It is also used as anthelmintic, antihypertensive, diuretic, hair growth promoter, brain, liver and spleen tonic. [4]

Sulforhodamine B (SRB) assay method is rapid, sensitive, reliable and inexpensive ideal method for *in vitro* anticancer drug discovery screening which gives a colorimetric end that is nondestructive, stable and visible to the naked eye. Thus, providing a sensitive measure of drug-induced cytotoxicity useful in quantitating clonogenicity and suitability for high volume, automated drug screening.[5]

The present study, aims to study the effect of crude leaves extracts of TD on human colon cancer cell Line HCT15, human breast cancer cell line MCF7 and human leukemia cell line MOLT4 by Sulforhodamine B (SRB) assay method.

## MATERIALS AND METHODS

### *Collection and Authentication*

Fresh leaves of *Tabernaemontana divaricata* (TD) were collected from Mumbai local market. The leaves and the aerial parts of the plant were authenticated at the

Agharkar Research Institute, Pune with voucher specimen No.3/187/2013/Adm.1692/13/079.

***Preparation of extracts***

The leaves of TD were air dried under shade and grinded to coarse powder. The leaf powder (200g each) were individually extracted by ultra-sonication process on ultrasonic bath with petroleum ether (40°C-60°C) / ethanol (500 ml) for 72 hours. The petroleum ether extract (PETD) and ethanolic extract (ETD) were then filtered using Whatman Filter No.1 and concentrated by evaporating the solvents on evaporating dish. The crude leaf extracts were then stored in amber colored air tight containers.

***Procurements of cell lines, Standard drug and chemicals***

All the cell cultures and media used in studies such as human colon cancer cell Line HCT 15, human breast cancer cell line MCF7, and human leukemia cell line MOLT4. Standard drug Adriamycin (Doxorubicin) were procured and maintained at ACTREC, Kharghar, Mumbai, during the studies. All the chemicals were procured from local suppliers throughout the experimentation with the help of ACTREC, Kharghar, Navi Mumbai.

***In vitro anticancer screening by Sulforhodamine B (SRB) assay method***

The crude petroleum ether extract (PETD) and ethanolic extract (ETD) of *Tabernaemontana divaricate* leaves were screened for *in vitro* cytotoxicity activity against human colon cancer cell Line HCT15, human breast cancer cell line MCF7 and human leukemia cell line MOLT4. All the cell cultures and media used in studies and standard drug Adriamycin (ADR) were procured and maintained at ACTREC, Kharghar, Mumbai.

The cell lines were grown in RPMI 1640 medium comprising 10% fetal bovine serum and 2 mM L-glutamine. For the current screening experiment, cells were inoculated into 96 well micro titer plates in 100 µL at plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the micro titer plates were incubated at 37 °C, 5% $CO_2$, 95% air and 100% relative humidity for 24h before addition of extract and Adriamycin.

The plant extracts of TD were initially solubilized in DMSO at 100mg/ml and diluted to 1mg/ml using water and stored frozen until use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100, 200, 400 and 800 µg/ml with complete medium containing test sample. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro titer wells already containing 90 µl of medium, resulting in the required final drug concentrations *i.e.*10, 20, 40, 80 µg/ml.

After addition of the extracts and standard, plates were incubated for 48h at standard conditions and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated at 4°C for 60 minutes. The supernatant was discarded; the plates were washed five times with tap water and air dried. SRB solution (50 µl) at 0.4 % (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature.

After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was then eluted with 10 mM trizma base, and the absorbance was read at a wavelength of 540 nm with 690 nm reference wavelength on a plate reader.

Calculation of percent growth was done on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated using formula,

$$[Ti/C] \times 100\ \%$$

## RESULTS AND DISCUSSION

The *in vitro* cytotoxicity activity results against human colon cancer cell Line HCT15, human breast cancer cell line MCF7 and human leukemia cell line MOLT4 were analyzed as 50% growth inhibition ($GI_{50}$).

Percent Control Growth at varying concentrations was observed for all the cell lines of ETD, PETD extracts and ADR (Adriamycin) as depicted in **Table 1**. **Figure 1** represents Growth Curve of ETD, PETD extracts and ADR (Adriamycin) for the cell lines. Both ETD and PETD extracts did not produce significant effect on all the human cancer cell lines used in this study **(Table 2)**. ETD and PETD showed significant activity on breast MCF7 and leukemia MOLT4 human cancer cell lines with $GI_{50}$ values of 19 µg/mL, 41.7 µg/mL and 23.9µg/mL, 33.7 µg/mL respectively. ETD extract with $GI_{50}$ value of 19 µg/mL is considered to demonstrate cytotoxic activity on human breast cancer cell line MCF7. Both the extracts showed concentration dependent cytotoxic activity on all the cell lines.

Since ancient time, plants have being used as a rich source of therapeutic agents and as a basis for synthetic drugs. 60% of current chemotherapeutic drugs are derived from or based upon natural phytochemicals. Thus, plants can be considered as one of the main natural resource agents in the research and development of cancer chemo-preventive drugs. As the number of cancer cases is increasing worldwide, many plant extracts and active principles are being studied in *in vitro* and *in vivo* cancer models and are considered as new sources of therapeutic anticancer agents in future.[6,7]

**Table 1:** % Control Growth and Drug Concentrations (µg/ml) for Human Colon cancer cell line HCT 15, Human Breast cancer cell line MCF7 and Human Leukemia cancer cell line MOLT4 of ETD, PETD extracts and ADR (Adriamycin)

| Concentration (µg/ml) | % Control Growth | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Human Colon cancer cell line HCT 15 | | | Human Breast cancer cell line MCF7 | | | Human Leukemia cancer cell line MOLT4 | | |
| | ETD | PETD | ADR | ETD | PETD | ADR | ETD | PETD | ADR |
| **Experiment 1** | | | | | | | | | |
| **10** | 98.4 | 100 | 18.1 | 47.7 | 95.4 | -7.6 | 32.7 | 42.8 | -12.4 |
| **20** | 96.6 | 100 | 18.2 | 29.1 | 76.2 | -15.2 | 19.7 | 39.4 | -19.8 |
| **40** | 82.6 | 99.6 | 18.6 | 18.8 | 22.4 | -37.7 | 18.9 | -5 | -30.3 |
| **80** | 67.1 | 90.8 | 10.9 | 13.6 | 19.4 | -64.5 | 2.1 | -12.9 | -37.9 |
| **Experiment 2** | | | | | | | | | |
| **10** | 95.8 | 99.5 | 18.3 | 35.1 | 90 | -12.7 | 56.2 | 89.8 | -27.1 |
| **20** | 94.7 | 99 | 17.4 | 33.9 | 79.3 | -21.1 | 53.1 | 89.2 | -27.4 |
| **40** | 82.9 | 96.9 | 17.9 | 19.1 | 22.2 | -41 | 43.9 | 69.7 | -27.5 |
| **80** | 63.2 | 88.6 | 13.2 | 14.2 | 22.4 | -63.7 | 16.2 | 17.7 | -30.7 |
| **Experiment 3** | | | | | | | | | |
| **10** | 97.4 | 100 | 18.8 | 35.5 | 88.8 | -15.3 | 50.6 | 63.1 | -29.6 |
| **20** | 96.7 | 99.8 | 17.5 | 29.3 | 71.9 | -19.8 | 28.8 | 63.3 | -36.9 |
| **40** | 86.9 | 97.5 | 17 | 14.9 | 20.3 | -45.2 | 20.4 | 25.6 | -37.7 |
| **80** | 71.3 | 92.5 | 16.3 | 10.3 | 17.5 | -65.8 | 16.1 | 12.2 | -40.2 |
| **Average Values** | | | | | | | | | |
| **10** | 97.2 | 99.8 | 18.4 | 39.4 | 91.4 | -11.9 | 46.5 | 65.3 | -23.1 |
| **20** | 96 | 99.6 | 17.7 | 30.8 | 75.8 | -18.7 | 33.8 | 64 | -28 |
| **40** | 84.1 | 98 | 17.9 | 17.6 | 21.6 | -41.3 | 27.7 | 30.1 | -31.8 |
| **80** | 67.2 | 90.6 | 13.5 | 12.7 | 19.8 | -64.7 | 11.5 | 5.7 | -36.2 |

Results in triplicate with average for ETD – Ethanolic extract of *Tabernaemontana divaricata,* PETD – Petroleum ether extract of *Tabernaemontana divaricata* and ADR – Adriamycin

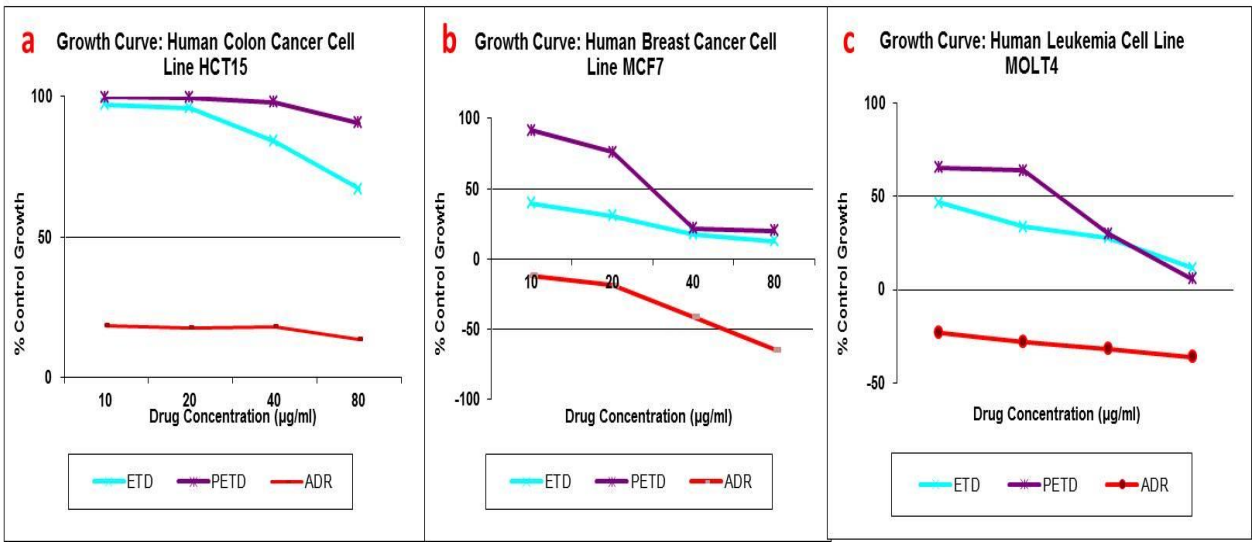


**Figure 1:** Growth Curve of ETD, PETD extracts and ADR (Adriamycin). a). Human Colon cancer cell line HCT 15. b). Human Breast cancer cell line MCF7. c). Human Leukemia cancer cell line MOLT4.

**Table 2:** Drug concentrations (µg/ml) calculated from graph for Human Colon cancer cell line HCT 15, Human Breast cancer cell line MCF7 and Human Leukemia cancer cell line MOLT4 of ETD, PETD extracts and ADR (Adriamycin)

| | Human Colon Cancer Cell Line HCT15 | | | Human Breast Cancer Cell Line MCF7 | | | Human Leukemia Cell Line MOLT4 | | |
|---|---|---|---|---|---|---|---|---|---|
| | LC50 | TGI | GI50 | LC50 | TGI | GI50 | LC50 | TGI | GI50 |
| **ETD** | >80 | >80 | >80 | >80 | 79.8 | 19.0 | >80 | >80 | 23.9 |
| **PETD** | >80 | >80 | >80 | >80 | >80 | 41.7 | >80 | 79.6 | 33.7 |
| **ADR** | >80 | >80 | <10 | 58.4 | 26.3 | <10 | 73.1 | 27.5 | <10 |

$GI_{50}$ value of ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds whereas $GI_{50}$ value ≤ 20µg/ml in extracts, Total growth inhibition (TGI).

In the present study, *Tabernaemontana divaricata* has shown mild cytotoxic activity on human breast cancer cell line MCF7 and human leukemia cell line MOLT4 except on human colon cancer cell line HCT15.

## CONCLUSION

*Tabernaemontana divaricata* shows different roles in CNS, cardiovascular, gonadotropic, anti-tumor, antinfectious, neurodegenerative diseases and anti-oxidative activity. The present study explores the anti-cancer potential of *Tabernaemontana divaricata.* The obtained result provides a support for the use of *Tabernaemontana divaricata* as an ethanomedicine and gives the overall perception about importance of *Tabernaemontana divaricata* in medicine and nutraceuticals purposes.

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