



Anticancer Efficacy of *Thevetia peruviana* (Pers.) K. Schum. Extracts on Triple Negative Human Breast Cancer Cell Line

S. Rajhans^{1*}, K. Bhadresha², D. K. Yadav², R. M. Rawal², H. A. Pandya¹
and A. U. Mankad¹

¹Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University, Ahmedabad, Gujarat, India.

²Department of Lifesciences, Gujarat University, Ahmedabad, Gujarat, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1130206

Editor(s):

(1) Dr. Daniela Rigano, Department of Pharmacy, via Domenico, Montesano 49, Naples Italy and University Federico II of Naples, Italy.

(2) Prof. Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.

Reviewers:

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(3) Saturnine Carmela, University of Basilicata, Italy.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54621>

Original Research Article

Received 05 December 2019
Accepted 10 February 2020
Published 19 February 2020

ABSTRACT

In this 21st century cancer has become the second big reason for death next to heart attack in both developed and the developing countries. Worldwide, there are almost 2.1 million newly diagnosed breast cancer cases in 2018, accounting for 1 in 4 cancer cases among women. Triple Negative Human Breast Cancer is one of the types of human breast cancer. The plant extracts of *Thevetia peruviana* (Pers.) K. Schum. has been used against the Triple Negative Human Breast Cancer cell line MDA-MB-231 for the current experiment All the 5 parts (leaf, stem, flower, fruit and seed) had been selected for the experiment and the extracts of the various parts were made in hydro-alcoholic solvent i.e. methanol: Water.

*Corresponding author: E-mail: sanjuktarajhans@gmail.com;

Keywords: *Thevetia peruviana* (Pers.) K. Schum; triple negative human breast cancer; MDA-MB-231; plant parts; soxhlet extraction.

1. INTRODUCTION

Presently, cancer has become the second big reason for death next to heart attack in both developed and the developing countries. Cancers originating from breast tissue, commonly from the inner lining of milk ducts or the lobules that supply milk in ducts are known as breast cancers. Worldwide, there are almost 2.1 million newly diagnosed breast cancer cases in 2018, accounting for 1 in 4 cancer cases among women [1].

In different areas of breast, development of several types of tumors may take place. Any cancer test that is negative for the three main criteria i.e., the hormone estrogen, the hormone progesterone and a protein called HER-2 (Human Epidermal Growth Factor-2) is known as Triple Negative Human Breast Cancer (TNBC). The signs and symptoms of the TNBC is similar to the other breast cancer types. The three main symptoms of the TNBC (Triple Negative Breast Cancer) are (a) development of a lump or mass in the breast (b) breast pain or redness and (c) turning of the nipple inwards along with some discharge [2,3].

Over the years, use of natural products as anticancer agents is widely in practice and they are widely incorporated with ayurvedic and allopathic medicine [4], as medicinal plants are renowned sources for phytochemicals with therapeutic values. Many studies have been done on bioactive plant products as anticancer, anti-inflammatory, and antimicrobial agents. One of the plant families i.e. Apocynaceae genus *Thevetia* and its other genera like *Catharanthus*, *Nerium*, *Strophanthus*, are well known for their anticancer activities because they are considered as a storehouse of numerous secondary metabolites especially cardiac glycosides [5,6]. *Thevetia peruviana* (Pers.) K. Schum. commonly called yellow oleander has been traditionally used for the treatment of gastrointestinal and inflammatory diseases as well as heart failures and skin tumors [7,8]. Studies have revealed the potent cytotoxic and antiproliferative effects of cardiac glycosides in several cancer cell lines, including breast, prostate, lung and renal cancers, melanoma, leukemia and neuroblastoma [9]. Therefore, present study aimed to investigate the role of *Thevetia peruviana* (Pers.)

K. Schum as anticancer agents in triple negative breast cancer line.

2. MATERIALS AND METHODS

2.1 Plant Material

Collection of the plant material was done from the Gujarat University area. Different parts of the plant such as leaves, stems, flowers, fruits and seeds were collected. The parts were cleaned using water and kept for shade drying. After the drying process the parts were subjected to Soxhlet extraction. The solvent system taken was in the ratio of 70:30, methanol: Water (hydro-alcoholic). The qualitative screening of hydroalcoholic extracts of *Thevetia peruviana* (Pers.) K. Schum. (leaves, stems, flowers, fruits and seeds) was done for evaluating the presence of the secondary metabolites. The result of the phytochemical analysis exhibited that the plant is rich in phytochemicals like alkaloids, phenolic compounds, tannins, glycosides, cardiac glycosides, flavonoids, diterpenes, steroids and saponins [10]. On this basis of the phytochemical analysis it was further decided to evaluate the anticancer activities of the extracts of the plant parts.

2.2 Cell Lines and Cell Culture

Breast cancer cell line (MDA-MB-231) was used in the current study (NCCS Pune, India). It is a highly aggressive, invasive and poorly distinguished triple-negative breast cancer (TNBC) cell line. Cell lines were cultured in DMEM (himedia) supplemented with 10% (v/v) fetal bovine serum (Gibco). The cells were cultured at 37°C under a humidified atmosphere containing 5% CO₂.

2.3 In vitro Cell Cytotoxicity Assay

The cell lines which were in exponential growth phase were washed, trypsinized and re-suspended in complete culture media i.e., DMEM (Dulbecco's Modified Eagle Medium), 10% FBS (Fetal Bovine Serum), Penicillin (100 IU/ml), Streptomycin (100 µg/ml), Amphotericin-B (5 µg/ml), The cells were seeded at 2×10⁴ cells/well in 96 well microtiter plate and incubated for 24 hrs. During this time period partial monolayer formation takes place. Following these procedures, the cells were then exposed to the different extracts as mentioned above. The

control set wells only received the maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% CO₂ and 75% of relative humidity for a time period of 24 hrs. An inverted microscope (zeiss) was used to examine the morphological changes of the cells that were treated with the drugs. The examination was done at different time intervals. The cells serving as the control set were compared with the treated cells. At the end of 24 hrs, cellular viability was determined. After 24 hrs 10 µl of MTT was added and incubated for another 4 hrs. 100 µl of DMSO was further added to each well. Finally, the absorbance of the samples was measured using a microplate (ELISA) [Thermo Fisher Scientific] reader at the wavelength of 570 nm.

The % cell viability was calculated with the following formula: Cell viability % = Mean OD of wells receiving each plant extract dilution / Mean OD of control wells x 100

2.4 Determination of IC₅₀

IC₅₀ value is a measure of the efficacy of a substance in obstructing a specific biological or biochemical function. A line drawn from the 50 % value on the Y axis meets the curve and interpolate to the X axis. The X axis value gives the Log (concentration of the compound). The antilog of that value gives the IC₅₀ value. Percentage inhibition of compound against cell line was calculated using the following formula: % cell survival = (At - Ab) / (Ac - Ab) * 100 [11].

where,

At = Absorbance of Test, Ab= Absorbance of Blank (Media), Ac= Absorbance of control (cells)
% cell inhibition = 100 - % cell survival

3. RESULTS

Assessment of the cytotoxic effect of *T. peruviana* hydroalcoholic extract on breast cancer line (MDA-MB-231) was performed by MTT assay (Fig. 1). An online tool has been used for calculating the IC₅₀ value and for plotting the graphs.

The formula used here is

$$Y = \frac{\text{Min} + \text{Max} - \text{Min}}{1 + (X/IC_{50})^{\text{Hill coefficient}}}$$

A Sigmoid graph was obtained for all the 5 samples that had been examined for the

presence of the anticancer activity using the MTT assay [12].

IC₅₀ values indicate the concentration of the extract that inhibits the growth of 50% of the cell population. All extracts against breast cancer cell line, showed a cytotoxic effect with IC₅₀ values lower than 20 µg/ml. The strong anticancer activity was observed in flower and seeds extract which shows the IC₅₀ value of 0.007 and 0.002 µg/ml respectively at incubation for 24 hrs (Fig. 4 and 6). Leaves, fruits and stem extract shows the IC₅₀ value of 1.19, 1.464 and 5.474 µg/ml respectively (Figs. 2,3,5).

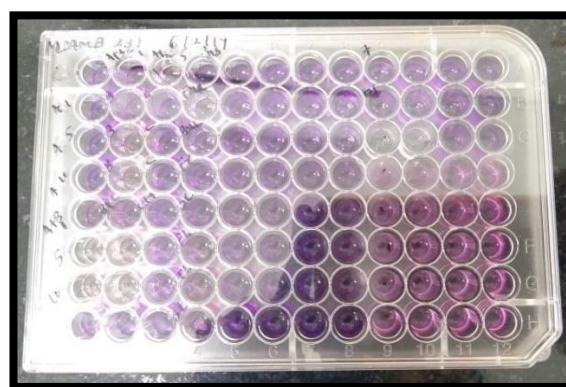


Fig. 1. 96 well plate with cell line and MTT

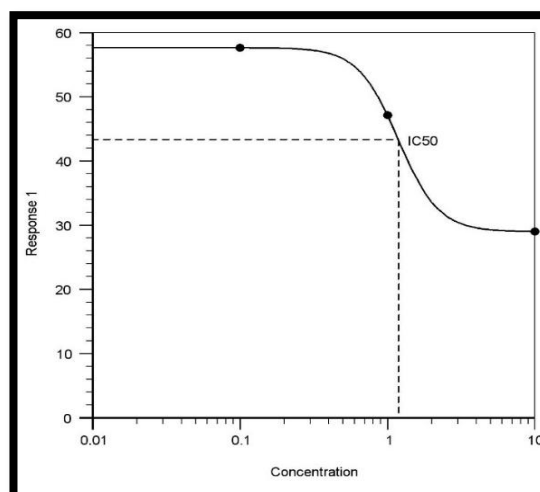


Fig. 2. IC₅₀ Graph of hydroalcoholic extract of leaves of *Thevetia peruviana* (Pers.) K. Schum on MDA-MB-231 cell line reveals response (% survival) on Y-axis and concentration in log transformed scale on X-axis. The MTT result for the leaves extract with sequential dilution of 10 µg, 1 µg, 100 ng, 10 ng and 1 ng against MDA-MB-231 presents IC₅₀ value of 1.19

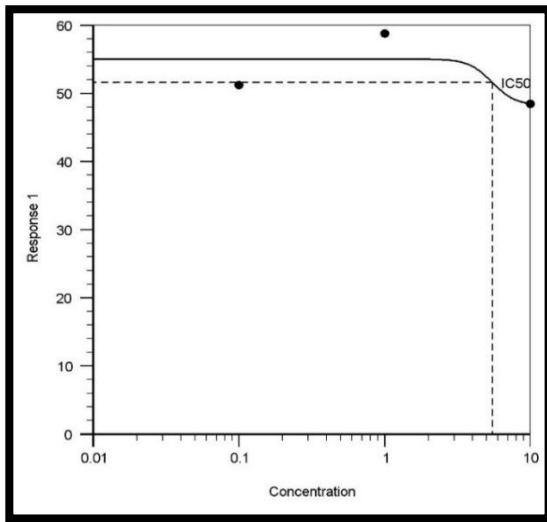


Fig. 3. IC₅₀ Graph of hydroalcoholic extract of stems of *Thevetia peruviana* (Pers.) K. Schum on MDA-MB-231 cell line reveals response (%survival) on Y-axis and concentration in log transformed scale on X-axis. The MTT result for the stems extract with sequential dilution of 10 µg, 1µg, 100 ng, 10 ng and 1 ng against MDA-MB-231 presents IC₅₀ value of 5.474

4. DISCUSSION

The overall result suggests that the extract of the flowers and seeds are potent inhibitors against the MDA-MB-231 cell lines. In previous research works many researchers have reported that the extracts of the plant parts of *Thevetia peruviana* (Pers.) K. Schum. possess potential anticancer activities. Managit C et al. [13] reported that the ethanolic extract of flowers were effective against the HeLa cells. Silva AR et al. [14] Testified that the methanolic extract of the fruits possess anticancer potential against the human breast, colorectal, prostate and lung cancer cell lines. Save SA et al. [15] reported that from the twigs of the plant strong anticancer drugs had been formulated, which were effective against breast and lung cancer cell lines. In the current experiment the hydro alcoholic extract (70:30) (methanol: water) of the seeds have exhibited the best result against the human breast cancer cell line MDA-MB-231. The IC₅₀ value was 0.002. In the order next to the seeds the flowers with IC₅₀ value of 0.007 have shown anticancerous activity. In accordance to our finding Nesy EA [16] showed 100% cell death on cell lines DLA and EAC at a concentration of 1.2 mg/ml. [10]. The leaves, fruits and the stems have also exhibited anticancer activity with the IC₅₀ value of

1.19, 1.464 and 5.474 respectively. It can be said that almost every part of the plant *Thevetia peruviana* (Pers.) K. Schum. possess anticancer property.

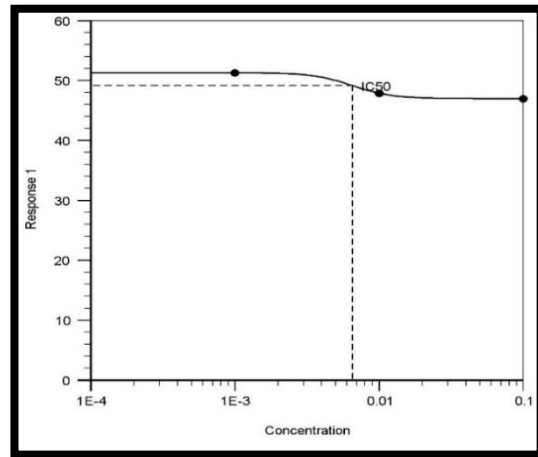


Fig. 4. IC₅₀ Graph of hydroalcoholic extract of flowers of *Thevetia peruviana* (Pers.) K. Schum on MDA-MB-231 cell lines reveals response (%survival) on Y-axis and concentration in log transformed scale on X-axis. The MTT result for the flowers extract with sequential dilution of 10 µg, 1 µg, 100 ng, 10 ng and 1 ng against MDA-MB-231 presents IC₅₀ value of 0.007

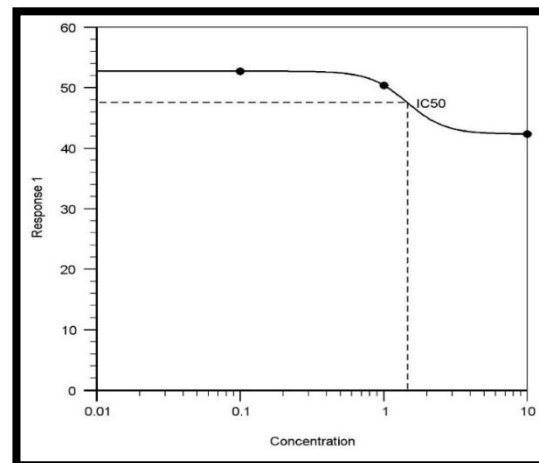


Fig. 5. IC₅₀ Graph of hydroalcoholic extract of fruits of *Thevetia peruviana* (Pers.) K. Schum on MDA-MB-231 cell lines reveals response (%survival) on Y-axis and concentration in log transformed scale on X-axis. The MTT result for the fruits extract with sequential dilution of 10 µg, 1 µg, 100 ng, 10 ng and 1 ng against MDA-MB-231 presents IC₅₀ value of 1.464

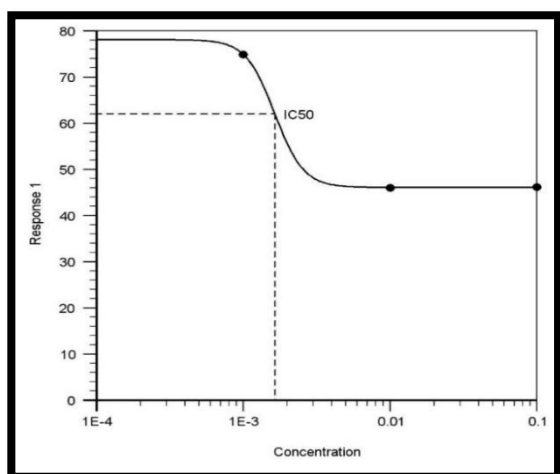


Fig. 6. IC₅₀ Graph of hydroalcoholic extract of seeds of *Thevetia peruviana* (Pers.) K Schum. on MDA-MB-231 cell lines reveals response (%survival) on Y-axis and concentration in log transformed scale on X-axis. The MTT result for the seeds extract with sequential dilution of 10 µg, 1µg, 100 ng, 10 ng and 1 ng against MDA-MB-231 presents IC₅₀ value of 0.002

5. CONCLUSION

On the basis of the phytochemical analysis by Rajhans S et al. [10] and the above MTT assay result it can be concluded that the leaves, stems, flowers, fruits and seeds all the five parts of the plant possess anticancerous property. The best result was obtained from the flowers and the seeds, showing effective anticancerous property. The Triple Negative Human Breast Cancer can be treated via medicines. The chief constituents of the medicines will be the chemicals derived from the different parts of the plant *Thevetia peruviana* (Pers.) K. Schum.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

Authors would like to acknowledge the software services and timely support of the department of Botany, Bioinformatics and Climate Change Impacts Management and department of Lifesciences, Gujarat University for this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;68:394-424.
2. Dey B, Kumar A. A review article on breast cancer. International Journal of Pharmacy and Pharmaceutical Research. 2018;11: 284-298.
3. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: An overview. J Adv Pharm Technol Res. 2010;1:109-126.
4. Valko V, Fickova M, Pravdov E, Nagya M, Grancai D, Czige S. Cytotoxicity of water extracts from leaves and branches of *Philadelphus coronarius* L. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2006;150:71-73.
5. En S, Chen Y, Lu Y, Wang Y, Ding L, Jiang M. Cardenolides from the Apocynaceae family and their anticancer activity. Fitoterapia. 2016;112:74-84.
6. Iriti M, Kubina R, Cochis A, Sorrentino R, Varoni EM, Kabala-Dzik A, Azzimonti B, Dziedzic A, Rimondini L, Wojtyczka RD. Rutin, a quercetin glycoside, restores chemosensitivity in human breast cancer cells. Phytotherapy Research. 2017; 31(10):1529-1538.
7. Onso-Castro AJ, Villarreal ML, Salazar-Olivo LA, Gomez-Sanchez M, Dominguez F, Garcia-Carranca A. Mexican medicinal plants used for cancer treatment: Pharmacological, phytochemical and ethnobotanical studies. J Ethnopharmacol. 2011;133:945-72.
8. Oji O, Okafor QE. Toxicological studies on stem bark, leaf and seed kernel of yellow oleander (*Thevetia peruviana*). Phytother Res. 2000;14:133-135.
9. Prassas I, Diamandis EP. Novel therapeutic applications of cardiac glycosides. Nat Rev Drug Discovery. 2008;7:926-935.
10. Rajhans S, Mankad UA, Pandya AH. Screening *Thevetia peruviana* (Pers.) K. Schum. for its bioactive phytochemicals. International Journal of Research and Analytical Reviews. 2019;6:932-35.
11. Thakkar K, Prasad AK, Nayak J, Iyer SV, Kumar S. Antioxidant and *in vitro* cytotoxic activity of extracts of aerial parts of

- Cocculus hirsutus* (L) using cell line cultures (breast cell line). The Journal of Phytopharmacology. 2014;3:395-399.
12. Quest Graph™ IC50 Calculator. AAT Bioquest INC; 2020. Available:<https://www.aatbio.com/tools/ic50-calculator>.
 13. Managit C, Sakurai H, Saiki I. Ethanolic extract of *Thevetia peruviana* flowers enhances TNF- α and TRAIL- Induced apoptosis of human cervical cancer cells via intrinsic and extrinsic pathways. Oncology Letters. 2017;13:2791-2798.
 14. Silva AR, Carreón FT, Figueroa M, Zavala SDLT, Arellanez AG, Rodriguez-Gracia A, Wong LJG, Arnaut HA. Anticancer potential of *Thevetia peruviana* fruit methanolic extract. BMC Complementary and Alternative Medicine. 2017;241:1-11.
 15. Save SA, Lokhande RS, Chowdhary AS. *Thevetia peruviana*: The good luck tree. Innovations in Pharmaceuticals and Pharmacotherapy. 2015;3:586-606.
 16. Nesy EA, Mathew L. *In vitro* cytotoxicity and antimicrobial efficacy of *Thevetia peruviana* seed kernal extracts. Int J Pharm Sci. 2016;8:47-50.

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