www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(3): 618-621

www.thepharmajournal.com
Received: 28-01-2020
Accepted: 29-02-2020

Sindhu Jose
Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

T Sivak Kumar
Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

R Ramprasad
Department of Pharmaceutics, Arulmigu Kalasalingam College of Pharmacy, Anandnagar, Krishnankoil, Tamil Nadu, India

Corresponding Author:
Sindhu Jose
Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Determination of anti-proliferative effect of methanol extract of *Begonia trichocarpa* Dalz leaf on cultured Hela cells

Sindhu Jose, T Sivakkumar and R Ramprasad

Abstract
Traditional medicinal plants have been used in the treatment of various diseases for centuries. A number of plant-derived compounds have been proposed as anticancer agents and are currently undergoing medical development. *Begonia trichocarpa Dalz* of *Begoniacae* family is wild, vulnerable, endemic species found in Kerala. Therefore, in this study, Methanol extract of *Begonia trichocarpa* (MEBT) was investigated for the apoptotic effect on human cervical cancer cells (HeLa).The *In vitro* anti-proliferative effect of MEBT on cultured HeLa cells was performed by colorimetric MTT assay. It was assessed that MEBT has anti proliferative effect on cultured HeLa cells and the IC_{50} value was found 24.05µg/ml. Anti-proliferative effect of methanol extract exhibited 84.06% inhibition at 100µg/ml concentration, whereas fraction BGTM1 from methanol extract of *Begoina trchocarpa* Dalz showed its anti-proliferative effect 56.65 % at 100µg/ml concentrations on cultured HeLa cells.

Key word: *Begonia trichocarpa,* anti-proliferative, HeLa cells.

Introduction
In recent years there has been a renewed interest in herbal medicines and the use of herbal medicine as Nutraceuticals. The importance of Nutraceuticals has been increasing daily due to its preventive nature; it may be due to its anti-oxidant property of medicinal plants. Use of nutraceuticals as a preventive increased especially in the case like diabetics, cancer, etc. Cancer is the common term used for all malignant tumors. Hippocrates (460-377 $_{BC}$) used term *karkinos* for breast cancer means crab, reflecting the character of crab on the nature of disease. Neo-plasis and Malignant are the other terms used for cancer, but there is a difference between malignant and neoplasia. Neoplasia "benign" are slow growing and localized without creating more difficulties to the host, but malignant are proliferating rapidly, causing difficulties by spreading all over the body and finally causing death of the host. The basic flat form of cancer is parenchyma and supportive stroma of fibrous connective tissue and blood vessels where parenchyma cells grow. The cause of this shift may be related to multiple factors including sex, age, race and exposure to environmental carcinogenic agents, of these factors last is the most important. Daily numerous carcinogenic factors encroach in our body and generate free radicals to stimulate carcinoma, but the defense mechanism of the body fight with the carcinogenic agents and reverse the boy mechanism to normal. Certain herpes and papilloma group DNA virus and certain type RNA virus have also been implicated as causative agents in an animal (Harsh Mohan 2010, Causes of cancer Wikipedia).
Many of the known anti- cancer agents used today in cancer therapy are secondary plant products or its derivatives. The use of herbs as medicines had been increased after the discovery of anticancer agent vinca alkaloids vinblastin and vincristine from *vinca rosea* in 1950s.Methanol extract of Begonia trichocarpa and its isolated compound showed anti-proliferative effect.

Material and methods
Determination of anti-proliferative effect of methanol extract of *Begonia trichocarpa* Dalz leaf on cultured HeLa cells
In vitro anti-proliferative effect of methanol extracts of *Begonia trichocarpa* Dalz leaf on cultured HeLa cells was determined by the percentage difference in viability by standard MTT assay method after 24 hours of incubation [22].

Preparation of cultured HeLa cells

The HeLa cervical cell line was purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluence at 37 °C in 5% CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, GERMANY) [23].

***Estimation of in-vitro anti proliferative effect of methanol extract of Begonia trichocarpa* Dalz leaf**

The methanol extract of *Begonia trichocarpa* Dalz leaf selected for *in-vitro* study, the selection was done on the basis of Preliminary phytochemical evaluation, TLC analysis and estimation of flavonoid content.

Cytotoxicity evaluation

The cell line was cultured in 25cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100IU/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37 ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated by direct observation of cells by inverted phase contrast microscope and followed by the MTT assay method.

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x104 cells/well) was seeded in 96 well tissue culture plate and incubated at 37 ºC in a humidified 5% CO_2 incubator. 1 mg of plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After 24 hours the growth medium w HeLa (cervical cancer) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen) as removed, freshly prepared each plant extract in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37 ºC in a humidified 5% CO_2 incubator [24].

Cytotoxicity assay by direct microscopic observation

The entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vocalization in the cytoplasm of the cells were considered as indicators of cytotoxicity [25].

Cytotoxicity assay by MTT method and evaluation of IC_{50}

About 15mg of MTT (Sigma, M-5655) was reconstituted in 3ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in the wells was removed and 3.0µl of the reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37 ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilization solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals [26, 27]. The absorbance values were measured by using the micro plate reader at a wavelength of 570nm (Laura B. Talarico *et al.* 2004) (ELISASCAN, ERBA).

% viability = (OD of Test/ OD of Control) X 100.

Result and discussion

Determination of *In vitro* anti-proliferative effect of methanol extract of *Begonia trichocarpa* Dalz leaf on cultured HeLa cells

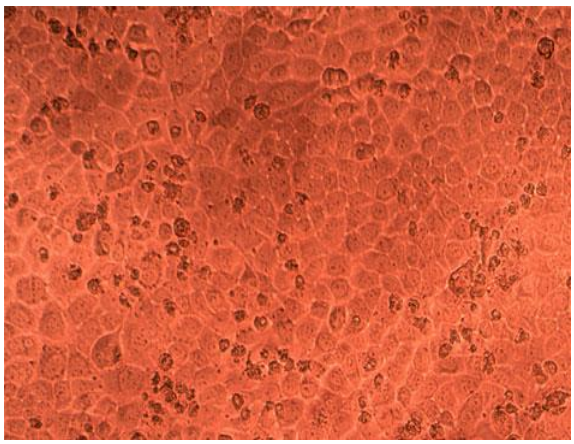
On the basis of above studies methanol extract of *Begonia trichocarpa* Dalz exhibited an increased antioxidant activity as compared with EABT. So methanol extract was selected for *In vitro* study of anti-proliferative effect of *Begonia trichocarpa* Dalz. *In vitro* anti-proliferative effect of methanol extract on cultured HeLa whole cells was performed and the observation was given in the Table. Cytotoxicity assay by direct microscopic observation are given in Fig 1.

Table 1: Percentage Viability and percentage inhibition of methanol extract of *Begonia trichocarpa Dalz* leaf by MTT assay

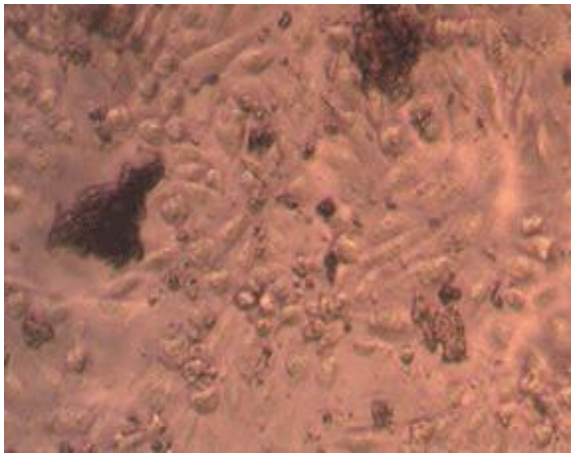
Sample volume (µg/ml)	Average Absorbance 540nm	Percentage Viability	% Inhibition
6.2	0.3413	66.28	33.8
12.5	0.2978	55.783	42.22
25	0.2189	42.51	57.49
50	0.1572	30.53	69.47
100	0.0821	15.94	84.06
IC_{50} value			24.05µg/ml

A dose dependent % inhibition of viability of HeLa cell exhibited by methanol extract of *Begonia trichocarpa* Dalz leaf was observed. IC_{50} value was found to be 24.05µg/ml.

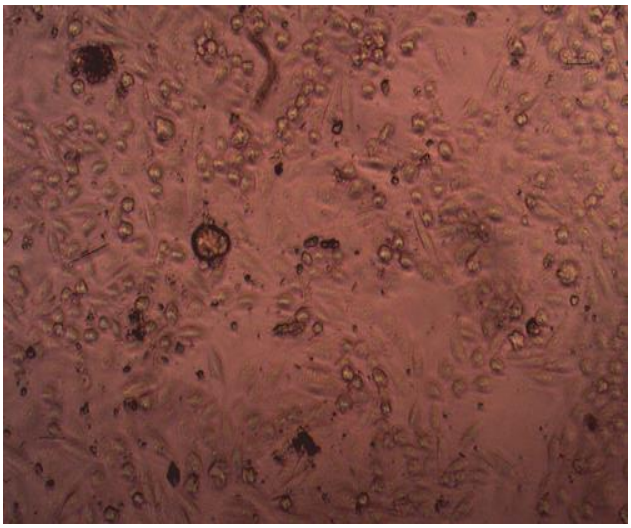
Cytotoxicity assay by direct microscopic observation



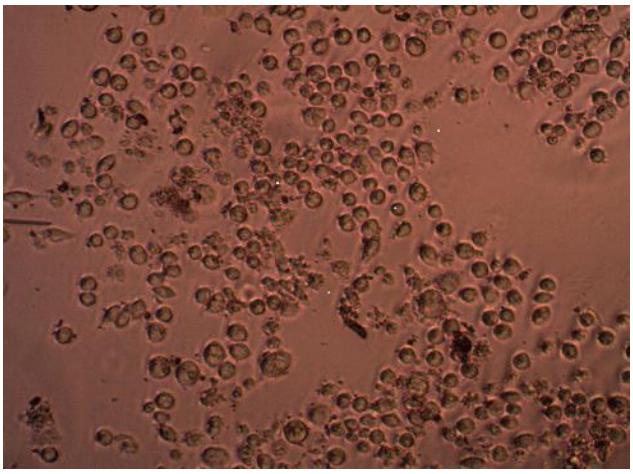
(A)



(B)



(C)



(D)

Fig 1: Effect of methanol extract of *Begonia trichocrpa* Dalz leaf on HeLa cells by direct microscopic observation of cytotoxicity assay. Microscopic observations of (A) control, (B) 6µ/ ml, (C)12µ/ ml, (D) 24µ/ ml of methanol extract of *Begonia trichcarpa* Dalz leaf

Microscopic observation shows rounding, shrinking, granulation in the cytoplasm of the cells were found, that indicate the Cancer cell cytotoxicity was produced by methanol extract of *Begonia trichocarpa* Dalz leaf.

Conclusion

The *In vitro* anti proliferative effect of MEBT on cultured HeLa cells was performed by colorimetric MTT assay, it was assessed that MEBT has anti-proliferative effect on cultured HeLa cells and the IC_{50} value was found 24.05µg/ml. *Begonia trichocarpa Dalz belong* to the Begoniacea *family* is a venerable plant selected on the basis of its non-popular traditional remedy in the treatment of throat infection was also show to proliferative effect.

Reference

1. Kamboj VP. Herbal medicine. Current science. 2000;78(1):35-9.
2. Houghton PJ. The role of plants in traditional medicine and current therapy. The Journal of Alternative and Complementary Medicine. 1995;1(2):131-43.
3. Jain S, Buttar HS, Chintameneni M, Kaur G. Prevention of cardiovascular diseases with anti-inflammatory and anti-oxidant nutraceuticals and herbal products: an overview of pre-clinical and clinical studies. Recent patents on inflammation & allergy drug discovery. 2018;12(2):145-57.
4. Sachdeva V, Roy A, Bharadvaja N. Current prospects of nutraceuticals: A review. Current pharmaceutical biotechnology. 2020;21(10):884-96.
5. Tsuchiya R, Fujisawa N. Historical survey of carcinoma of the pancreas. Journal of hepato-biliary-pancreatic surgery. 1999;6(2):165-70.
6. Nandhini S. Evaluation of Anticancer Potential of Leaves and Stem of *Azima Tetracantha Lam* (Doctoral dissertation, College of Pharmacy, Madras Medical College, Chennai).
7. Rous P, Kidd JG. Conditional neoplasms and subthreshold neoplastic states: a study of the tar tumors of rabbits. The Journal of experimental medicine. 1941;73(3):365-90.
8. Tan ML, Choong PF, Dass CR. Cancer, chitosan nanoparticles and catalytic nucleic acids. Journal of Pharmacy and Pharmacology. 2009;61(1):3-12.
9. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research. 2008;25(9):2097-116.
10. Shariat SF, Sfakianos JP, Droller MJ, Karakiewicz PI, Meryn S, Bochner BH. The effect of age and gender on bladder cancer: a critical review of the literature. BJU international. 2010;105(3):300-8.
11. De Aguiar Saldanha Pinheiro AC. Development of new molecular methods for the diagnosis and the study of viral diseases of fish. DOI **10.6092/unibo/amsdottorato/6939**.
12. Hausen H. Human genital cancer: synergism between two virus infections or synergism between a virus infection and initiating events?. The Lancet. 1982;320(8312):1370-2.
13. Luo H, Wang F, Bai Y, Chen T, Zheng W. Selenium nanoparticles inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest. Colloids and Surfaces B: Biointerfaces. 2012;94:304-8.
14. Kaba SI, Egorova EM. In vitro studies of the toxic effects of silver nanoparticles on HeLa and U937 cells. Nanotechnology, science and applications. 2015;8:19.
15. Krishnakumar N, Sulfikkarali N, RajendraPrasad N, Karthikeyan S. Enhanced anticancer activity of naringenin-loaded nanoparticles in human cervical (HeLa) cancer cells. Biomedicine & Preventive Nutrition. 2011;1(4):223-31.
16. Singh R, Nawale LU, Arkile M, Shedbalkar UU, Wadhwani SA, Sarkar D, Chopade BA. Chemical and biological metal nanoparticles as antimycobacterial agents: A comparative study. International journal of antimicrobial agents. 2015;46(2):183-8.
17. Veeralakshmi Ramu Ramprasad. In Vitro Anti-Plasmodial Activity of Ethanolic Extract of Begonia Trichocarpa Dalz International Journal of Chemistry and Pharmaceutical Sciences, 7(6), 149-151
18. Nadri S, Soleimani M, Kiani J, Atashi A, Izadpanah R. Multipotent mesenchymal stem cells from adult human eye conjunctiva stromal cells. Differentiation. 2008;76(3):223-31.
19. Pichitsiri W. Renal allograft failure: a study of the drivers of epithelial cell de-differentiation (Doctoral dissertation, Newcastle University).
20. Ciapetti G, Stea S, Cenni E, Sudanese A, Marraro D, Toni A, Pizzoferrato A. Cytotoxicity testing of cyanoacrylates using direct contact assay on cell cultures. Biomaterials. 1994;15(1):63-7.

21. Talarico LB, Zibetti RG, Faria PC, Scolaro LA, Duarte ME, Noseda MD, Pujol CA, Damonte EB. Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds Gymnogongrus griffithsiae and Cryptonemia crenulata. International Journal of Biological Macromolecules. 2004;34(1-2):63-71.