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Evaluation of *in vitro* anticancer activity of *Trianthema decandra* extract

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Abstract
Medicinal plants and its products have been the backbone of traditional system of medicine throughout the world for over thousands of year. It is continued to provide new remedies to human being without any side effects. In the present study, ethanol extract of *Trianthema decandra* leaves were evaluated for their *in-vitro* anticancer activity by making use of cancer cell line (MCF-7 cell line) cytotoxicity by MTT assay. The results obtained indicated that the plant extracts has potent cytotoxic activity on MCF-7. The results of the present findings strengthen the potential of the selected plants as a resource for the discovery of novel anticancer agents.

Keywords: *Trianthema decandra*, leaf, anticancer, MTT assay

1. Introduction

Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases. It is estimated that more than 1300 Indians die due to cancer. Mortality rate due to cancer is was increased up to 6% (Rajan *et al* 2011) [1]. Breast cancer is the most common cancer among women worldwide. It is a type of cancer where cells in the breast divide and grow without normal control. The incidence of breast cancer has doubled during the past 30 years. 50 to 75 per cent of breast cancers begin in the ducts, 10 to 15 per cent begin in the lobules and a few begin in other breast tissues (Dillon *et al* 2010) [2]. Fortunately, the mortality rate from breast cancer has decreased in recent years with an increased emphasis on early detection and more effective treatments (Sunil *et al* 2014) [3].

Several commonly used herbs have been identified by the National Cancer Institute as possessing cancer-preventive properties. These include members of the Allium sp. [garlic, onions and chives], members of the Lamiacea family [basil, mints, oregano, rosemary, sage, and thyme], members of the Zingiberaceae family [turmeric and ginger] and members of the Umbelliferae family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley). In addition, many herbs contain a variety of phytosterols, triterpenes, flavonoids, saponins, and carotenoids, present in the plants are also prevent to be cancer chemoprotective Jaikumar and Jasmine (2016) [4]. Present study aimed to investigate ethanolic extract of *Trianthema decandra* leaf (Tamil: Sakthi saranai) for their potential anticancer activity against human breast cancer cell line viz.MCF-7.

2. Materials and Methods

Collection, identification, and authentication of the selected medicinal plants

Trianthema decandra leaf was collected from the nearby regions of Kumbakonam, Thanjavur district (Tamil Nadu). The plants were identified and authenticated by Dr. John Britto, Director, Rabinath Herbarium, St. Josephs College, Tiruchirappalli. India. Voucher specimens of the collected plants were deposited in the herbarium center of the host institute. The plant materials were dried under shade at room temperature pulverized by a mechanical blender and sieved through 40 meshes then stored in airtight closed bottle until required.

Extraction

The shade-dried, powdered leaf sample (100 g) was extracted in ethanol by using Soxhlet apparatus. The resultant extracts were filtered by using Whatman No 1 filter paper and then concentrated in a rotary evaporator and were stored in a refrigerator at 4ºC in small sterile glass bottles for further analysis.

Anticancer assay

Anticancer assay was evaluated by the MTT reduction assay [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium] (Mosmann 1983; Monks *et al* 1991) [5, 6]. The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediamine tetraacetic 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% CO_2, 95% air and 100% relative humidity. Aliquots of 100 µl of different concentrations of leaf and bark extracts (25, 50, 100 and 200µg/ml) dissolved in DMSO (1%) were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations for 48h at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 48h of incubation, to each well 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) phosphate- buffered saline solution was added and incubated at 37°C for 4 h. Then, 100µl of 0.1% DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for inhibition Concentration (IC_{50}) was determined graphically. The absorbance at 570nm was measured with a UV- Spectrophotometer. The medium without samples served as control and triplicate was maintained for all concentrations. The effect of the samples on the proliferation of MCF-7 was expressed as the % cell viability & % Cell growth inhibition using the following formulas: % Cell viability = Abs 570 of treated cells / Abs 570 of control cells × 100%.

% of Cytotoxicity = [100 - Abs (sample) /Abs (control)] x100.

3. Results and Discussion

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line and useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen via estrogen receptors. The cell growth inhibition of *Trianthema decandra* leaf extract and AgNPs the tested against MCF-7 cell line at different concentrations (25, 50, 100 and 200µg/ml). The results of the study observed that the concentrations increases there are an increase in the cell growth inhibition (Cytotoxicity) and represent in table 1 and figure 1. The cell growth inhibition of *Trianthema decandra* leaf extract was found to be lowest growth inhibition was 9.92 % at 25µg/ml and highest growth inhibition was 67.25% at 200 µg/ml. Photomicrograph of MCF-7 cell line at various concentrations (25, 50, 100 and 200µg/ml) of *Trianthema decandra* leaf extract are shown in Plate1. The IC_{50} value was more than 142.18µg/ml.

Table 1: Percentage cell growth inhibition of *Trianthema decandra* extract on MCF 7cell line by MTT assay

S.No.	Concentrations (µg/ml)	Absorbance (Optical density)	Cell Viability (%)	Cytotoxicity (%)
1.	25	0.342	90.07	9.92
2.	50	0.311	81.91	18.08
3.	100	0.224	59.17	40.82
4.	200	0.124	32.74	67.25
	Cell Control	0.379	100	0
Half Inhibition Concentration (IC_{50})				142.18µg/ml

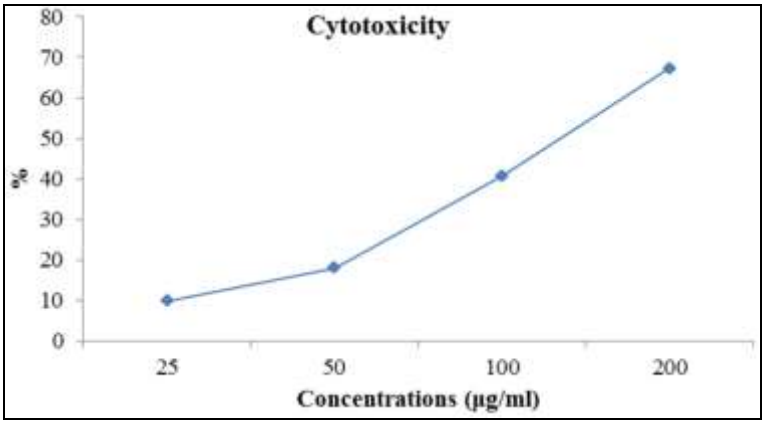

Fig 1: Percentage of cell growth inhibition (Cytotoxicity) of *Trianthema decandra* extract on MCF 7cell line by MTT assay

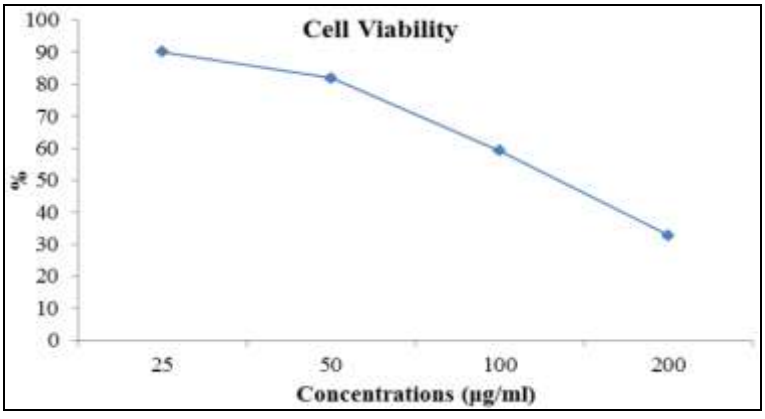

Fig 2: Percentage of cell viability of *Trianthema decandra* extract on MCF 7cell line by MTT assay

Normal cells showing surface architecture. Cytotoxic cells shows the cells became rounder, shrunken and showed signs of detachment from the surface of the wells denoting cell death (Round pebbles).

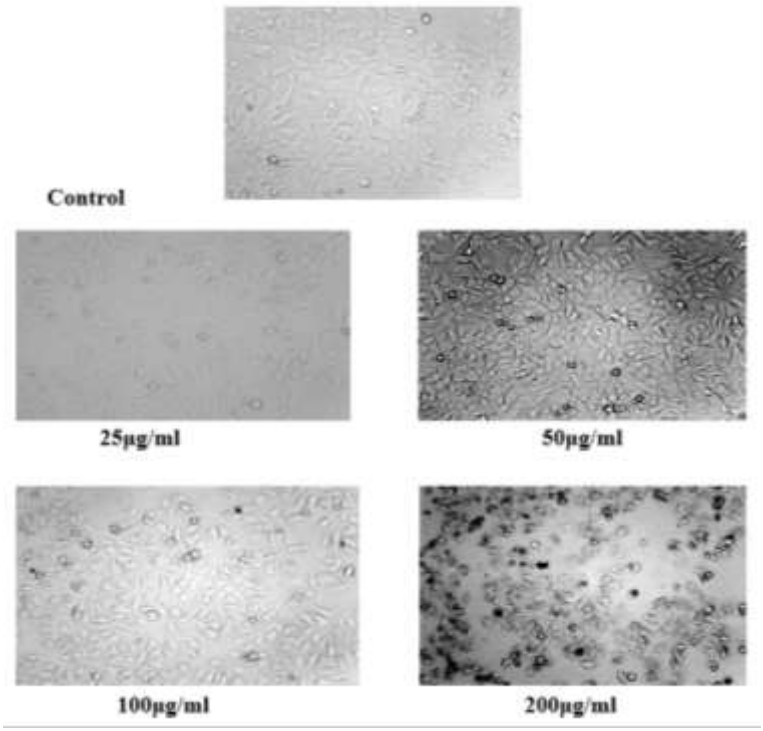

Plate 1: Photomicrograph of MCF-7 cell line of plant extract

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers (Mosmann 1983) [5]. The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute (Nagamine *et al* 2009) [7].

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line that was first isolated in 1970 from the malignant adenocarcinoma breast tissue of a 69-year old woman. MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cells to process estrogen via estrogen receptors. MCF-7 cells are also sensitive to cytokeratin. When grown *in vitro*, the cell line is capable of forming domes and the epithelial like cells grow in monolayers. Growth can also be inhibited using tumor necrosis factor alpha (TNF alpha) (Son *et al*., 2009) [8].

These results are in agreement with those reported by Ranjit *et al* (2015) [9] who observed a significant anti-cancer property of various ornamental flowers (*Ixora coccinia, Allamanda cathartica, Hibuscus rosa-sinensis* and *Tecoma stans*) against MCF-7 cell lines by using MTT assay. Umapriyatharshini *et al* (2018) [10] studied the anti-cancer effects *Garcinia quaesita* Pierre and *Garcinia zeylanica* on breast cancer stem cells isolated from MCF-7. Hexane and chloroform extracts of *Garcinia quaesita* and *Garcinia zeylanica* barks showed dose dependent reduction in proliferation and stemness in MCF-7 cells.

Earlier reports explained that phenolic compounds and its congeners are known to show cytotoxicity against various cancer cell lines and capable of inducing caspase-mediated apoptosis activity (Owen *et al* 2000; Nandi *et al* 2007) [11, 12]. The mechanism of action of anticancer activity of phenolics could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to anticancer activity. In addition, the presence of 2,3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death (Plochmann *et al* 2007) [13]. The main objective of this assay is to check the cytotoxicity brought about by the extract and to find the toxicity levels in terms of IC_{50} dose when live and dead cell percentages are equal, which is considered as the optimum dose for the various assays. It has been shown that the ethanolic extract of *Trianthema decandra* leaf possesses activity at higher concentration.

Data of the results indicate that the cytotoxic effect strengthens with increase in the concentration of the extracts. Due to the mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan produced is directly proportional to the number of viable cells. Polyphenol (flavonoid) compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells (Wali *et al* 2019) [14].

4. Conclusion

Chemoprevention studies are underway to identify promising candidates for reduced cancer risk. Based on the results obtained in this study, in which the *in vitro* cytotoxicity assay of MCF 7 was used. It is concluded that

the ethanolic extract of *Trianthema decandra* leaf possess cytotoxic efficacy against breast carcinoma MCF-7 cell line. Results of the present study provided new evidence for anticancer activity of *Trianthema decandra* leaf which could be useful for developing new anticancer therapies.

5. References

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