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Research Article

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Anti-cancer activity of Aya Thambira Chendooram (ATC) in *in-vitro* cell line against Breast Carcinoma

S. Mohamed Ajmal[1], R.Raseeha[1], R.Rajarajeswari[*]
[1]Final year BSMS, Sivaraj Siddha Medical College, Salem
[*]Lecturer, Department of Sool Magalir Maruthuvam, Sivaraj Siddha Medical College, Salem
E-mail: *mohamedajmal955@gmail.com*

Abstract

The Siddha medical science is one of the ancient Indian Traditional medicines nearly followed by the Tamil speaking people. Our Siddha system of medicine not only focuses on prevention and cure but also emphasises in Kayakarpam (Rejuvenation). Cancer (Putru) is one among the leading causes of human death worldwide and the incidence continues to increase. Breast cancer is the leading cause of death in women worldwide among other cancers. The risk is more at urban areas (one in twenty) and comparatively low in rural areas(one in sixty).Since an increasing proportion of cancer patients are acquiring resistance to chemotherapeutic agents, it is necessary to search for new compounds that provide suitable specific anti-proliferative effect that can be developed as anti-cancer agents. Aya Thambira Chendooram is a herbomineral medicine and it is tested against MCF-7 cell lines. The result showed that ATC possess cytotoxic effect against Breast Carcinoma.

Keywords: Aya Thambira Chendooram, Siddha, Tamil, MCF-7 cell lines, Breast cancer.

Introduction

Cancer, a dangerous disease which stands tall in the research activity of many scientists giving the fact that no medicine has been found to completely cure, at least 14 million people all over the world are affected by cancer and at least 8.2 million people become victim to this disease by causing death. Junk food consumption, alcohol, smoking, stress, plastic usage and tremendously adapted life are considered to be the cause for cancer[(1)]. Siddha is the first and foremost system of medicine to possess remedies that help in cure if not alleviation of the troublesome symptoms of this condition. Breast cancer remains the most common cancer in women and is second only to lung cancer as the leading cause of cancer related death in the United States as well as India[(2)].Some of the cells begin to grow abnormally. These cells divide more rapidly than healthy cells do and may spread through the breast, to the lymph or to other parts of the body (metastasize). The most common type of breast cancer begins in the milk-production ducts, but cancer may also occur in the lobules or in other breast tissues[(3)]. Aya Thambira Chendooram (ATC) is one of the Siddha herbo-mineral medicines mentioned in the book Siddha Anuboga Vaidhya Thirattu by Abdullah Shahib[(4)]. The purpose of this study was to assess the growth inhibitory effect of Aya Thambira Chendooram (ATC) in the human breast cancer cell line (MCF-7).

Materials and Methods

1. CO_2 incubator- Sanyo, Japan
2. Multimode microplate reader- BioTek, USA
3. Refrigerated centrifuge- Remi, India
4. Cell: MCF-7 cell line - NCCSPune
5. MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) from Sigma
6. Fetal bovine serum from Genetix Biotech, India
7. Trypsin from SRL Chemicals
8. Penicillin/Streptomycin from Sigma
9. DMEM medium from Genetix Biotech, India
10. DMSO from SRL chemicals.

Methodology

Cell culture and MTT assay procedure [5]

1. The breast cancer cell line, MCF-7 was purchased from NCCS Pune. The cells were grown in a DMEM medium supplemented with 10% fetal bovine serum and antibiotics as mentioned earlier. Cell proliferation (MTT) assay was performed following the method described by Carmichael et al., (1987) and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated controls.

2. For the MTT assay, the cells were grown in 25 cm ×25 cm ×25 cm tissue culture flasks containing DMEM medium as culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2. Cells were regularly passaged and maintained before including for the experiment.

3. When a cell density in a culture flask reached 70-80% confluence, they were trypsinized and seeded in 96-well plates in the density of 5000 cells per well in 100 µL and incubated for 24 hours at CO2 incubator.

4. Next day, Aya Thambira Chendooram (ATC) was prepared as 10 mg/ml stocks by adding directly into phosphate buffered saline, PBS (1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mMNaCl, 2.7 mMKCl. pH 7.4),. The working stock of 2X (2000, 600, 200, 60, 20, 6, 2 and 0.6 µg/ml) concentration to the cell in 100 µL volume and the final concentration range were: 1000, 300, 100, 30, 10, 3,1 and 0.3 µg/ml. 100 µl of diluted stocks were added to the cell and the plate was further incubated for 48 hours in the co_2 incubator at 37°C.

5. MTT solution was composed of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in PBS . From this solution 50 µl was pipetted out into each well to achieve 1mg/mL as final concentration. The plate was further incubated for 3 hours in incubator and the medium was carefully decanted. The formazan crystals were air dried in dark place and dissolved in 100 µL DMSO and the plates were mildly mixed at room temperature and the OD was measured using Synergy HT microplate reader at 570 nm.

6. From the optical densities the percentage growths were calculated using the following formula:

Percentage Growth (%) = $100\times [(T-T_0)/(C-T_0)]$

Where,

T is optical density of test,
C is the optical density of control,
T_0 is the optical density at time zero (at the time of compound addition will serve as blank to assess the cytotoxicity).
From the percentage growths a dose response curve was generated and GI_{50} values were calculated.

Cell imaging

After 48 hours before adding MTT solution treated cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

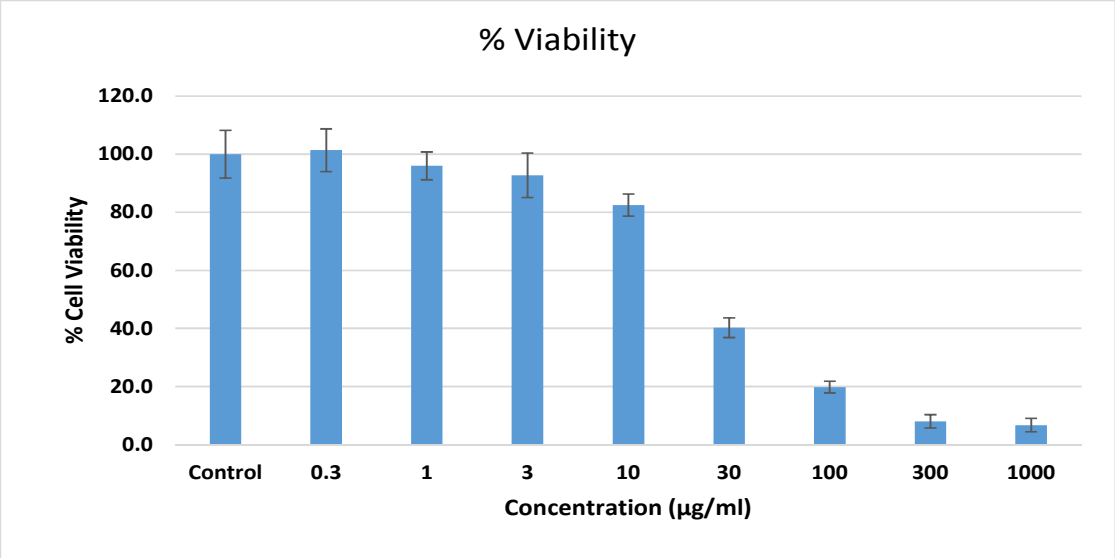
Results and Discussion

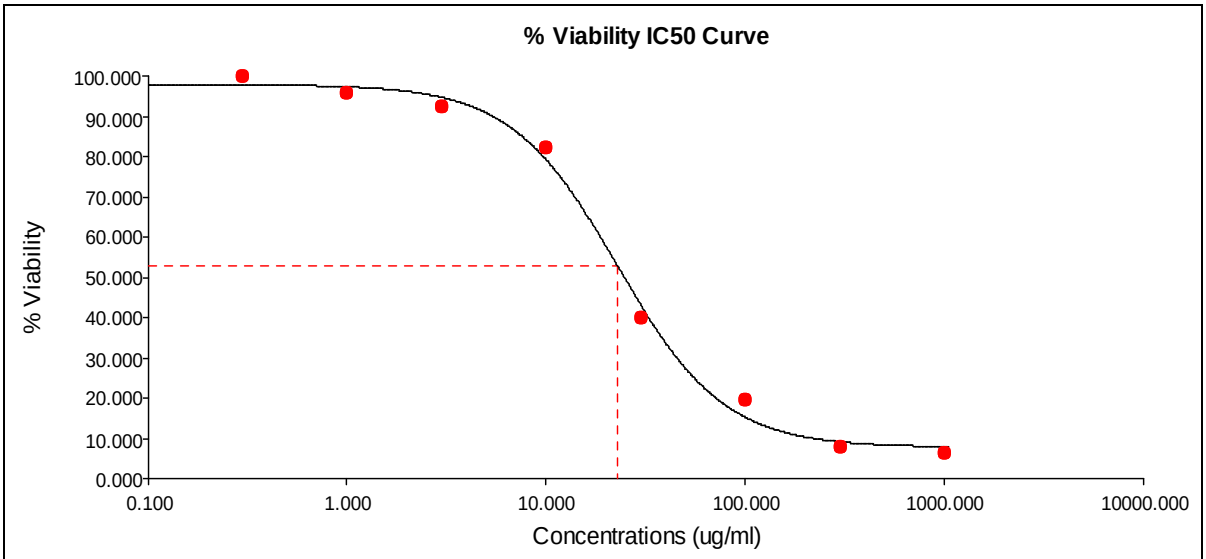
Cell growth inhibition property

The Aya Thambira Chendooram (ATC) was tested against MCF-7 cell line. The Aya Thambira Chendooram (ATC) concentration ranging from 1000, 300, 100, 30, 10, 3, 1 and 0.3µg/ml in semi logarithmic range. The Aya Thambira Chendooram (ATC) of each concentration was performed in Quadruplicate and cumulative variation were maintained less than 20% between the data points.

Table 1: % Viability of cells

Conc (ug/ml)	% Viability	SD
1000	6.7	2.3
300	8.0	2.3
100	19.9	2.0
30	40.2	3.4
10	82.5	3.8
3	92.7	7.7
1	96.0	4.8
0.3	101.3	7.4
Control	100.0	8.2

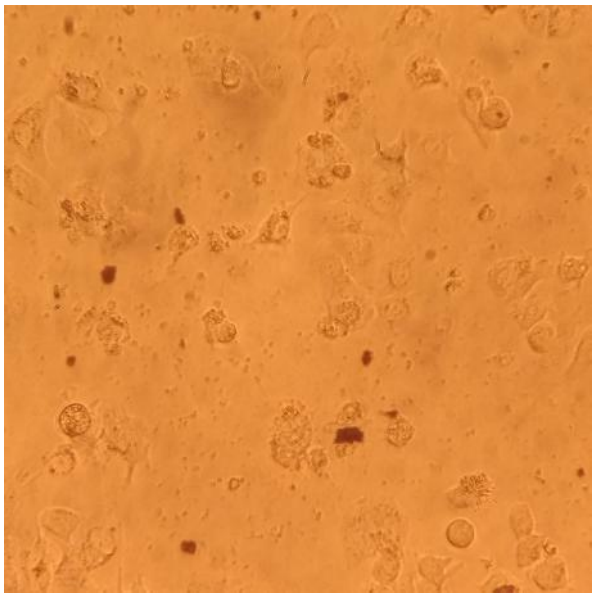




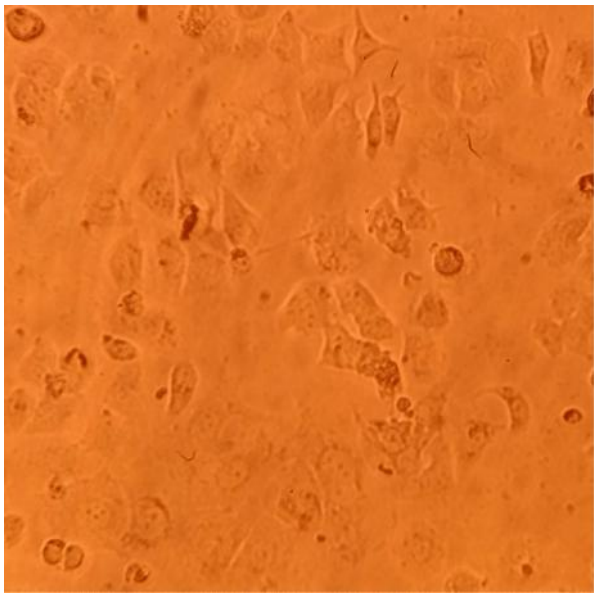
Curve Name	Well ID	Curve Formula	A	B	C	D	R2
IC50 curve_MCF-7	MCF-7	Y = (A-D)/(1+(X/C)^B) + D	98	1.63	**22.9**	7.96	0.996

The results acquired from the in-vitro studies achieved via the MCF-7 cell lines reveals that the unique Siddha medicine Aya Thambira Chendhooram (ATC) has a potent anticancer activity. There was increase in the cell growth inhibition when concentration of sample was increased, the IC50 value was 22.9 µg/ml for the cell line studies as exposed by the MTT assay method. Hence the level of cytotoxicity of the Aya Thambira Chendhooram can be concluded to be more effective.

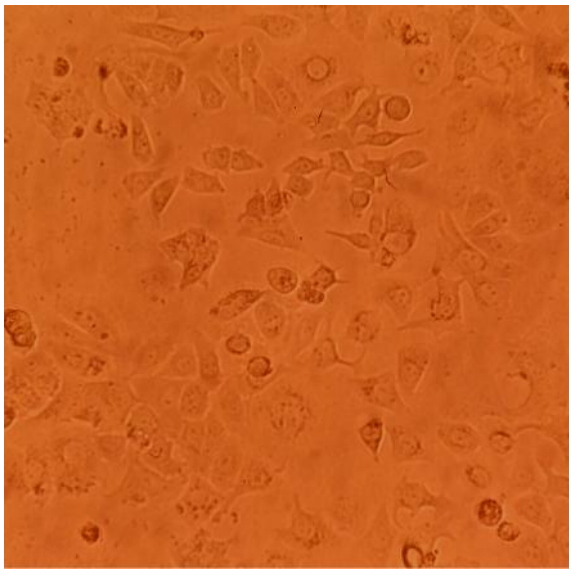
Cell imaging



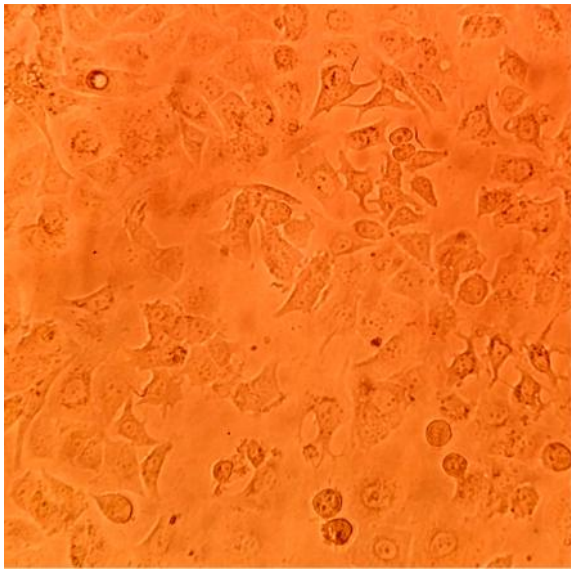
10 µg/ml



3 µg/ml



1 µg/ml



Control

Conclusion

The current study enumerates anti-cancer effect of the drug Aya Thambira Chendhooram (ATC) which is indicated in the Siddha classical text. In future, the component responsible for the anti-cancerous activity will be analysed and further studies will help to lift the medicine globally.

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