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S Deborah
PG & Research Department of Biotechnology, National College (Autonomous & CPE), Tiruchirappalli, Tamil Nadu, India

SP Anand
Assistant Professor, PG & Research Department of Botany, National College (Autonomous & CPE), Tiruchirappalli, Tamil Nadu, India

G Velmurugan
PG & Research Department of Botany, National College (Autonomous & CPE), Tiruchirappalli, Tamil Nadu, India

Correspondence
S Deborah
PG & Research Department of Biotechnology, National College (Autonomous & CPE), Tiruchirappalli, Tamil Nadu, India

Evaluation of *In vitro* anticancer activity of *Tarenna asiatica* (L.) fruits ethanolic extract against human breast cancer

S Deborah, SP Anand and G Velmurugan

Abstract
Tarenna asiatica (L.) is a medicinal plant from Kolli hills, Eastern Ghats, India. Fruit the *Tarenna asiatica* is an edible one and it has been used traditionally for treatment of a number of diseases. In the present study the ethanol extract of the edible fruit of the plant have been tested for anticancer activity. The extract was prepared by soxhlet separation and vacuum evaporator method. The *in-vitro* anticancer studies were performed against human breast cancer cell line and Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) was used to analyze the cell growth inhibition. The results showed that the ethanol extract of wild edible fruit *Tarenna asiatica* (L.) possessed a high amount of anticancer activity and the IC_{50} value also high as 237.08 µg/ml. The plant investigated possesses remarkable anticancer activity and hence isolation of the compound contributing to the activity may lead to develop at a novel and natural phytomedicine for the disease.

Keywords: human breast cancer cell line, eagles minimum essential medium, ic_{50}, *tarenna asiatica*, wild edible fruit

1. Introduction
Plants plays essential role in the folklore medicine from ancient cultures. In addition to the make use of as food and spices, plants have also been utilized as medicines for over 5000 years [1]. It is estimated that 85% of the population in developing countries continues to use traditional medicines even today [2]. Cancer is one of the major chronic human diseases and causes large suffering and economic loss of world-wide [3, 4]. Various new strategies are being developed to control and treat several human cancers [5, 6]. Over 60% of anticancer drugs available in the market are of natural product. Natural products are also the leads for formulation of many drugs [7]. Therefore, the phytochemicals present in several herbal products and plants may have the potential to act as preventive or therapeutic agents against various human cancers [5]. The increased popularity of herbal remedies for cancer therapy perhaps believes that herbal drugs provide benefit over the allopathy medicines while being less toxic [8, 9]. Since the conventional therapies have devastating and destructive side effects, there is a continuous search of new herbal cures of cancer [10]. *Tarenna asiatica* fruit is used as an herbal remedy for various ailments, including eye infection, skin problems and abdominal pain. The parts of plants are traditionally used to promote suppuration, as anthelmintic and antiulcer agent [11, 12, 19]. In the present study *in-vitro* anticancer studies were performed against human breast cancer cell line and Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) was used to analyze the cell growth inhibition. The results showed that the ethanol extract of wild edible fruit *Tarenna asiatica* (L.) possessed a high amount of anticancer activity and the IC_{50} value also high as 237.08 µg/ml.

2. Materials and Methods
2.1. Plant collection and identification
Tarenna asiatica, wild edible mature fruits were collected in the month of April to June at Kolli hills. The collected fruits specimen was authenticated by Botanical survey of India (BSI), Coimbatore, Tamil Nadu, India.

2.2 Preparation of Extract
The fresh fruits were dried in shade for about 3 weeks and ground using a mixer to a coarse powder. The powder of dried solvent using a water bath maintaining at 60-80ºC at ambient conditions to get a crude hydro alcoholic extract devoid of solvents. The extract was prepared by using soxhlet and vacuum evaporator method.

2.3 *In-vitro* evaluation of anticancer activity by MTT assay

2.3.1 Cell line

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37 ^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

2.3.2 Cell Treatment

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37ºC, 5% CO_2, 95% air and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat Dimethyl Sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hour at 37 ºC, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.3.3 MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15 µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

$$\% \text{ Cell viability } = \frac{[A] \text{ Test}}{[A] \text{control}} \text{x } 100$$

2.4 Statistical analysis

The absorbance values were denoted as mean ± SEM. The IC_{50} is half the maximal inhibitory concentration of the toxic compound which results in the reduction of biological activity by 50%. IC_{50} was determined using Graph Pad Prism software.

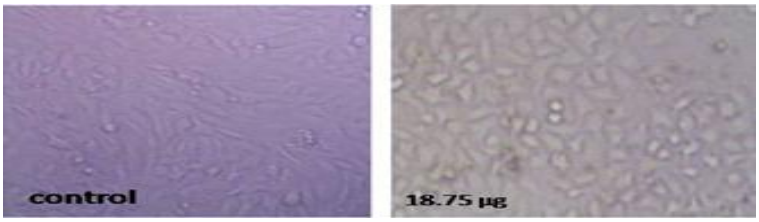
3. Result and discussion

3.1 *In vitro* anticancer activity

The results in cell growth inhibition by the ethanolic extract against human breast cancer cell lines for various concentrations are shown in table-1 and figure-1. The ethanolic extract at 18.75, 37.5, 75, 150 and 300 µg/mL had shown dose dependent inhibition of cells. As the concentration increases, there is an increase in the cell growth inhibition. 300 µg/mL ethanol extract of *Tarenna asiatica* inhibited 57.00% of human breast cancer cell lines. The estimated IC_{50} of ethanolic extract of *Tarenna asiatica* against human breast cancer cell lines was 237.08 µg/mL respectively. The results showed that an ethanolic extract of *Tarenna asiatica* has a high anticancer activity using MTT assay and the other extracts had moderate to weak cytotoxic activity on both the cell lines. In the study traditional edible fruit extracts in ethanol solvent were tested for cytotoxic activity against MCF7 cell lines and extract showed less significant activity against other cell lines. Agents capable of inhibiting cell proliferation, inducing apotosis or modulating signal transduction are currently used for the treatment of cancer [13, 14, 15]. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment [16]. Breast cancer is the most common malignancy among women. MCF-7 cell has become a prominent model system for the study of breast cancer as it relates to the susceptibility of the cells to apoptosis. Despite the fact that many tumors initially respond to chemotherapy, breast cancer cells can subsequently survive and gain resistance to the treatment [2, 17]. In this research the ethanolic extract of *Tarenna asiatica* was tested for anticancer activity by MTT assay on cell lines MCF-7. The IC_{50} value was found to be 237.08 µg/ml by MTT assay against the extract had a high activity against on MCF-7 cell lines. The anti-proliferative agent on human breast cancer cells (MCF-7) which was due to the presence of lignins and flavonoids [18, 19]. The present study shows that the ethanolic extract have significantly increased the percentage of cells with condensed nuclei when compared to other solvents.

Table 1: MCF-7 cells treated with ethanolic fruits extract of *Tarenna asiatica*

S. No.	Concentration of extract (µg/mL)	Absorbance	Inhibition of cell growth (%)	IC_{50} value µg/ml
1.	18.75	0.033±0.001633	5.02	237.08
2.	37.5	0.083±0.001247	12.69	
3.	75	0.186±0.000816	28.32	
4.	150	0.264±0.001247	40.30	
5.	300	0.373±0.001247	57.00	



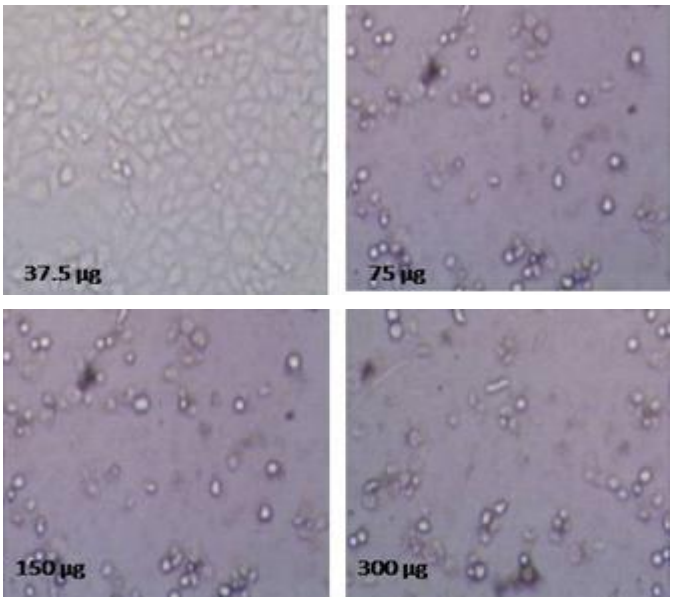


Fig 1: Inhibiting the activity of Breast Cancer cell

4. Conclusion

The present investigations find, out of an ethanolic extract of *Tarenna asiatica* fruits have a potent of cytotoxic activity against MCF-7 cells. The results obtained from the *in-vitro* studies performed using the MCF-7 cell lines reveals that the ethanolic extract of fruits of *Tarenna asiatica* has a higher anticancer activity. There was increase in the cell growth inhibition when concentration of samples was increased; The IC_{50} value was 237.08 µg/ml for the cell line studies as shown by the MTT assay method. The upshot of this study encourages to carrying out further studies to be extended for other cell lines and *in vivo* cytotoxicity investigations are required to identify anticancer activity.

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6. References

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