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In Vitro Cytotoxic Activity of Leaf Extracts of Selected Medicinal Plants

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A B S T R A C T

In the present study the ethanol extracts of *Capparis sepiaria*, *Euphorbia heterophylla* and *Tamilnadia uliginosa* leaf were tested for cytotoxic activity against three different human cancer cell lines such as HeLa cervical cancer cell line, MCF-7 human breast adenocarcinoma cell line and A-549 Human lung adenocarcinoma epithelial cell line, and compared with vero normal cell line. These studies revealed that *Tamilnadia uliginosa* crude extracts exhibited potent cytotoxic activity towards A-549 cell line with CTC_{50} value of 164.3 µg/ml followed by 179.4 µg/ml against MCF-7 cell lines and 208.2 µg/ml against HeLa cell lines. The leaf extracts of *Euphorbia heterophylla* showed least cytotoxicity against the tested cell lines. The results indicate the feasible anticancer nature of the crude ethanolic extract of *Tamilnadia uliginosa* leaves.

Introduction

The use of herbal medicines in the developed world is widespread and is increasing. Herbal medicines, which include a wide spectrum of substances ranging from homemade teas to the national regulatory bodies – approval medicinal substances, are defined as plant-derived products that are used medicinal substances, are defined as plant-derived products that are used for medicinal and /or nutritional purposes (KuczKowski, 2006). Medicinal plants are most important source of life saving drugs for a majority of the world's population (Debnath *et al.*, 2006). Many natural chemo preventive agents are capable of protecting against some forms of human cancer (Rajeshwar *et al.*, 2005). The strong association between the increasing of the consumption of these natural products and human diseases prevention has been explained by the content of the phytonutrients (Halliwell and Gutteridge, 1984). For these reasons, the search for antioxidant and cytotoxicity as chemoprevention agents is a continued process.

The medicinal plants are providing several drug components including cytotoxic characteristics. The results of the screening of plant extracts for anti-proliferative activity have shown that higher plants are a potential

source of antioncogenic agents which can participate favorably with chemotherapy and hormonal treatments. *In vitro* studies have offer proof that chemotherapeutic agents such as extracts may encourage apoptotic tumor cell death *in vivo*. The objective of this study is to determine the *in vitro* cytotoxicity activities of the ethanolic crude leaf extracts of *Capparis sepiaria, Euphorbia heterophylla* and *Tamilnadia uliginosa* against HeLa cervical cancer cell line, MCF-7 human breast adenocarcinoma cell line and A-549 Human lung adenocarcinoma epithelial cell line, and compared with vero normal cell line.

Materials and Methods

Collection of leaves

The leaves of the medicinal plants selected for the present study were collected from Sirumalai hills (Eastern Ghats), Dindigul, Tamil Nadu and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The names of the plants identified were *Capparis sepiaria* L. (Capparaceae), *Euphorbia heterophylla* L. (Euphorbiaceae) and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (Family: Rubiaceae). The leaves collected were shade dried and powdered using mortar and pastle. A fine powder obtained was stored in air tight poly bags and used for preparation of extract.

Preparation of extract

The cold extraction procedure was used for extracting leaves with solvents as per the procedure adopted by Prakash and Karmegam (2012) and Vigneshwari *et al.,* (2014). The leaves collected were transported to the laboratory for further processing. The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over newspaper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant leaves was taken in a 250 ml conical flask and added with 100-200 ml of ethanol individually. The solvent-leaf powder mixtures were kept at room temperature for 48 hrs and rapidly stirred using glass rod every 8 hrs. After 48 hrs, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

In vitro cytotoxicity studies

Chemicals

3-(4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai, India. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell lines and culture medium

HeLa (Epithelial cervical cancer, Human) cell culture was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37^0C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02%

EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

The human breast carcinoma MCF-7 and human lung adenocarcinoma cell culture was procured from National Centre for Cell Science (NCCS), Pune, India. Stock cells were cultured in MEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml),streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

A-549 (Human, small cell lung cancer) and Vero (African green monkey, normal kidney) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, each weighed test samples (leaf extracts) were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carried out cytotoxic studies. The extract concentrations of 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were prepared for the assay.

Determination of cell viability by MTT Assay (Francis and Rita, 1986)

Principle

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination

was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^{o} C in 5% CO_2 atmosphere.

The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan.

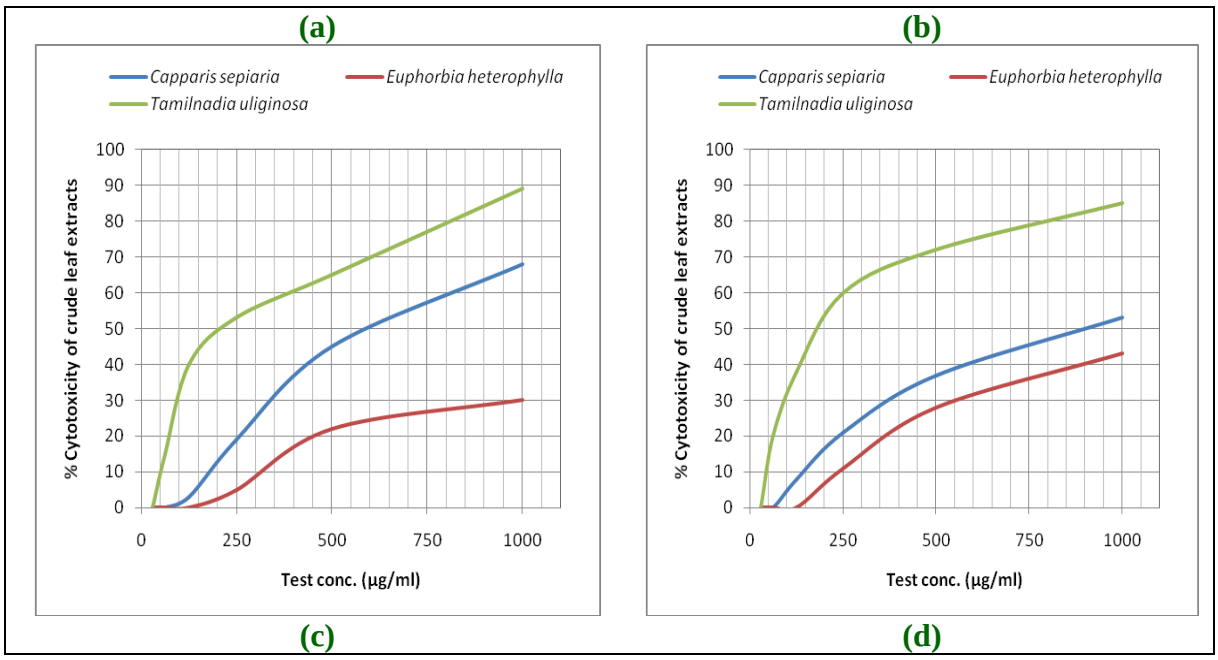
The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% ($CTC_{50:}$ Cytotoxic Concentration 50) values were generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Results and Discussion

The cell viability test against different cell lines with ethanolic leaf extracts of *Capparis sepiaria*, *Euphorbia heterophylla* and *Tamilnadia uliginosa* showed variations in activity (Fig. 1). The viability of the cell lines was found to decrease towards the increase of concentration of the leaf extracts. Among the three different extracts tested against HeLa cervical cancer cell line, MCF-7 human breast adenocarcinoma cell line, A-549 Human lung adenocarcinoma epithelial cell line, and vero normal cell line, *Tamilnadia uliginosa* leaf extracts showed higher activity than in *Euphorbia heterophylla* and *Capparis sepiaria*. The least cytotoxic activity was found in *Euphorbia heterophylla* against all the cell lines tested. The percent cytotoxic inhibitory activity of 96, 78, 63, 42, 21 and 0 against A-549 cell lines was observed in the ethanolic leaf extracts of *Tamilnadia uliginosa* at 1000, 500, 250, 125, 62.5 and 31.25 µg/ml concentrations, respectively.

Fig.1 Cytotoxic activity of ethanolic crude extracts of selective medicinal plants against (a) HeLa cell line, (b) MCF-7 cell line, (c) A-549 cell line and (d) Vero cell line



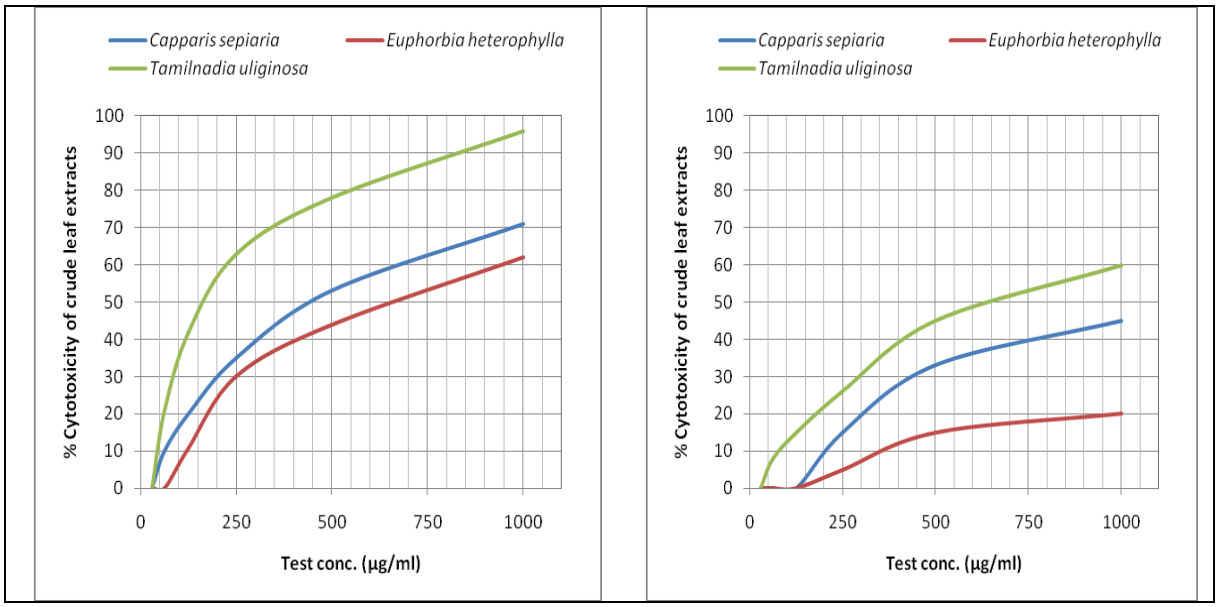


Table.1 CTC_{50} activity of selected medicinal plant extracts against cell lines

Sl. No.	Ethanolic leaf extract used	CTC_{50} (µg/ml)			
		HeLa cell line.	MCF-7 cell line	A-549 cell line	Vero cell line
1.	*Capparis sepiaria*	576.5	891.2	463.6	<1000
2.	*Euphorbia heterophylla*	<1000	<1000	654.8	<1000
3.	*Tamilnadia uliginosa*	208.2	179.4	164.3	673.1

The CTC_{50} values observed in the leaf extracts of *Tamilnadia uliginosa* was 208.2, 179.4, 164.3 and 673.1 µg/ml against HeLa, MCF-7, A-549 and vero cell lines respectively (Table 1). Against normal cell lines (vero cells), the leaf extracts of *Tamilnadia uliginosa* showed very low cytotoxic activity (673.1 µg/ml); whereas, the ethanolic leaf extracts of *Euphorbia heterophylla* and *Capparis sepiaria* showed a CTC_{50} above 1000µg/ml. Cytotoxic compounds from plant sources are one of the vital classes of drugs used for cancer treatment.

There have been several researches to get new cytotoxic agents from natural resources. The present study is attempted to identify the cytotoxic compound present in the selected medicinal plants, *Capparis sepiaria*, *Euphorbia heterophylla* and *Tamilnadia uliginosa*. The ethanolic crude extracts of selected medicinal plants conifers the presence of cytotoxic compounds or tumor degrading substances. The plants possess compounds known to inhibit a series of human cancer lines- Hela (cervical cancer), MCF-7 (Human breast adenocarcinoma) and A-549 (Matsukawa *et al.*, 1993, Duker-Eshun *et al.*, 2004; Pater and Pater, 1985). The results showed a potent cytotoxicity effect (CTC_{50}) of *Tamilnadia uliginosa* leaf extract against A-549 cell line in lowest concentration of 164.3µg/ml. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC50 value ≥30 µg/ml (Suffness and Pezzuto, 1999). In this point of view the plant is potent to inhibit cell viability. This study

implies vital basis for further studies into the isolation, characterization and mechanism of cytotoxic compounds from crude extract.

In vitro cytotoxicity studies revealed that ethanolic extracts of leaves of *Tamilnadia uliginosa* exhibited cytotoxicity towards A-549 cell line with CTC_{50} value 164.3 µg/ml, which was found to be the lowest CTC50 concentration among the extracts tested. The observations in the present study indicate the specific cytotoxic nature of ethanol extract of *Tamilnadia uliginosa* leaves against cancerous A-549 cell line.

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