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Original Article

INVITRO CYTO TOXICITY OF *TRICHODESMA INDIUM* LINN.R.BR., EXTRACTS AGAINST THREE HUMAN CANCER CELL LINES.

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ABSTRACT

Objectives: *Trichodesma Indicum* Linn.R.Br. of Boraginaceae family was traditionally used as an anticancer plant material. The present study was aimed to evaluate the cytotoxic activity of the plant against three human cancer cell lines-Breast cancer cell line (MCF-7), Cervical cancer cell line (HeLa) and laryngeal epithelial carcinoma cell line(HEp2),since a few clinical trials are needed to confirm its anticancer activity.

Methods: The chloroform& ethanol extracts of the plant were used for in-vitro screening. The cytotoxic effect of the plant was analysed by MTT assay.

Results: The results obtained are statistically analysed by two way analysis of Variance(ANOVA)and it was found that the ethanol extract has stronger cytotoxic activity than chloroform extract against all the tested cell lines. However higher degree of cyto toxicity was found to be in ethanol extract against human breast cell line (MCF-7) (IC_{50} 57.41µg/ml).

Conclusion: The traditional use of the plant material as anticancer was correlated with its invitro cyto toxicity results. The plant has a remarkable cytotoxic activity against the tested cancer cell lines.

Keywords: Boraginaceae, Cytotoxic activity, IC_{50} value, MTT assay, *Trichodesma Indicum.*

INTRODUCTION

Over the past decades, herbal drugs have been accepted universally for the treatment of a range of human diseases. Conventional medicine is widely used in many developing countries like India. Use of herbal products has increased dramatically in the last two decades[1]. According to WHO, 80% of world's health problems are treated by herbal drugs only[2,3]. Medicinal plants can be promising source of noval chemotherapeutic agents including cancer. Out of 25,000 plant species existing on the earth, approximately one thousand species have anticancer potential[4]. A large number of plant species have been screened through bioassays for the search of noval herbal anticancer drugs[5]. Several medicinal plants all over the world are being used traditionally for the prevention and treatment of cancer. However, only few medicinal plants have attracted the interest of scientist in investigating the remedy for neoplasm (tumour or cancer)[6].

Trichodesma indicum Linn, R.Br. is a perennial herb, commonly known as Adhapushpi that is, flowers are bent downward[7]. It is distributed in India, Burma and Pakistan[8]. It belongs to the family Boraginaceae. In general, it is called as Indian borage. It is an annual herb with pale blue flowers, found as weed throughout the greater part of India. The whole plants and roots are reportedly used to treat arthritis, anorexia, dysentery, skin diseases, snakebite poisoning and fever[9]. In ayurveda, the plant is beneficial for diseases of the eye. It is also prescribed for expulsion of the dead fetus[10]. The plant is useful in vitiated conditions of Vata and kapha, arthralgia, inflammations, dyspepsia, dysentery and leprosy[11].

Some of the constituents of the plant have been identified so far are non-steroidal compounds-Hexacosane,Ethyl hexa cosanate and hexacosa dienoicacid ethyl Esters from leaves[12,13] and oleic, linoleic,palmatic and steric and linoenic acid from seed oil[14].It inhibits diarrhea[15] and sulphur dioxide induced cough reflux in mice[16].In Chhattisgarh state, tribal people are using it for the treatment of breast cancer[12] which makes this study to carry out the analysis of the plant extracts against human cancer cell lines-breast cancer cell line(MCF-7), Cervical cancer cell line (HeLa) and laryngeal epithelial carcinoma cell line(HEp2).

Breast cancer is the most common form of cancer in women worldwide [17].Cervical cancer is the second most common cancer among women worldwide as reported in 2002[18].The HEp2 cell line has been used for experimental studies of tumor protection in rats, hamsters, mice, embryonated eggs and terminal cancer patient volunteers.HEp2 cell line has a high proliferation rate and a 23hours cell cycle[19]. The present study is carried out to determine the possible cyto toxic action of the chloroform and ethanol extracts of the selected plant.

MATERIALS AND METHODS

Collection of the Plant material: The plant *T. indicum Linn,* R.Br is collected during the month of January-February 2013 from Anaimalai hills region of Western Ghats, South India and is authenticated by Botanical survey of India, Coimbatore. A voucher specimen is preserved there with the specimen number: 11CH213.

Chemical used: All the chemicals used were of A.R grade (Merck brand).

Preparation of Extraction: The plant Extracts were prepared by maceration method. The aerial parts of the plant were washed, air-dried for two days. The dried plants parts were ground into coarse powder. About 1 kilogram of the plant powder were defatted by soaking in petroleum ether for about 48hrs.The solvent was removed under reduced pressure at a controlled temperature of 40-50^{0}C by rotary evaporator. The extract collected was again macerated with chloroform and ethanol respectively. The maceration procedure was repeated 3 times for 48hrs for each extract. Then the extracts were vacuum-evaporated and stored in vacuum desiccator.

Collection of Cancer cell lines: The human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa) and laryngeal epithelial human cell line(HEp2) were obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum

Essential Medium (EMEM) containing 10% fetal bovine serum (FBS)[20,21]. All cells were maintained at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure: The monolayer cells were detached with trypsin-ethylene diamine tetra acetic 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 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. After 24 hrs the cells were treated with serial concentrations of the test samples. They were initially dissolved in dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold 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, resulted the required final sample concentrations. Following drug addition, the plates were incubated for an additional 48 hrs at 37^0 C, 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.

MTT Assay: 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow colored 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 was directly proportional to the number of viable cells which was measured spectro photometrically[20,22]. Since the reduction in MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.5-Fluorouracil was used as a positive control. Control cells were treated with the highest concentration of DMSO (0.1%) as vehicle control.

After 48hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^{0}C for 4hrs. The medium with MTT was then flicked off and the formed formazan crystals are solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula[20,22-24].

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Statistical analysis: The Results of the study were based on experimental values that were performed in triplicate. The statistical analysis was performed using the analysis of variance (ANOVA) to determine the effect of extracts concentration on treated cells cyto viability. Non linear regression graph was plotted between % Cell inhibition and Log_{10} concentration. The IC_{50} was determined using Graph Pad Prism software (version 3.00).

RESULTS

The cell viability of the two extracts against 3 cell lines were reported in fig1&2, graphically by plotting % of cell viability against concentration of the extract. The IC_{50} values determined were tabulated (Table:1). Further statistical analysis by ANOVA was shown in tables1&2.

Table 1: ANOVA summery of the effect of Chloroform extract concentration on the tested cancer cell lines

H_0: There is no significant relationship between concentration & cell viability. H_1: There is a significant relationship between concentration & cell viability.

	df	sum of squares	mean square	F
Between groups	4	11185.87	2796.47	115.99
Within groups	11	265.5	24.11	

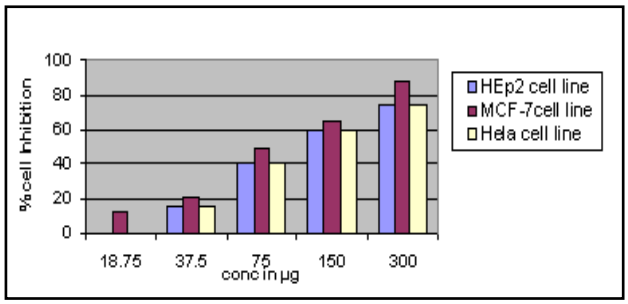

Fig.1: MTT assay of $CHCl_3$ extract against 3 cell lines:

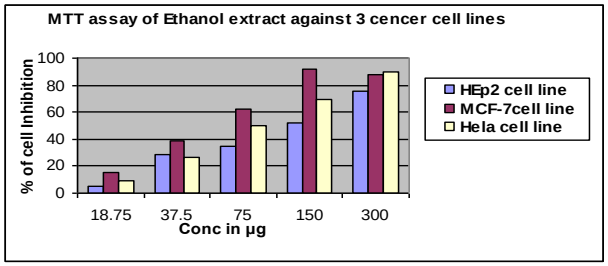

Fig.2: MTT assay of Ethanol extract against 3 cell lines :

Table 2: ANOVA summery of the effect of Ethanol extract concentration on the tested cancer cell lines:

	df	Sum of squares	mean square	F
Between Groups	4	11185.87	2713.29	27.02
Within Groups	11	1104.41	100.40	

Table 3: IC_{50} values of *Trichodesma Indicum*Linn.R.Br., extracts :

S.No	Extract	IC_{50} value(µg/ml)		
		HeLa	HEp2	MCF-7
1.	$CHCl_3$ extract	115.3	123.9	87.09
2.	Ethanol extract	78.52	143.8	57.41
3.	5-Fluorouracil (standard Drug)	0.50	0.30	0. 38

DISCUSSION

The present study evaluated the cytotoxic effect of chloroform and ethanol extracts of *T. indicum* Linn. R.Br, by performing MTT assay which is a simple and reliable technique. The cell viability of the two extracts against 3 cell lines were reported in fig1&2, graphically by plotting % of cell viability against concentration of the extract. From the data, it has found that incubation with different concentrations of the extract affects the viability of the cell lines.

IC_{50} values of the plant extract was found to be 87.09µg/ml for chloroform extract against MCF-7 cell line and 57.4109 µg/ml for ethanol exact against the same cell line. The best potential was obtained at certain optimal concentration of the extract. It has been exhibited that the plant appeared to have a potential cytotoxic activity. A common cancer drug 5-Fluorouracil was used as a standard.

Further statistical analysis by ANOVA was shown in tables1&2. $P < 0.05$ was set to be the limit of significance. From the ANOVA tables, it was inferred that there is a significant influence of both the treated extracts concentrations on the cyto viability of the tested human cancer cell lines, since the calculated value is higher than the table value, H_1 is accepted and H_0 is rejected. (Tables1&2).

The potential use of *T. indicum* Linn.R.Br, as therapeutic agent holds a great promise as the isolation of one or more cytotoxic chemicals

from the crude extract and the judicious use of such chemicals can control the progression of cancer.

CONCLUSION

The present study concluded that the chloroform &ethanol extracts of *T. indicum,* Linn. R.Br, have shown a potential cyto toxic activity against all the tested human cancer cell lines. Sensitivity varied according to the cell lines. The IC_{50} values showed that the ethanol extract has a significant anticancer activity particularly against human breast cancer cell line (MCF-7). The cytotoxic activity of the plant is correlated with its traditional use in folk medicine. It is vital to explore the medicinal properties of herbal products used traditionally by practitioners in Indian medicine. This can be followed by carefully carried out control clinical trials. Further investigations are needed to prove some additional insight into the *in vivo* cytotoxic activity of the plant extracts with a view to obtain remarkable chemotherapeutic agents.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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