



ISSN: 0975-766X
Research Article

Available Online through
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ANTICANCER ACTIVITY OF CHLOROFORM EXTRACT OF *HELICTERES ISORA*

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Received on 29-03-2011

Accepted on 15-04-2011

Abstract

Helicteres isora belongs to the family Sterculiaceae is an ayurvedic herb .It is distributed widely in forest throughout India and are commonly known as East India Screw tree ¹, is a medicinally important sub-deciduous shrub or small tree .Almost all parts of the plant are used in traditional medicinal system for curing various diseases. Its fruit, root and bark possess significant antidiabetic, antioxidant, antimicrobial and hypolipidemic activity. The fruit of *Helicteres isora* after collection, authentication and drying was extracted with chloroform using soxhlet extractor.The present study was aimed to determine the preliminary phytochemical evaluation and cytotoxicity study of the chloroform extract of *Helicteres isora* on MCF – 7 cell line (Human breast cancer) by MTT assay method. The preliminary phytochemical evaluation showed the presence of carbohydrates, flavonoids, tannins and alkaloids².Its cytotoxic evaluation showed a potent action against to MCF – 7 (Michigan Cancer Foundation - 7) cell line.

Keywords: Cytotoxicity, *Helicteres isora*, MCF 7 cell line, MTT assay method.

Introduction

WHO has estimated that about 80% of world's population depends on traditional medicines for meeting their primary health care needs. The areas of cancer have a leading position in utilization of medicinal plants as source of drug discovery. 60% of FDA approved anticancer preparations are natural drugs. Cancer is still a major cause of mortality and morbidity in developing as well as developed countries. Molecular targeted agents are currently being studied in all treatment settings including the chemoprevention. *Helicteres isora* Linn (*sterculiaceae*) is a shrub or small tree available in forests throughout central and western India. In traditional

medicine the root juice is used in diabetes. The roots and bark are expectorant and demulcent .Fruits are astringent, stomachic, and have antispasmodic effect. In this study the fruit extract was used to determine its activity against human breast cancer.

Materials and Methods

The plant was collected from Tamilnadu in the month of september. It was authenticated by taxonomist. The fruits of the plant were dried and powdered .The dried powder was extracted and it is used for further investigation.

Extraction Process

50 gm of dried powdered drugs of *Helicteres isora*³ was extracted with chloroform by soxhlation process⁴. The dried extracts were used for phytochemical analysis and cytotoxic activity.

Priliminary phytochemical screening

Various chemical test⁵ was performed as per the standard procedure. It showed the presence of carbohydrates, flavonoids, tannins and alkaloids

Cell lines and culture medium

MCF-7 (Human, breast cancer) cell culture⁶ was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of MCF-7 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% CO₂ 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 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solution

Each weighed test drug was 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 carrying out cytotoxic studies⁷.

Determination of cell viability by mtt assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 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 μ l of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO₂ 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 μ l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO₂ atmosphere. The supernatant was removed and 100 μ l of propanol was added and the plates were gently shaken to solubilize the formed formazan⁸. 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% (CTC₅₀) values is generated from the dose-response curves for each cell line.

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

RESULT AND DISCUSSION

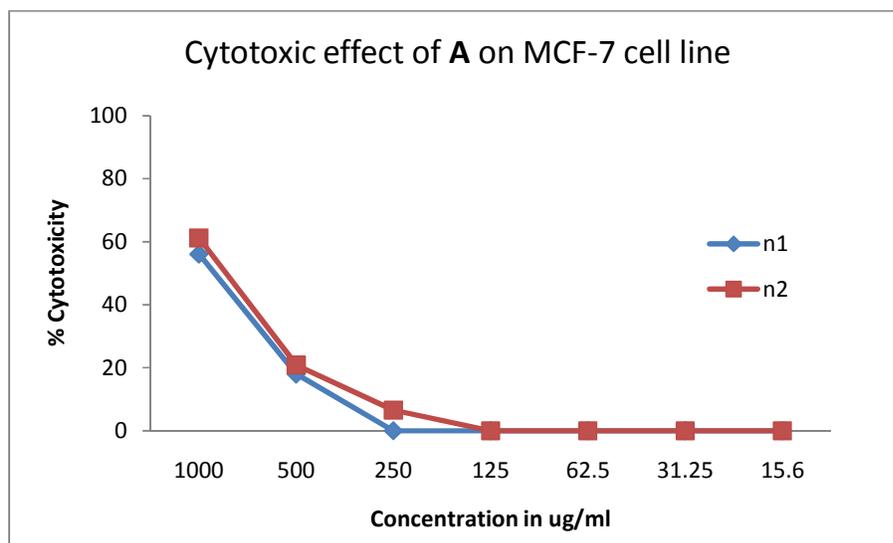
The preliminary phytochemical study showed the presence of carbohydrates, flavonoids, tannins and alkaloids. The drug was showed a potent cytotoxicity towards MCF-7 cell. The test drug exhibited cytotoxicity with CTC₅₀ values 875.00 ± 35.3 . So the extract have a potent action against to human breast cancer.

Table: Cytotoxic property of test drug on MCF-Cell line.

Sl. No	Name of Drug	Test Conc. (μ g/ml)	% Cytotoxicity		CTC ₅₀ (μ g/ml)		Average CTC ₅₀ (μ g/ml) \pm SEM
			n1	n2	n1	n2	

1	CHLOROFORM EXTRACT OF <i>HELICTERES</i> <i>ISORA</i>	1000	56.08	61.25	900.00	865.00	875.00 ± 35.3
		500	17.99	20.85			
		250	0.00	6.50			
		125	0.00	0.00			
		62.5	0.00	0.00			
		31.25	0.00	0.00			
		15.6	0.00	0.00			

Figure: Graphical representation of cytotoxic effect of drugs.



Conclusion

The drug has a potent action against human breast cancer. The cytotoxic activity of the drug is due to the presence of alkaloids and flavonoids. Our further plan is to isolate and evaluate these active principles and elucidate exact mechanism of action.

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