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## ***In vitro* Cytotoxic Evaluation of *Hugonia mystax* Linn. Leaf and Stem Bark Extracts**

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**Abstract:** In the present study, the methanol extracts of *Hugonia mystax* Linn. leaves and stem bark were tested for cytotoxic effect against three different human cancer cell lines, HeLa cervical cancer cell line, MCF-7 human breast adenocarcinoma cell line and A-549 human lung adenocarcinoma epithelial cell line, in comparison with normal vero cell line by MTT assay. Results revealed that the methanolic extracts of leaves stem bark exhibited cytotoxicity towards the cancerous cell lines. CTC<sub>50</sub> concentration of 228.12, 181.01, 343.75 and 875.0 µg mL<sup>-1</sup> against HeLa, MCF-7, A-549 and vero cell lines was recorded. Methanolic stem bark extract showed the lower CTC<sub>50</sub> concentration against HeLa and MCF-7 cell lines and higher CTC<sub>50</sub> concentration against A-549 and vero cell lines. The results indicated us the feasible anticancer nature of the leaf and bark methanolic crude extracts of *H. mystax*.

**Key words:** Cytotoxicity, plant extracts, *Hugonia mystax*, CTC<sub>50</sub>, MCF-7

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### **INTRODUCTION**

Medicinal plants are important source of valuable drugs. Traditional systems of medicines continue to be widely practiced on many accounts. In recent years, discovery of novel therapeutic agents of origin has been targeted. The beneficial effect of compounds from natural sources attributes among other things to the high content of bioactive compounds (Rafter, 2002). The cytotoxic effects of medicinal herbs and their phyto-constituents against different cell lines are the evidences of positive utility of medicinal plants for variety of ailments (Uddin *et al.*, 2003; Aini *et al.*, 2008; Sunilson *et al.*, 2009; Siddiqui *et al.*, 2010; Aisha *et al.*, 2011). The Linaceae is a relatively small plant family that consists of only three genera, among which is the genus *Hugonia* (Hutchings, 1996). About 30 of whose 34 species of *Hugonia* are found in the tropical regions of continental Africa, Madagascar and Mauritius. Some of these species, including *H. castaneifolia* are used as herbal remedies (Hutchings, 1996). Some *Hugonia* species yielded lignans related to the antitumour agent podophyllotoxin expectant a few years ago to investigate the root bark of *H. castaneifolia* that occurs in East Africa, for cytotoxic and other constituents. *H. mystax* is distributed in tropical Asian countries, often in isolated patches. It is found in different habitat such as foot hills, dry and wet lands. Native plants are distributed in southern part of India and Sri Lanka Malaysia, Indonesia, the West Indies

and world wide. *H. mystax* is scrambling shrub with spreading branches, set with numerous short stiff woody tendrils (modified peduncles, sometimes bearing flowers).

Many natural chemopreventive agents are capable of protecting against some forms of human cancer (Rajeshwar *et al.*, 2005; Saha *et al.*, 2011; Aisha *et al.*, 2011). 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. Several medicinal plants including *Hugonia* species are providing several drug components including cytotoxic characteristics. In the field of anticancer drug research several higher plant compounds are shown to possess *in vitro* and *in vivo* antitumor activity. The results of the screening of plant extracts for antiproliferative activity have shown that higher plants are a potential source of antioncogenic agents which can participate favorably with chemotherapy and hormonal treatments. As far as the literature is concerned, the cytotoxicity studies with *H. mystax* extracts is unavailable. Hence the present study has been carried out with the objectives to determine *In vitro* cytotoxicity activities of the methanolic crude extracts of *H. mystax* leaf and stem bark against HeLa cervical cancer cell line, MCF-7 human breast adenocarcinoma cell line and A-549 human lung adenocarcinoma epithelial cell line.

## MATERIALS AND METHODS

**Plant collection:** The leaves and stem barks of *Hugonia mystax* L. (Fig. 1) were collected from foothills of Sirumalai hills, Dindigul District, Tamil Nadu, India and the plant specimen was identified by Dr. S. Karuppusamy, Department of Botany, Madura College, Madurai, Tamil Nadu. The identification was confirmed with Botanical Survey of India, Coimbatore, Tamil Nadu, India (Ref. No: BSI/SRC/5/23/10-11/Tech-1522). The leaves and stem bark were collected and shade dried for a week and powdered using mortar and pestle. A fine powder obtained was stored in air tight polythene bags and used for preparation of extract.

**Preparation of extract:** For methanol extraction, the powdered plant materials, leaf and stem bark, weighing 100 g each was taken in a thimble of Soxhlet apparatus and kept on round bottom flask containing 250 mL of methanol. The extraction was done for 24 h and finally methanol was separated. The final greasy crude extract was collected, air dried and stored in refrigerator until use.

**In vitro cytotoxicity studies:** Following were the chemicals used in the present study. 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 and Dimethyl Sulfoxide (DMSO) and propanol from EMerck Ltd., Mumbai, India.

**Cell lines and culture medium:** HeLa (Epithelial cervical cancer, Human), human breast carcinoma MCF-7, human lung adenocarcinoma cell culture, 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 MEM (excepting A-549 and vero cell lines which were cultured in DMEM) supplemented with 10% inactivated FBS, penicillin ( $100 \text{ IU mL}^{-1}$ ), streptomycin ( $100 \mu\text{g mL}^{-1}$ ) and amphotericin B ( $5 \mu\text{g mL}^{-1}$ ) in an humidified atmosphere of 5%  $\text{CO}_2$  at  $37^\circ\text{C}$  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  $25 \text{ cm}^2$  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 Methanolic Leaf (MLE) and stem bark (MBE) extracts were separately dissolved in distilled



Fig. 1(a-b): The plant used in the study-*H. mystax* L. showing (a) vegetative twig with hooks and (b) a fruiting branch

DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of  $1 \text{ mg mL}^{-1}$  concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carried out cytotoxic studies.

**Determination of cell viability by MTT assay:** 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 (Denizot and Lang, 1986). The monolayer cell culture was trypsinized and the cell count was adjusted to  $1.0 \times 10^5 \text{ cells mL}^{-1}$  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°C for 3 days in 5% CO<sub>2</sub> 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°C in 5% CO<sub>2</sub> 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<sub>50</sub>: Cytotoxic Concentration 50) values is generated from the dose-response curves for each cell line. The results obtained were subjected to the analysis of variance at p<0.05 using SPSS computer software.

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

**RESULTS AND DISCUSSION**

The cytotoxicity was concentration dependent for all the cell lines used. The highest cytotoxicity was 95.45% observed in 500 g mL<sup>-1</sup> MLE against A-549 followed by MCF-7 (82.78%). Whereas, vero cell lines showed least cytotoxicity (58.67%) and it was significantly (p<0.05) lower from the cytotoxicity levels recorded for HeLa,

MCF-7 and A-549 cell lines (Table 1). Cytotoxicity was not observed in 31.25 µg mL<sup>-1</sup> MLE against HeLa cell lines, 15.60 µg mL<sup>-1</sup> MLE against MCF-7 cell lines, 62.50 µg mL<sup>-1</sup> MLE against A-549 and vero cell lines. CTC<sub>50</sub> of MLE against tested cell lines is shown in Fig. 2. The lowest CTC<sub>50</sub> concentration of MLE against MCF-7 cell line was 181.01 µg mL<sup>-1</sup> followed by 228.12 µg mL<sup>-1</sup> (HeLa)<343.75 µg mL<sup>-1</sup> (A-549)<875.0 (vero cell line).

The highest cytotoxicity of 81.85% in 500 µg mL<sup>-1</sup> MBE was recorded against MCF-7, followed by HeLa (77.02) and A-549 (72.85), respectively (Table 2). Similar to MLE, MBE also showed concentration dependent cytotoxicity against different cell lines. The least cytotoxicity recorded for MBE was 62.50 µg mL<sup>-1</sup> for HeLa, 31.25 µg mL<sup>-1</sup> for MCF-7 and vero cell lines and 250 µg mL<sup>-1</sup> for A-549. The lowest cytotoxicity of MBE at 500 µg mL<sup>-1</sup> was recorded against vero cell lines (38.85%)

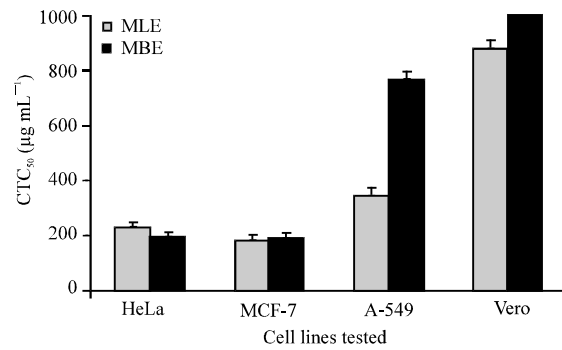


Fig. 2: CTC<sub>50</sub> (Cytotoxic Concentration 50) of methanolic leaf (MLE) and stem bark (MBE) extracts of *H. mystax* L. Error bars indicate ±SD

Table 1: Cytotoxic effect of methanolic leaf (MLE) extracts of *H. mystax* L. on HeLa, MCF-7, A-549 and vero cell lines

Test concentration (µg mL <sup>-1</sup> )	% Cytotoxicity against cell lines			
	HeLa	MCF-7	A-549	Vero cell
500	76.41±1.03 <sup>c</sup>	82.78±2.29 <sup>b</sup>	95.45±1.22 <sup>a</sup>	58.67±3.66 <sup>d</sup>
250	54.29±2.85 <sup>c</sup>	76.66±2.93 <sup>b</sup>	83.41±0.63 <sup>a</sup>	19.42±2.02 <sup>d</sup>
125	32.28±1.21 <sup>b</sup>	61.81±2.14 <sup>a</sup>	29.91±0.22 <sup>b</sup>	3.25±4.60 <sup>c</sup>
62.50	29.96±1.09 <sup>b</sup>	44.12±5.52 <sup>a</sup>	-	-
31.25	-	29.81±0.60	-	-
15.60	-	-	-	-

Values are Mean±SE. Same letters in values between columns did not differ significantly at p<0.05 by ANOVA

Table 2: Cytotoxic effect of methanolic stem bark (BME) extracts of *H. mystax* L. on HeLa, MCF-7, A-549 and vero cell lines

Test concentration (µg mL <sup>-1</sup> )	% Cytotoxicity against cell lines			
	HeLa	MCF-7	A-549	Vero cell
500	77.02±2.10 <sup>a</sup>	81.85±0.64 <sup>a</sup>	72.85±2.12 <sup>b</sup>	38.85±0.83 <sup>c</sup>
250	52.68±4.66 <sup>b</sup>	79.39±0.64 <sup>a</sup>	25.57±1.43 <sup>c</sup>	29.35±0.25 <sup>c</sup>
125	43.92±6.36 <sup>b</sup>	67.76±0.68 <sup>a</sup>	-	27.07±1.11 <sup>c</sup>
62.50	36.47±4.23 <sup>a</sup>	38.79±0.70 <sup>a</sup>	-	19.95±0.08 <sup>b</sup>
31.25	-	30.05±0.23 <sup>a</sup>	-	16.33±1.68 <sup>b</sup>
15.60	-	-	-	-

Values are Mean±SD. Same letters in values between columns did not differ significantly at p<0.05 by ANOVA

which was significantly lower ( $p < 0.05$ ) than the values recorded for HeLa, MCF-7 and A-549. The average  $CTC_{50}$  for MBE was higher than  $1000 \mu\text{g mL}^{-1}$  for vero cell lines, whereas, it was least in MCF-7 ( $187.52 \mu\text{g mL}^{-1}$ ) followed by HeLa ( $191.02 \mu\text{g mL}^{-1}$ ) and A-549 ( $762.25 \mu\text{g mL}^{-1}$ ) (Fig. 2).

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 an attempt to identify the cytotoxic compounds present in the selected medicinal plant *H. mystax*. The crude extracts of selected medicinal plants confer the presence of cytotoxic compound or tumor degrading substances in many occasions. A wide range of such kind of medicinal plants from Asia has been reviewed recently by Hafidh *et al.* (2009) and Bisht *et al.* (2011), implies the scope of utilizing plant resources for anticancer drugs. The compounds from medicinal plants have been shown to inhibit a series of human cancer lines HeLa, MCF-7 and A-549 (Matsukawa *et al.*, 1993; Duker-Eshun *et al.*, 2004). It has been reported that hexane and dichloromethane extracts of root bark of *H. casteneifolia* exhibit strong activity against brain shrimp (Meyer *et al.*, 1982). The fact that previously some *Hugonia* species yielded lignans related to the antitumour agent podophyllotoxin (Konuklugi, 1996). The plants are the best resources possessing As best of knowledge in *H. mystax* species cytotoxicity work is not found. The result shows a potent cytotoxicity effect against MCF-7 cell line with lowest concentration of  $181 \mu\text{g mL}^{-1}$  MLE. A closer range of  $IC_{50}$  concentration of  $170 \mu\text{g mL}^{-1}$  crude methanolic extracts of *Cayratia carnos* leaves against MCF-7 has been reported by Sunilson *et al.* (2009). Siddiqui *et al.* (2010) reported that vindoline and catharanthine from *Catharanthus roseus* showed 200 and  $60 \mu\text{g mL}^{-1}$  of  $IC_{50}$  values, respectively against human colorectal carcinoma cell line. Even purified compounds are more effective at lower doses as reported by Gansane *et al.* (2010) who observed that the semi purified fractions of *Zanthoxylum zanthoxyloides* at  $IC_{50}$  range of  $1.91$  to  $4.32 \mu\text{g mL}^{-1}$  showed good cytotoxic activity. The present study implies vital basis for further studies into the isolation, characterization and mechanism of cytotoxic compounds from crude extracts of *H. mystax*.

## CONCLUSION

*In vitro* cytotoxicity studies revealed that the methanol extract of *H. mystax* leaves and stem bark possess potent cytotoxicity towards MCF-7 cell line with  $CTC_{50}$  value  $181.01$  and  $187.52 \mu\text{g mL}^{-1}$ , respectively.

Methanolic stem bark extract of *H. mystax* showed poor cytotoxicity against vero cell lines with  $CTC_{50}$  value above  $1000 \mu\text{g mL}^{-1}$ . These observations indicate the specific cytotoxic nature of methanol extract of *H. mystax* against cancerous MCF-7 cell line.

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