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ANTICANCER ACTIVITY OF WATER HYACINTH [*EICHHORNIA CRASSIPES* (MART) SOLMS] ON HUMAN CERVICAL CANCER CELL LINE

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Abstract: *Eichhornia crassipes* (Mart) Solms commonly known as water hyacinth is native to Brazil. It is one of the most productive plants on earth and is considered the world's worst aquatic weed which causes serious environmental issues. In the current study the methanol extract of this plant have been tested for anticancer activity. The *in-vitro* anticancer studies were performed against human cancer cell line (HeLa). MTT assay was used to analyze the cell growth inhibition. The methanol extract of *E. crassipes* resulted in mild anticancer activity on HeLa cell line.
Keywords: Aquatic weed, *Eichhornia crassipes,* Anticancer, HeLa cell lines, leaf extracts.
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INTRODUCTION

Cancer is the second leading cause of death worldwide. Conventional cancer therapies cause serious side effects and hence there is a demand for utilising alternative source. Healing with plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past. There is evidence that Phytochemicals reduce the risk of colon cancer and inhibit spread of tumours in experimental animals. More than 3000 plant species have been reportedly used in the treatment of cancer. Aqueous fraction of *Eichhornia crassipes* leaf exhibited 44% cytotoxic potential against NCI-H322 (lung) cell line and 20–31% cytotoxic activity against T47D (breast) cell line (Kumar *et al*., 2014). It is evident that the crude extract of *E. crassipes* showed highly significant effect than the isolated compounds. Some fractions of the extract exhibited very potent anticancer activity against liver cancer cell line while other fractions exhibited high anticancer activity against hormone dependent tumor types (breast cancers) (Aboul-Enein *et al.,* 2011). *E. crassipes* is said to be the most productive plant on earth and a worst aquatic weed causes severe environmental problems. It is an eye opening for us to study on anticancer activity of methanol extract of *E. Crassipes* on human cervical cancer cell lines, He La. Water hyacinth, *E. crassipes* is native to South America, one of the world's worst aquatic weeds (Hill *et al*., 2011). Due to its high noxious nature, it has been enlisted in 100 of the world's worst invasive alien species (Lowe *et al*., 2000). This weed is an aquatic macrophyte, a monocotyledon and belongs to the family Pontederiaceae. This aquatic plant is free floating with beautiful clusters of violet and yellow flowers and bulbous green leaves. Its rapid growth rate and high adaptability to extreme conditions contributes to its high degree of invasion (Hill *et al*., 2011). It is particularly dominant in the tropics and subtropics due to improper waste water management in these areas (Villamagna and Murphy, 2010). It spreads in the form of dense mats due to its complex root system and thus

interfere with boat navigation, fishing and also leads to blockage of canals (Malik, 2007). Once introduced in an area it can easily compete with native vegetation and thus is very difficult to eradicate or control (Villamagna and Murphy, 2010). Various programmes on management of water hyacinth are being run throughout the globe, but no effective control strategy has been developed till date. However, Water hyacinth has long been used to treat Goitre in India. The formulation contains water hyacinth in equal quantity with table salt and *Piper congum* (Oudhia, 1999). Ogunlesi *et al*. (2010) extracted vitamin C from water hyacinth and its relevant medicinal use like skin care. Thus the effective utilization of water hyacinth with respect to Bioprospecting values is warranted; hence study has been conducted to understand the anticancer potential of water hyacinth against cervical HeLa cell lines.

EXPERIMENTAL

Collection of plant material and extraction: Leaves of *E. crassipes* were collected from Kurichi Kulam (Kurichi lake), Coimbatore city, Tamil Nadu, India. It is situated between 10°57'57.6"latitude and 76°57'48.96" longitude. Kurichi Kulam in Coimbatore city is one of the major water bodies enhances the ground water level around this area. It is often polluted due to invasion of alien weed, water hyacinth. The Coimbatore city municipal corporation made efforts to remove them periodically with considerable budget expenditure. Hence attempt was made to use it against cancerous cells to check its in-vitro anti-cancerous activity. The whole plant was removed from the lake and brought into the laboratory at Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamilnadu. The aerial parts of the plant were washed thoroughly under running tap water. The leaves of the fresh plant material was chopped into small pieces and air dried. The aerial parts of the plant were dried, ground, extracted with methanol in heating mantle using soxhlet apparatus. After completion of extraction, the solvents were removed by distillation in rotary evaporator. The crude extracts were fractioned using methanol. The extracts were concentrated to dryness at reduced pressure. Fractionates of the extracts were refrigerated for future use (Dantu *et al*., 2012).

***In-vitro* interpretation of anticancer activity by MTT assay**

Cell line: The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune, Maharashtra, India and grown in Eagles Minimum Essential Medium containing 10% Fetal Bovine Serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity, and the culture medium was changed twice a week.

Cell treatment procedure: The monolayer cells were detached with trypsin-ethylenediamine 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 allowed to incubate for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. Five sample concentrations viz. 12.5, 25, 50, 100 and 200 µg/mL were obtained by adding 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. After addition of sample, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity (Dantu *et al*., 2012). 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 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, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then discarded and the formed formazan crystals were solubilised in 100µL of DMSO and then measured the absorbance at 570 nm using micro plate reader (Thavamani *et al*., 2013). The percentage cell viability was then calculated with respect to control as follows:

% Cell viability = [A] Test / [A]control x 100

Statistical analysis: The absorbance values were denoted as mean ± SE. 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 by probit analysis using SPSS software (version 16).

RESULTS AND DISCUSSION

The result for cell growth inhibition by the extract against HeLa cell lines for various concentrations was given in the Table 1. As the concentration of the extract increases the cell growth inhibition also increased and it was found to be with 17% growth inhibition at 200µg/mL (Figure 1). Choudhury *et al*. (2016) were found the anticancer activity of *Garcinia morella* at 200µg/mL on T-Cell Murine Lymphoma. The results obtained in the present study showed that methanol extract of *E. crassipes* had a very moderate anticancer activity (Figure 2). This phenomenon has been supported by a number of studies such as, various solvent extracts of root of *Clerodendrum phlomidis* tested on Mouse embryonic fibroblasts cell line (NIH 3T3) (Kumar *et al.,* 2014) and flower of *Tabernaemontana divaricata* against HeLa cell lines (Dantu *et al*., 2012) using MTT assay. Alcoholic extract of *C. phlomidis* and *T. divaricata* had weak cytotoxic activity on both the cell lines (Kumar *et al*., 2014). Fridlender *et al*. (2015) reported plant derived anticancer substances. Mild cytotoxic activity has been described for *C. spinosa* and *C. ambrosioides* (Hmamouchi *et al*., 2000). In an another study four trifoliate plant extracts in different solvents were tested for cytotoxic activity against HeLa cell lines and MCF7 cell lines and extract showed less significant activity against HeLa cell lines but showed good activity against MCF7 (Dogra *et al*., 2009). In a research the methanolic extracts of *Artocarpus heterophyllus* was tested for anticancer activity by MTT assay on different cell lines like HEK293, A549, HeLa and MCF-7. In the present study the IC_{50} value was found to be 32.33µg/mL by MTT assay against HeLa cell lines. Probit analysis (Table 1) was also worked out to arrive at regression equation (Probit (p)=-0.328+0.010x) and the chi square value is 172.74. However, IC_{50} value of extracts of *Artocarpus heterophyllus* was 35.26 µg/mL against A549 cell lines and no activity against HeLa and MCF-7 cell lines (Patel, 2011). In addition, Lenora *et al*. (2016) extracted sikhimic acid, a precursor for Tamiflu® from *E. crassipes*. Reducing power of various solvent extracts of *E. crassipes* was evaluated, compared to standard antioxidant L–Ascorbic acid and found greater than that of the standard suggesting the potential of development of useful natural antioxidants (Jayanthi and Lalitha, 2011). Lalitha and Jayanthi (2014) also demonstrated the potential of extracts of *E. crassipes* in antiaging. Two skin creams of the ethyl acetate extract were evaluated for its antiaging efficacy by DNA damage inhibition assay and DPPH radical scavenging assay. There was an increase in DNA damage inhibition and DPPH radical scavenging ability with increase in concentration for both the creams promising the future of water hyacinth in cosmeceutical industry. Water hyacinth has also been reported to contain compounds with anticancer properties (Aboul-Enein *et al.,* 2014). The present study also confirmed mild anticancerous activity of *E. crassipes* against human HeLa Cell lines.

Table 1. Probit analysis-IC_{50} value of the extract of *E. crassipes* extract against HeLa cells

S.No.	Concentration µg/mL	Number of Subjects	Observed Responses	Expected Responses	Probability
1.	0.000	100	0	37.136	0.371

S.No.	Concentration µg/mL	Number of Subjects	Observed Responses	Expected Responses	Probability
2.	50.000	100	98	57.120	0.571
3.	100.000	100	89	75.400	0.754
4.	200.000	100	83	95.567	0.956

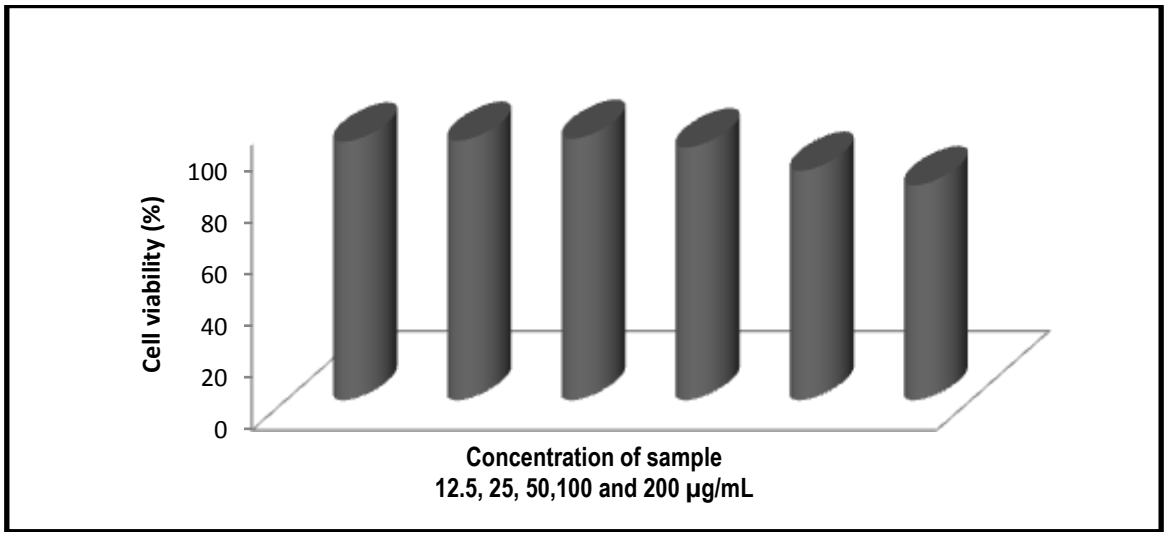
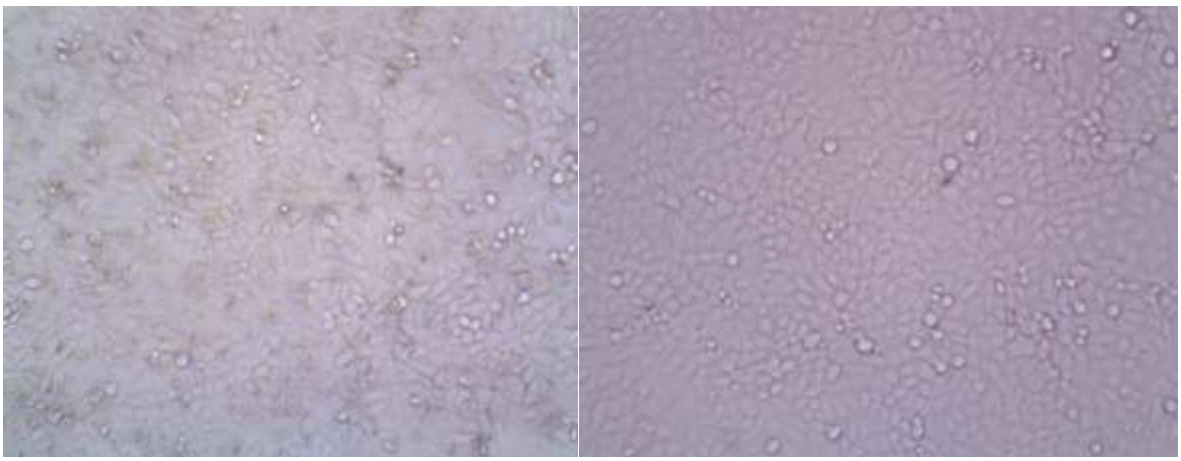


Figure 1: Anticancer activity of *E. crassipes* extract against HeLa cells



a. 200µg/mL extract treated cells **b.** Control cells

Figure 2: Anticancer activity of *E. crassipes* extract against HeLa cells

CONCLUSION

The results obtained from the *in-vitro* studies using the HeLa cell lines revealed that the methanol extract of *E. crassipes* has a mild anticancer activity. It has also been observed that there was increase in the cell growth inhibition when the concentration of the extract was increased. Hence, it is concluded that the methanol extract of *E. crassipes* may be considered as anti-cancerous potent at early malignant stage. Therefore *E. crssipes* confirms as a valuable resource for natural compounds of desirable medicinal properties like antioxidants, antiaging and anticancer. Hence, commercial use of water hyacinth could be an alternative for the management of this weed, contributing to solve environmental and economic problems caused by it.

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