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Research Article

EVALUATION OF IN-VITRO CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF *IPOMOEA BATATAS*

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ABSTRACT

The ethanolic extract of *Ipomoea batatas* was evaluated for its in vitro cytotoxic and antioxidant activities. The extract showed potent cytotoxic activity in trypan blue dye exclusion method using DLA cell lines with EC_{50} value of 305µg/ml and exhibited a dose dependent decrease in cell count for all the concentrations tested. The antioxidant activity was evaluated by DPPH free radical method. The extract exhibited potent antioxidant activity with an EC_{50} of 36.5µg/ml.

Keywords: *Ipomoea batatas,* DLA cell lines, Cytotoxic activity, Antioxidant activity, DPPH.

INTRODUCTION

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of body's own cells .[1] Combating cancers is of paramount importance today. An alternative solution to western medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease. Of the 92 anticancer drugs commercially available prior to 1983 in the United States, approved worldwide between 1983 and 1994, approximately 62% can be related to natural origins.[2]

Free radical damage may lead to cancer. Antioxidants interact with radicals and may prevent some of the damage by free radicals. Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may slow or possibly prevent the development of cancer.[3]

Ipomoea batatas L. Commonly known as sweet potato belongs to the family convulvulaceae is a plant cultivated throughout India.[4] Its Antidiabetic,[5] anti-mutagenicity,[6] immunostimulatory[7] and memory enhancing[8] properties have been reported. In the present study the in vitro cytotoxic and antioxidant activities of the plant was evaluated.

MATERIALS AND METHODS

Plant material

The leaves of Ipomoea batatas have been collected from Malappuram dt, Kerala, India during the month of November 2008 and were dried under shade. The plant was identified and authenticated by Dr.P.S.Udayan, senior scientist, taxonomy division,Centre for medicinal plants research, Arya Vaidya sala, Kottakkal,Kerala.

Preparation of extract [9]

The coarsely powdered shade dried leaves of *Ipomoea batatas* was charged in an aspirator bottle and extracted with ethanol by cold maceration method for 3 days. After decantation and filtering, nearly 80% of the solvent was removed by distillation over boiling water bath and the remaining under reduced pressure. The extracts so obtained, were further dried in vacuum desicator and the extract so obtained was used for further studies. The extracts were dissolved in distilled water using 1% CMC as suspending agent.

DPPH free radical photometric assay[10]

DPPH (Diphenyl picryl hydrazine) is a free radical at room temperature which produces violet colour in ethanol. It is reduced in the presence of an anti-oxidant molecule, giving rise to uncoloured solution. The use of DPPH provides an easy and rapid way to evaluate anti oxidants. **S**ample stock solutions (10mg/ml) were diluted to final concentration of 250,125,50,25,10 and 5µg/ml in ethanol. One ml of 0.6mm DPPH ethanol solution was added to 2.5ml of sample solution of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted in to the percentage antioxidant activity (AA) using the following formula.

AA%=100-{[Absorbance of sample -absorbance of blank]×100/Absorbence of control}.

Ethanol (1.0 ml) and blank extract solution (2.5 ml) was used as the blank. DPPH solution (1.0 ml, 0.3 mM) and ethanol (2.5 ml) was used as the negative control. The EC_{50} value was calculated graphically.

Trypan blue dye exclusion method [11]

10mg of the extract was taken in an eppeandorf vial of capacity 1ml and diluted to 6 different concentrations with its duplicate and its control (50%) using alcohol as a solvent and mixed with the help of vortexing machine. Aspirated tumor cells from the peritoneal cavity of mice was obtained from Amala Cancer Research Centre, Thrissur. The procedure was approved by the institutional animal ethics committee. The tumour cells were added to the test tube containing phosphate buffer solution (PBS) which was then dipped in ice. Washed the cells with PBS and centrifuged 3 times. Cells were then suspended in 1 ml 'PBS' and adjusted the cell number to 10 million i.e. $10x10^6$ cells/ml.

Checked the cell viabihty using 'trypan blue' stain (1%) Counted the cells in counting chamber. The experiment was set by incubating the suspension in different concentrations of samples at 37° C for 3 hours. After incubation, added 0.1 ml trypan blue and determined number of dead cell using haemocytometer.

RESULTS

In the DPPH photometric assay method, the ethanolic extract of *Ipomoea batatas* exhibited a comparable anti oxidant activity with that of standard ascorbic acid at varying concentration tested (5, 10, 25, 50,125,250 µg /ml). There was a dose dependant increase in the percentage antioxidant activity for all concentrations tested (Table 1).The extract at a concentration of 5µg/ml showed a percentage inhibition of 18.96±2.02 and for 250 µg/ml it was 88.38±1.64.

Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied from 1 to 50 µg/ml. Ascorbic acid at a concentration of 1µg/ml exhibited a percentage inhibition of 36.2282±3.5146 and for 50 µg/ml 95.1439±0.2826 (Table 2). A graded increase in percentage of inhibition was observed for the increase in the concentration of ascorbic acid. The EC_{50} value of ascorbic acid was found to be 6.1 µg/ml. All determinations were

done in duplicate and the mean values were determined. An increased EC_{50} value was observed (36.5µg/ml) for the plant extract when compared with standard drug ascorbic acid (6.1µg/ml) (Table 1 & 2)

Estimation of the cytotoxic activity was done by try pan blue dye exclusion method using DLA cell lines. The various concentrations of plant extracts used were 10, 50, 100, 200, 500 µg/ml and control (without extract). A decrease in the cell count was observed with the increase in the concentration of the extract. There was a dose depended increase in the cytotoxic activity for all the concentrations tested. The extract at a concentration 10 µg/ml showed 1% reduction in cell count, and 95% reduction was observed for 500 µg/ml concentrations. The IC_{50} value was found to be 305 µg/ml (Table 3).

Table 1: Anti oxidant activity of *Ipomoea batatas* extract by DPPH free radical method

Concentration (µg/ml)	% AA	EC_{50}
5	18.96 ± 2.02	
10	26.62±1.14	
25	7.12 ±0.53	36.5µg/ml
50	65.62±1.24	
125	88.31±2.24	
250	88.58±1.64	

Values are mean ± SD of three parallel measurements

Table 2: Anti oxidant activity of ascorbic acid by DPPH free radical method

Concentration(µg/ml)	% AA	EC_{50}
1	36.23±3.51	
5	41.63±0.0	
10	84.48±4.52	6.1 µg/ml
25	94.31±0.79	
50	95.14±0.28	

Values are mean ± SD of three parallel measurements

Table 3: Cytotoxic activity using DLA cell lines by trypan blue dye exclusion method

Concentration (µg/ml)	DLA (%) cyto toxic activity	IC_{50}
10	1±0.02	
50	2±0.05	
100	12±0.03	305 µg/ml
200	25±0.08	
500	95±0.06	
Control	0	

Values are mean ± SD of three parallel measurements

CONCLUSION

On the basis of the above results it can be concluded that the ethanolic extract possess significant anticancer and antioxidant activities studied by in vitro models. The presence of flavonoids and related phytoconstituents may be responsible for the activity. Further investigations are required to find active component of the extract and to confirm the mechanism of action. Further studies warranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity.

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