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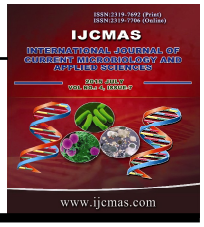


Original Research Article

In vitro Cytotoxic Activity of Methanolic Extract of *Parthenium hysterophorus* Flowers on MCF-7 and HeLa Cell Lines

R.Anup[1], P.T.Sainath[1], S.Padma[2] and M.K.Sukumaran*

[1]Bhavan's Sri Ramakrishna Vidyalaya, Sainikpuri, Secunderabad, Telangana, India
[2]Department of Biochemistry, Bhavan's Vivekananda College, Sainikpuri, Secunderabad, India
**Corresponding author*

A B S T R A C T

Keywords

Parthenium hysterophorus flowers, Methanolic extract, *In-vitro* cytotoxicity, HeLa, MCF-7

The present study has been designed to evaluate the cytotoxic activity of methanolic extract of *Parthenium hysterophorus* flowers. The *in-vitro* cytotoxic activity of methanolic extract was performed by MTT assay method against MCF-7 and HeLa cancerous cell lines. MTT assay is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple blue insoluble formazan precipitate which is then quantified spectrophotometrically at 570 nm. The methanolic extract of *Parthenium hysterophorus* flowers was cytotoxic to both MCF-7 and HeLa cell lines. IC_{50} values for MCF-7 and HeLa were found out to be 30.81±0.09 and 5.35±0.03ng/mL respectively. Methanolic extract of *Parthenium hysterophorus* flowers separated as a single brown coloured spot with an R_f value 0.53.

Introduction

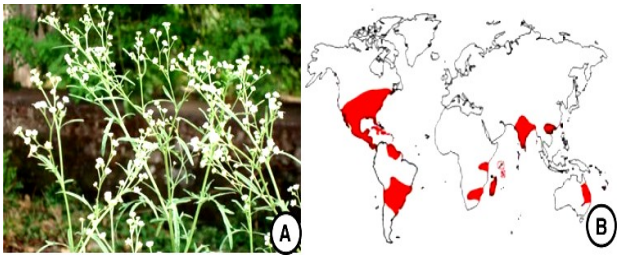
Since ancient times, plants are used as medicines for the treatment of various health complications. Although, synthetic drugs are readily available, plants still remain the choice even today in health care. WHO has already declared that a large proportion of the World's population depends on plant based therapies to face the needs of the primary health care (WHO, 1999) (Dikshit *et al.,* 2004). *Parthenium hysterophorus* is one such medicinal plant (Towers *et al.,* 1977). It is native to America (Navie *et al.,* 1996) but has spread in many countries. It is commonly known as congress grass in India. This weed is believed to have been introduced into India as contaminants during the import of PL 480 wheat (1950s). Presently, this invasive weed is widely prevalent in India (Shah *et al.,* 2009).The weed normally grows to a height of about 1 to 1.5m. Sesquiterpenoids from this plant spread in air and creates contact dermatitis (direct or indirect sensitization). Its leaf extracts were found to have a role in the fecundity, fertility and behavioral response (Kumar *et al.,* 2011). The toxic property of this plant has been exploited to use it as a remedy against various diseases. For example, the extract is used against skin diseases, ulcerated sores, facial neuralgia, fever, anemia and anti-inflammatory in folk remedies (Recio, 2000). Since, toxic property of this plant has been exploited as a remedy against various diseases. The present

study was undertaken to screen the methanolic extracts of *Parthenium hysterophorus* flowers for their cytotoxic activity.

Materials and Methods

Plant material

The plant was identified based on the leaves, which are lobed with fine soft hairs; the flowers on the top are small creamy colour with black coloured seeds. Based on these features the plant was identified as *Parthenium hysterophorus*. The identification was confirmed by a botanist.



A. *P. hysterophorus* weed; **B.** geographical distribution of *P. hysterophorus*

The flowers of *Parthenium hysterophorus* were collected from different areas in Sainikpuri, Secunderabad and air dried in shade. The air dried flowers were ground into a fine powder in a mechanical grinder.

Preparation of methanolic extract

Methanolic extraction of dried *Parthenium hysterophorus* flowers was carried out according to the methods described by Narasimhan *et al.* (1984) and Basarkar and Saoji (2013) with slight modifications. 25g of fine powder was taken into a porous cellulose thimble and extraction was carried out with methanol by soxhlet apparatus (8 h at 70°C). The resulting dark brown extract was concentrated in a flash evaporator at 35°C.

The concentrated sample thus obtained was centrifuged at 18,000 rpm for 30 min. The supernatant was discarded and the pellet was dissolved in minimum volume of methanol. All the assays were carried out with this extract (methanolic extract).

Estimation of protein content in the methanolic extract

Protein concentration in the methanolic extract was determined in triplicate by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

TLC of methanolic extract

TLC was carried out according to the method outlined by Basarkar and Saoji (2013) with slight modification. 5 µL of the methanolic extract was separated on TLC with the mobile phase, benzene: acetone in the ratio 4:1.

The separated compounds were detected by exposing the plates to iodine vapours. After plate development, R_f value of the separated compound was calculated.

Determination of *in vitro* cytotoxicity by MTT assay

Culture media

Dulbecco Modified Eagle's Medium containing 2mM L-glutamine, 100Units/mL penicillin and 100µg/mL streptomycin supplemented with 5% foetal bovine serum (complete media).

Preparation of cell suspension for the assay

The desired human cancer cell lines, MCF-7 (breast cancer cell line) and HeLa (cervical cancer cell line) were grown at 37^0C, 5% CO_2 and 90% relative humidity till sub-

confluent stage. The cells were then harvested by treatment with trypsin-EDTA solution. The number of cells was counted in a haemocytometer and the cell density was adjusted to 75,000 cells/mL in complete media.

MTT assay

MTT assay was carried out according to method described by Slater *et al.* (1963), van de Loosdrecht *et al.* (1994) and Alley *et al.* (1988). Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetra zolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer.

The absorption maximum is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore, conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve.

Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

MTT assay was carried out in triplicates in 96 well microtiter culture plates.

100 µL of the cell suspension (7500 cells) was added into each well of the 96 well plates and incubated at 37^0C, 5% CO_2 and 90% relative humidity for 24 h. After 24 h**,** **c**ells were treated with different concentrations (5, 10 and 15µM) of methanolic extract and the plates were incubated for a further period of 24 h in the CO_2 incubator.

In control wells only medium was added. 20 µL of 5mg/mL MTT was added into each well and the plates were incubated for 3.5 h. At the end of incubation period, culture media was carefully removed and 150 µL MTT solvent was added into each well. After covering the plates with tinfoil, the plates were agitated on orbital shaker for 15 min. Absorbance was read at 590 nm with a reference filter of 620 nm.

Percent specific cytotoxicity is calculated as follows:

% Cell viability = [(O.D of control - O.D of test compound)/ (O.D. of control)] X 100

Results and Discussion

Estimation of protein content in the methanolic extract

The concentration of protein present in the methanolic extract was found out to be 2.125mg/mL.

TLC of methanolic extract

Methanolic extract on separation by TLC, showed a single brown coloured spot with an R_f value 0.53 (Figure 1) in benzene: acetone (4: 1) solvent system. This result is in accordance with those reported by Basarkar and Saoji (2013).

Determination of *in vitro* cytotoxicity by MTT assay

Effect of methanolic extract on *in-vitro* cytotoxic activity was determined by MTT assay. MCF-7 and HeLa cell lines were chosen for investigation of *in vitro* cytotoxic activity. The cytotoxicity is presented as percentage growth inhibition of cells (Table 1). The methanolic extract was cytotoxic to both the cell lines tested (Figure 2). The cytotoxic activity increased with an increase in the concentration of methanolic extract (36, 70 and 82% for MCF-7 and 42, 64 and 80% for HeLa cell lines at concentrations of 5, 10 and 15µM respectively) (Figure 2). IC_{50} values for MCF-7 and HeLa cell lines were 30.81±0.09 and 5.35±0.03ng/mL respectively. These results are somewhat comparable with those reported in literature with slight variations (Siva kumar Ramamurthy *et al.,* 2011; Raies Haq *et al.,* 2011).

Plants can serve as good source for bioactive compounds that exhibit anti-cancer activities. The methanolic extract was cytotoxic to both MCF-7 and HeLa cell lines tested. The protein concentration present in the methanolic extract was found to be 2.125 mg/mL. The R_f value of the separated compound was found to be 0.53.

Table.1 MTT assay for cytotoxic activity

Cell Lines	MCF-7	HeLa
IC 50	30.81±0.05ng/mL	5.35±0.03ng/mL
Concentration (µM)	% Inhibition	
5	36	42
10	70	64
15	82	80

Figure.1 TLC analysis of methanolic extract of *Parthenium hysterophorus* flowers

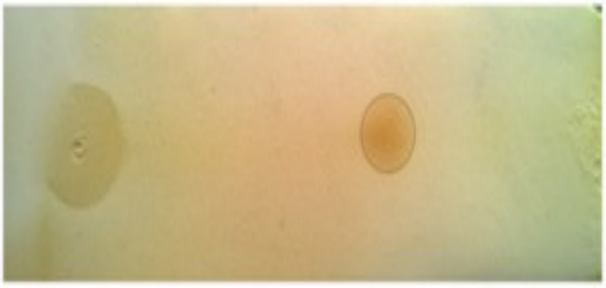
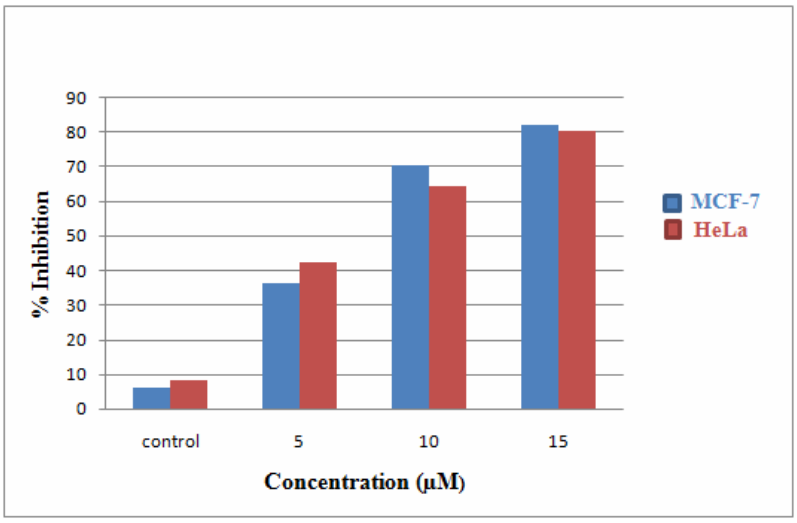


Figure.2 Cytotoxic activity of methanolic extract on MCF-7 and HeLa cell lines



Based on our results and those reported by others, *Parthenium hysterophorus* can be considered as an important source of natural products that have anti-cancer potentials. However, it is too early to reach a final conclusion and further investigations are required to include further cell lines and taking into account the allergy it causes to humans.

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