The Study of Antimicrobial, Anthelmintic and Cytotoxic activities of *Parthenium Hysterophorus* L.

Nitin Sharma, Mahesh Kumar, Usha Tiwari, Manoj Kalakoti

***Abstract*— The present study has been designed to evaluate antimicrobial, anthelmintic and cytotoxic activities of *Parthenium hysterophorus* L. plant leaves extract. Present work reports for the antimicrobial activities of *P. hysterophorus* leaf. Dried samples were sequentially extracted with many solvents. Methanol, acetone, chloroform, petroleum ether and water extracts of leaf showed considerable antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia* and also antifungal activity against *Candida albicans*. The *in-vitro* cytotoxic activity of methanolic extract was performed by MTT assay method against HeLa cell line. The methanolic, acetone, chloroform, petroleum ether, water extract (100 mg/ml concentration) of *Parthenium hysterophorus* plant leaves was taken for anthelmintic activity against *Pheretima posthuma*. Effect of inhibition of cell growth showed significant cytotoxicity against leaf methanolic extracts showed considerable inhibition (80–90%) of HeLa cell lines. The results obtained from the study indicate good anthelmintic activity against *Pheretima posthuma*. The present study concluded that the methanolic extract of *Parthenium hysterophorus* possess potent antimicrobial, anticancer and also anthelmintic activities.**

***Index Terms*— Antimicrobial activity, HeLa cells, leaf extract, Parthenium hysterophorus.**

I. INTRODUCTION

Parthenium hysterophorus is a species of flowering plant in the aster family, *Asteraceae*, that is native to the American tropics. Common names include Santa Maria Feverfew and White top Weed. In India, it is locally known as "carrot grass" *Congress Grass* or *Gajar Ghans*. The demand for the products and technologies based on plant to control plant pathogens has increased in recent years because the chemical control methods which are in practice are costly have hazardous consequences along with this various important pathogen have developed resistance to many of the currently available fungicides [1] and are also polluting soil and water, so the use of biodegradable material like fresh plant extract from different parts gained importance during last three decades for plant disease control [2]. Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in the modern medicine [3]. Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore the search for new drugs from plants continues to be a major source of commercial drugs. Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated [4]. The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [5]. In India and many other countries extracts of *P. hysterophorus* are used as ethno medicine against inflammatory, skin, neural, and female reproductive problems. In Maharashtra and Gujarat (India) the plant is used in the treatment of diabetes mellitus. *P. hysterophorus* has been found to be pharmacologically active as analgesic in muscular rheumatism and as vermifuge and therapeutic for neuralgia [6]. Here, we evaluate the potential of *Parthenium hysterophorus* plant extracts for antimicrobial activity against important human pathogenic bacteria, fungi *Candidia albicans* and anticancer activity against human cancer cells.

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II. MATERIALS AND METHODS

A. Collection and processing of plant materials

The whole plant leaves of *Parthenium hysterophorus* L. were collected on April 2015 from local areas of IBT, Patwadangar. The collected plant material was washed with tap water thrice and then with distilled water for 2-3 times. The plant was shade dried for few days and then kept in incubator at 37°C for 2-3days. The dried plant material was then crushed in mechanical grinder in order to make fine powder which was stored at room temperature.

B. Preparation of Plant Extract

The powdered material was weighed 10gm and is subjected to soxhlet extraction using Methanol, acetone, chloroform, petroleum ether, water as solvents in successive mode [7-8].

C. Microorganisms and Growth Conditions

For the Antibacterial activity against E*scherichia coli*, K*lebsiella pneumonia* and also antifungal activity against *Candida albicans*, culture was present in the laboratory. The bacterial culture was maintained at 4^0C on nutrient agar slants.

D. Evaluation of Antimicrobial Activity

Antimicrobial activity of plant extracts was determined using Kirby-Bauer disc diffusion and well diffusion method [9]. The inoculum suspension of bacterial strains was

swabbed on the entire surface of Mueller-Hinton agar (MHA). Sterile 6mm diameter paper discs (Himedia) saturated with 20 μL of extracts prepared in DMSO (2mg extract/disc) were aseptically placed on the upper layer of the inoculated MHA surfaces and plates were incubated at 37^0C for 18 hours. Antibacterial activity was determined by measuring diameter of the zone of inhibition (ZOI) surrounding discs. Standard antibiotic discs were used as positive controls. Discs containing 20 μL DMSO was used as a negative control.

E. Procurement of microorganism C. albicans

Freeze dried form of the microorganism *C. albicans* was available in the laboratory.

F. Preparation of culture media for the study

According to the manufacturer's instruction, Ampoule containing freeze dried form of the microorganism was opened and the contents were added to the Yeast Extract Peptone Dextrose (YEPD) broth which was incubated at 25 ± 2°C for 72 h. The 30 ml of molten sterile agar was poured aseptically in each four sterile petri plates and were allowed to solidify at room temperature. Hundred microliter of inoculum was spread with a sterile steel spreader to prepare a lawn of microorganism.

G. Ditch plate method

In three plates wells were prepared. The wells were filled with fixed volume (250 µl) of respective stock solution of plant extract using micropipette. Then on the 4th plate wells were prepared in the similar fashion. Flucanozole was used as positive control, and 50% DMSO and sterile distilled water were maintained simultaneously as negative controls in the same plate. Then all the plates were incubated in an upright position at 25 ± 2°C for 24h. The whole procedure was repeated twice. The inhibition zones were measured on the underside of the plates, using Hi-media zone scale after 24 and 48 h.

H. Anthelmintic Activity

The anthelmintic activity was performed on the adult Indian earthworm *Pheritima posthuma*, was avilabile in the laboratory. Albendazole, the standard drug, was diluted with normal saline to obtain 25, 50 and 100 mg/ml concentrations and was poured into Petri dishes. All extract of the plant was diluted with normal saline to obtain 100 mg/ml concentrations. Normal saline (0.9% NaCl) alone served as the negative control. All these dilutions were poured into the Petri dishes accordingly. Ten petri dishes of equal size were taken & numbered. Six earthworms (n=6) of similar sizes (about 8 cm) were placed in each petri dish at room temperature. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. Time of death for worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 ^{o}C). The paralysis time and lethal time were recorded in terms of minutes.

I. Cytotoxic activity

a) Chemicals

3-(4,5–dimethylthiazol–2–yl)–5–diphenyl tetra- zolium 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, Dimethyl Sulfoxide (DMSO). All chemicals were available in the laboratory.

b) *Cell lines and culture medium*

HeLa cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO_2 at 37^0C 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 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates.

c) Preparation of Test solutions

For Cytotoxicity studies, the mehanolic extract was separately 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 studies.

d) Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM 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, the monolayer was washed once with medium and 100 µl of different test concentrations of extracts were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the sample 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^o C in 5% CO_2 atmosphere. The supernatant was removed and 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 sample needed to inhibit cell growth by 50% (CC50) is generated from the dose-response curves for each cell line [10].

% Growth Inhibition = 100 – {(Mean OD of individual test group / Mean OD of control group) × 100}

III. RESULTS AND DISCUSSION

A. Antimicrobial activity

The antibacterial activities of the extracts derived from leaves of *P. hysterophorus* were evaluated against Gram negative *Klebsiella pneumonia* and Gram negative *Escherichia coli* bacterial strains. The antibacterial activity profiles of *P. hysterophorus* extracts are given in Table 1. *P. hysterophorus* leaf extracts prepared in Methanol, acetone, chloroform, petroleum ether and water exhibited bacterial growth inhibition potential at 2 mg/disc concentration against *E.coli, Klebsiella pneumonia.* However, inhibitory efficacy of leaf acetone, chloroform, petroleum ether and water were more pronounced. Methanol extract of leaf showed moderate activity (ZOI 18 mm) against *E.coli*. and (ZOI 16 mm) against *klebsiella pneumonia*. The antifungal activity of the leaf extracts derived from leaves of *P. hysterophorus* were

evaluated against *C. albicans* fungal strains. The means of the zones of inhibition of *C. albicans* by aqueous and alcoholic plant extracts at 24 h were measured. Methanolic curry leaves showed strongest growth inhibition of *C. albicans* with 26 mm [11].

Extracts	Diameter of zones of inhibition (mm)		
	Escherichia coli	*Klebsiella pneumonia*	*Candida albicans*
Petroleum ether	12	12	18
Acetone	14	10	22
Chloroform	16	14	20
Methanol	18	16	26
Water	NA	NA	NA

Table 1: Antimicrobial activity of *P. hysterophorus* L. leaf extracts

B. Anthelmintic Activity

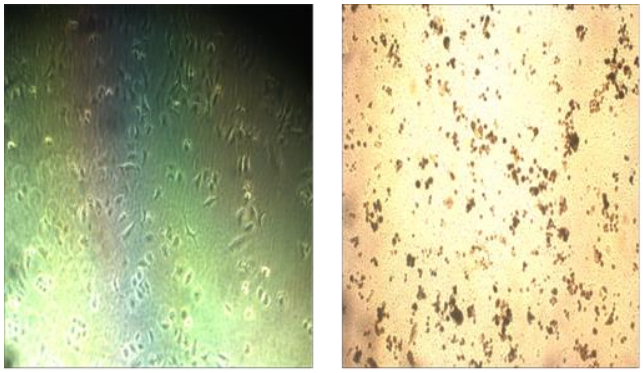
The result show that for the 100 mg/ml concentration, albendazole showed the best activity for death time 57.55±1.20 min and the methanolic extract of *Parthenium hysterophorus* showed a death time of 75.50±2.50 min. The paralysis and death times of the plant along with the standard are given in Table 2. The study revealed that the methanolic extract of *Parthenium hysterophorus* had significant activity (moderate) at the higher concentration (100 mg/ml) [12-14].

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Albendazole	100	28.62±0.50	57.55±1.20
Petroleum ether	100	41.20±1.52	82.60±2.20
Acetone	100	40.51±0.55	80.55±1.10
Chloroform	100	41.00±1.00	81.40±0.50
Methanol	100	38.50±1.48	75.50±2.50
Water	100	60.50±1.20	110.20±1.50

Table 2: Anthelmintic effect of *Parthenium hysterophorus L. against pheritima posthuma*

C. Cytotoxic activity

Through the MTT method, the median cytotoxic concentration (CC50) on HeLa cell line was established for methanolic extract of whole plant leafs of *Parthenium hysterophorus.* There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (30.00, 55.55, 78.32, 82.30 % for the concentrations 125, 250, 500, 1000 µg/ml, respectively) against the HeLa cell line (figure 1&2).



HeLa cells healthy HeLa cells transfected

Fig.1. HeLa cells transfacted with plant methanolic extract

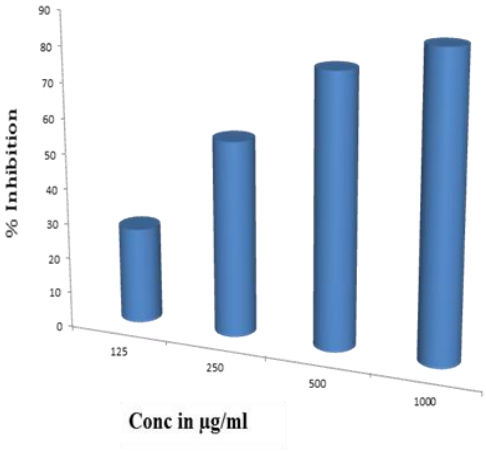


Fig.2. Cytotoxic effect on HeLa cell Line

IV. CONCLUSION

In conclusion, the present plant *Parthenium hysterophorus* can be considered as an important source of natural products that have antimicrobial, anti-cancer potentials and also potent anthelmintic activity.

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