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A STUDY ON THE CYTOTOXIC SCREENING AND ANTIHELMINTHIC ACTIVITY OF *PARTHENIUM HYSTEROPHORUS* LINNAEUS.

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ABSTRACT
This study was carried out to investigate the cytotoxic property and antihelminthic activity of the weed *Parthenium hysterophorus*. *Parthenium hysterophorus*, also known as Congress weed, is a common invasive and poisonous weed, which can disrupt the ecosystem as well as can cause vigorous spreading throughout the year due to its high germination capacity. Such a nuisance weed when studied properly can bring the new alternative source for the development of new medicines and also helps to manage it properly. The extracts were subjected for cytotoxic screening by using brine shrimp lethality assay and antihelminthic activity was conducted in earthworm (*Pheretima posthuma*). The brine shrimp lethality assay revealed that the methanolic extract possess moderate cytotoxic activity with LC_{50} value of 190.10µg/ml. The result obtained from the antihelminthic activity showed the best paralysis time (8, 5 and 3 minutes) and best death time (38, 22 and 14 minutes of hexane extract at concentration of 25, 50 and 100 mg/ml respectively and ethyl acetate extract showed the best paralysis time (6, 5 and 3 minutes) and best death time (37, 19 and 18 minutes) at same concentrations respectively whereas the methanolic extract showed best paralysis time (11, 7 and 5 minutes) at the similar concentrations mentioned above but did not cause death of any earthworm under our observation period. Hence, only hexane and ethyl acetate extract showed good antihelminthic activity at all the concentrations mentioned above. This study concludes that it could serve as a source of potent natural cytotoxic and antihelminthic agents. Therefore, this study gives the basis for further investigation in future.

KEYWORDS: *Parthenium hysterophorus*, *Pheretima posthuma*, brine shrimp lethality assay, antihelminthic activity, cytotoxic screening.

INTRODUCTION
Plants has been used as traditional medicines worldwide since early times for the control of different types of ailments of human as well as animals and has also given rise to modern medicines which are still in use.[1] According to World Health Organization (WHO) about 65-80% of the world's population in developing country depends upon these plant based drugs due to lack of access to current drugs and poverty.[2] The plant based drugs are the main and sometimes the only source of drugs for the millions of people living in the rural part of the developing countries.[3]

With the rise in population and globalization, the pattern of disease is changing resulting in inadequate supply of medicine along with increase in the side effects of allopathic drugs. Hence, the need of finding new drugs which are potent, safe and affordable are important for the development of future health strategies for the developing world.[4] As Cancer is one of the leading cause of illness and death, new anti-tumor drugs are needed to address the problem. Thus, the cytotoxic activity of this weed was studied by using brine shrimp lethality assay to find out alternative natural cost effective remedies. The brine shrimp lethality test is considered very simple, rapid, inexpensive and useful tool for preliminary investigation of toxicity. It utilizes very minimum amount of test material for performing the test.[5, 6] The level of toxicity is measured by the estimation of lethal drug concentration (LC50) which is defined as the ability to kill 50% of the laboratory cultured brine shrimp (*Artemia salina*) belonging to class Crustaceae and phylum Arthopoda within 24 hour.[6, 7]

The criteria of toxicity for extract is as follows.
LC_{50} values > 1000µg/ml (nontoxic), ≥ 500 ≤ 1000µg/ml (weak toxicity) and < 500µg/ml (toxic).[8]

The antihelminthic activity was studied by using the extract of this plant. Helminthiasis is defined as any macro parasitic disease of humans and animals in which a part of the body is infected with parasitic worms

known as helminthes. These helminthes mainly survive in the gastrointestinal tract of their hosts but can also present in any other organs where they can produce physiological damage. They are responsible for causing diseases in the wildlife resulting in the economic crisis in the livestock industry. They are the major cause of the economic problems in the developing countries.[9] Due to emergence of helminthes, which are resistant to commercial antihelminthic drugs, has increased the interest to create a need for alternative methods to control these parasite. Hence, plants with traditional medicinal value could provide access to affordable medicines to the people who can't afford present health care services.

MATERIALS AND METHODS

Study design: Experimental research design.

Plant material: The aerial parts of the plant *Parthenium hysterophorus* was collected from Nakhu area of Kathmandu Valley which stands at an elevation of approximately 1,400 meters (4,600 ft.) in month of June and July during rainy season and was duly identified as *Parthenium hysterophorus* at Pharmacognosy section, National Herbarium and Plant Laboratory, Godawari, Lalitpur, Nepal.

Chemicals and Apparatus: Hexane, ethyl acetate, methanol, brine shrimp eggs, artificial sea water, albendazole, normal saline, incubator, petri dishes, soxhlet extractor (Borosil) and rotary vacuum evaporator (ATICO India).

Plant processing: The aerial parts of *Parthenium hysterophorus* was cut into pieces and was shade dried at room temperature. Dried sample was crushed into powder by the help of electric blender and 20gm of the dried sample was subjected to extraction in soxhlet apparatus each time by using non-polar to polar solvents (hexane, ethyl acetate and methanol). The obtained extracts were dried by using Rotatory evaporator drier under vacuum at temperature 40ºC till the solid mass was obtained and thus obtained solid mass was poured into the glass vials. The extracts were preserved in refrigerator at 4ºC for further analysis.

Brine Shrimp Lethality Assay: The procedure for brine shrimp lethality assay was adapted from Himri et al. and Meyer et al.[7, 10]

Hatching of brine shrimp: Hatching of the brine shrimp eggs was done in the beaker containing artificial sea water. First of all, the brine shrimp eggs were sprinkled in the beaker which was under constant aeration with the help of an air pump. The air was placed in the bottom of the beaker to ensure complete hydration of the cysts. This was then placed in the incubator at 37ºC and was illuminated with torch light for 24 hours to maintain the temperature and to hatch nauplii. After 24 hour incubation at 37ºC, the air pump was turned off and the hatched nauplii free from egg shells were collected from the brighter portion of the beaker with the help of the dropper and were transferred into the glass test tubes containing the extracts of known concentrations.

Preparation of stock solution: 250mg of the crude extract of methanol to be tested was weighed and the final volume was made up to 250ml with sea water; called as stock solution (1000ppm).

Preparation of test solution: From the stock solution of 1000ppm i.e. 1000µg/ml, six different test solutions of concentrations (500µg/ml, 250µg/ml, 125µg/ml, 75µg/ml, 50µg/ml and 25µg/ml) were prepared by serial dilution in sea water. Then, 20 ml from each solution i.e. 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 75µg/ml, 50µg/ml were transferred, in triplicate, into clean test tubes.

Incubation: Ten active nauplii were drawn through the dropper in watch glass and were transferred to each test tube. The sea water was used as negative control. The test tubes were then incubated for 24 hours at 37ºC.

Counting of Nauplii: After 24 hours, the test tube were observed and the number of survived nauplii was counted against illuminated background using magnifying glass and percentage of deaths at each dose level and control were calculated using following formula.

% mortality = (Number of dead shrimps) / (Number of dead + alive shrimps) × 100

Nauplii were considered dead if they were lying immobile at the bottom of the test tubes.

Calculation of LC_{50} value: The value of the extract was calculated by plotting the mean percentage mortality against logarithm of concentration. The LC_{50} value was calculated from the linear regression equation. The regression equation was obtained by trend line plotting in MS-Excel 2007.

Antihelminthic property of the plant extracts: Antihelminthic activity of plant extracts was determined by experimenting in earthworm *Pheretima posthuma*. It is found in moist soil and used in the present study due to its similarity in anatomical features with the intestinal round worms present in the human and due to its easy availability.[11]

The procedure for antihelminthic activity was adapted from Durga et al.[12] The antihelminthic activity was performed on the adult earthworm *Pheretima posthuma*. The sample solutions of standard albendazole and different extracts of *Parthenium hysterophorus* of concentrations 25, 50 and 100mg/ml were prepared using DMSO as a solvent. Two controls were used in this experiment. One is Normal saline (0.9% NaCl) and another is 1ml DMSO mixed with 10ml Normal saline.

First of all, the adult earthworms were taken and washed with distilled water to remove its fecal matters. Then, petri dishes of equal sizes were taken and labeled properly with the help of permanent marker. The experiment was performed in duplicate for which 26 petri dishes were taken. Each petri dishes was numbered carefully. Then, 10ml normal saline was poured in petri dishes and one adult earthworm was placed in each petri dish and was weighed in the electronic balance. The earthworms with approximately similar weight were selected and were transferred in test tubes numbered appropriately. After selecting the earthworms of similar weight, one earthworm was placed in each petri dish. Then, 1ml of the sample solutions of standard drug albendazole and different extracts of plants of concentration of 25, 50 and 100mg/ml were poured into each petri dish containing 10ml normal saline solution except into the petri dishes used as control. The paralysis and death of the earthworms were observed. Time for paralysis was noted down when no movement of any sort could be observed, except when the worms were shaken vigorously. The time of death for worms was recorded after ascertaining that the worms did not move when shaken vigorously. The mean time for paralysis and death were recorded in terms of minute. The result of the test was compared with reference standard drug albendazole.

RESULTS

Brine Shrimp Lethality Assay: The result of brine shrimp lethality assay is as follows:

Table 1: Percentage mortality of brine shrimp at different concentration of methanol extract

S.N	Concentration (ppm)	Log concentration	% Mortality of Methanol extract	LC50value (µg/ml)
1	25	1.39	16.61	190. 10
2	50	1.69	26.62	
3	75	1.87	26.62	
4	125	2.09	30	
5	250	2.39	46.64	
6	500	2.69	63.4	
7	1000	3.00	100	
8	Control (sea water)	0	0	

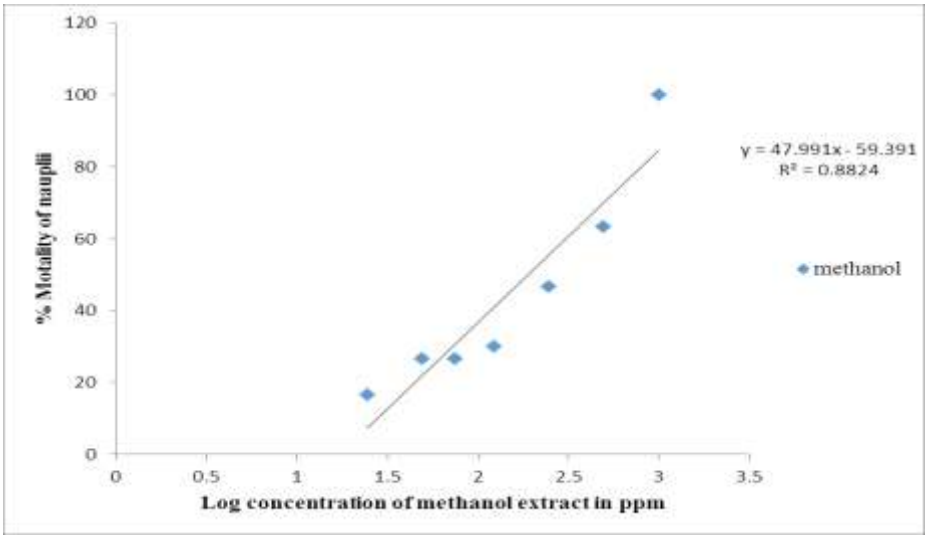

Figure 1: Percentage mortality of brine shrimp against methanol extract by logarithmic method

From the equation in the graph, the LC_{50} of methanol extract of *Parthenium hysterophorus* was found to be 190.10µg/ml obtained by plotting the curve of percentage mortality (y) of nauplii and log concentration (x) of methanolic extract in ppm.

Antihelminthic activity: The results of Antihelminthic activity of different extracts of plant are as follows.

Table 2: Antihelminthic activity of different plant extracts and standard drug albendazole.

S.N	Groups	Concentration (mg/ml)	Paralysis time (min)		Mean ± SD	Death time (min)		Mean ± SD
			1	2		1	2	
1.	Albendazole	25	9	9	9±0	–	–	–
		50	3	4	3.5±0.70	–	–	–
		100	1	2	1.5±0.70	–	–	–
2.	Hexane extract	25	8	8	8±0	36	40	38±2.82
		50	8	3	5.5±3.53	30	14	22±11.31
		100	3	3	3±0	14	15	14.5±0.70
3.	Ethylacetate extract	25	6	7	6.5±0.70	36	39	37.5±2.12
		50	8	3	5.5±3.53	22	17	19.5±3.53
		100	3	3	3±0	18	18	18±0
4.	Methanol extract	25	9	13	11±2.82	–	–	–
		50	7	8	7.5±0.70	–	–	–
		100	5	6	5.5±0.70	–	–	–
5.	Control (NS)	–	–		–	–		–
6.	Control (DMSO)	–	1hr20min		–	3hr10min		–

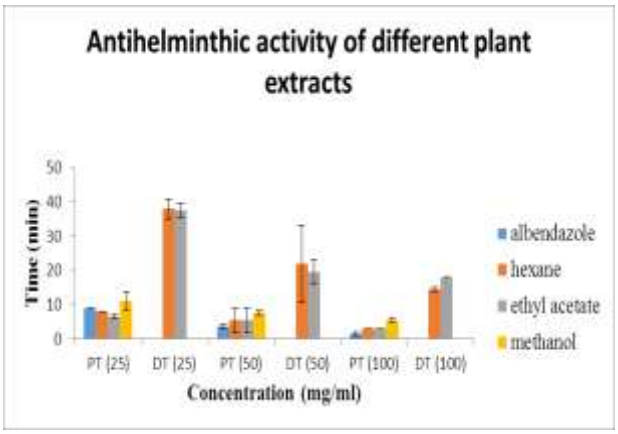

Figure 2: Antihelminthic activity of different plant extracts

Error bar in the figure 2 represents standard deviation from mean values.

Control (Normal saline):- Paralysis time = 0 Death time = 0

Control (DMSO):- Paralysis time = 1 hour 20 minutes Death time = 3 hour 10 minutes

Hexane and ethyl acetate extracts showed potent antihelminthic activity in comparison to the standard drug albendazole.

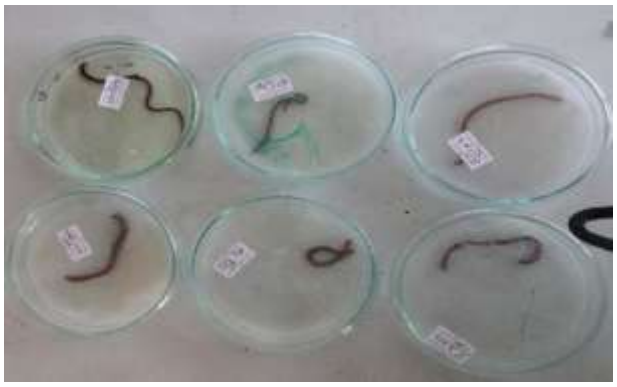

Figure 3: Antihelminthic activity of different extracts and standard drug albendazole at different concentration of 25, 50 and 100mg/ml

Figure 4: Weighing and exposure of earthworms in test tubes

STATISTICAL ANALYSIS

All the quantitative test was carried out in triplicate. Data obtained from the experiments was expressed as mean ± standard deviation (SD). Statistical difference of the results were evaluated using Microsoft excel 2007.

DISCUSSION

Today, the most common reason for using traditional medicines are its affordability and comparatively less side effects than the commercial medicines. When the modern synthetic medicines becomes ineffective, usage of traditional remedies increases in the treatment of different types of diseases such as infections, advanced cancer, etc.[13] This plant was studied to investigate their potential bioactivities for finding new medicines.

The brine shrimp lethality bioassay in this study showed the presence of moderate cytotoxic activity in the methanolic plant extract with LC_{50} value of 190.10µg/ml. In the previous study by Al-Mamum R; et al. (2010), the crude methanolic extract of leaves of *Parthenium hysterophorus* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was found to be 93.75µg/ml and Muhammad, B; et al. (2012) in their research have found remarkable cytotoxic activity of methanolic plant extract with LC_{50} value of 25±0.5µg/ml which differ from the result of the present study. This dissimilarity may be due to the method of extraction.[14, 15]

The result of antihelminthic activity of different plant extracts showed potent antihelminthic activity in comparison to standard drug albendazole. Hexane and ethyl acetate extracts showed good antihelminthic activity at all the concentration of 25, 50 and 100mg/ml than methanolic extract. Hexane extract showed best time for paralysis at 8, 5 and 3 minutes and best time for death at 38, 22 and 14 minutes at concentration 25, 50 and 100mg/ml respectively and ethyl acetate showed best time for paralysis at 6, 5 and 3 minutes and best time for death at 37, 19 and 18 minutes at concentration 25, 50 and 100mg/ml respectively. The standard drug albendazole and methanolic plant extracts showed the best time for paralysis at 9, 3 and 1 minutes and 11, 7 and 5 minutes respectively at similar concentrations mentioned above. The death of the earthworms did not occur in the albendazole and methanolic extract within the best death time of control DMSO i.e. 3 hour 10 minutes. However, the earthworms were found dead after 12 hours which did not occur under our observation period because the time period, after the death of earthworm in control DMSO, was not studied.

The previous study revealed the significant activity of methanolic extract at the higher concentration (100mg/ml) and showed least death time of 79.63 ± 3.26 minute which was comparable to the death time of standard albendazole which was 66.832 ± 2.16 minute.[12] The result of previous study was varied from that of the result of present study. The standard drug albendazole was not properly soluble in the solvent DMSO used for dissolving it which may be the reason behind the variation with the result of least death time shown in the previous study.

CONCLUSION

The finding in the present study revealed the potent antihelminthic activity of hexane and ethyl acetate extract of *Parthenium hysterophorus* whereas methanolic extract did not show good antihelminthic activity. The methanolic extract of the plant was found to be moderately toxic to *Artemia salina* with LC_{50} value of 190.10µg/ml. The present study concluded the plant as the reservoir of chemical constituents responsible for natural cytotoxic and antihelminthic property and also provides scientific evidence to its previous uses. Also, additional work is encouraged to find out the potential anticancer activity in in-vivo models as well for more detail study.

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