

## In Vitro Anthelmintic Activities of Crude Extraction of *Parthenium Hysterophorus* against *Haemonchus Contortus*

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### Abstract

This study was designed to evaluate anthelmintic activities of crude methanol extract of *Parthenium hysterophorus* against *Haemonchus contortus*. *Parthenium hysterophorus* leaves, stem, flower and roots crude extracts (1000mg/ml, 500mg/ml, and 250mg/ml) prepared in dimethyl sulfoxide then *in vitro* anthelmintic activity was evaluated against the *Haemonchus contortus* using Adult Motility Assay (AMA), Larvae Inhibition Test and Egg Hatch Test (EHT). *Parthenium hysterophorus* leaf crude extract show significant number (larvae L3) death in short time followed by flower when it is compared to other parts, positive and negative control groups ( $P < 0.05$ ). There is also significant number of adult death seen in groups treated with leaf and flower crude extracts with in short period of time when it is compared to other parts, even faster than the standard drug albendazole ( $P < 0.05$ ). The crude extract exhibited dose and/or time dependent larvaecidal and adulticidal effects against *H. contortus*. The best larvaecidal and adulticidal was demonstrated by leaf, 100% mean mortality percentage seen within 2hours for larvae and 3hours adult. Methanolic extract of *Parthenium hysterophorus* possess anthelmintic activities especially the leave and flower work best against adult and larvae (L3). But further studies need on toxicity, and *in vivo* test

**Keywords:** *Parthenium Hysterophorus*, *Haemonchus contortus*, Anthelmintic Activities

### Introduction

Helminthiasis by parasitic nematodes especially *Haemonchus contortus*, are among the most common and economically important and hence has an adverse effect on production of small ruminants especially in developing countries where mismanagement and poor control practices are prevalent (Githiori *et al.*, 2004). The problem of drug resistance has been limited the use of drugs (Mideo *et al.*, 2013). In addition drugs are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions (Alavijeh *et al.*, 2012). This leads to search for new drugs by pharmacological screening of medicinal plants. Study by McCorkle *et al.*, (1996) indicates that 80% of people in developing countries rely on phytomedicine for primary healthcare in both humans and animals.

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine (Sukanya *et al.*, 2009). Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Such plants should be investigated to better understand their properties, safety and efficacy (Prusti *et al.*, 2006). Today there is a continuous and urgent need to discover new drugs with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against various diseases.

*Parthenium hysterophorus* has been used as folk remedy for the treatment of infectious and degenerative diseases. The phytochemical investigation of *P. hysterophorus* revealed the presence of various chemical constituents which plays role in pharmacological effects viz., alkaloids, proteins, saponins, tannins, carbohydrate, glycosides, terpenoids, steroids, volatile oils, amino acids, amino sugars, lignans, phenolic compounds, flavonoids, metallic elements, organic acids, terpenoids and others (Lakshmanan *et al.*, 2013). Phytochemicals like alkaloids, tannins, and glycosides which have been associated with anthelmintic activity (Sarojini *et al.*, 2011). Tannins are known to produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite. They hinder energy production in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997).

Different extracts of *P. hysterophorus* tested for antimicrobial potential showed varying degree of

antimicrobial activities (Martin, 1997). But effects of *P. hysterophorus* against parasites is not well studied therefore this research address this gap.

## Materials and Methods

**Study Area:** The experimental study was conducted from September 2015 to April 2016 at Haramaya University

### Ethics approval

The experiment doesn't involve live animals, no ethical clearance was needed

**Study Plant Collection and Extracts:** Fresh *Parthenium hysterophorus* collected from locally available yards which were authenticated at Haramaya University. Approximately 1kg fresh *Parthenium* leaf, bark, flower, and root were separated and washed for 2-3 times with running tap water and then with sterile water and followed shade drying at room temperature for two weeks. The powdered in a mixer and fine powder is collected by passing through sieve. The fine powder is used for extraction.

**Preparation of solvent extractions:** Fine powder were soaked in methanol in the ration of dry powder to solvent (1:5) in separate flask and shaken for 24hrs by automatic orbital shaker (Sharma, and Gupta 2012). The mixture was later strained using a muslin cloth and filtered using a Whatman filter paper (No. 1: 125mm) and the filtrate was concentrated in a vacuum rotary evaporator and was evaporated to dryness in an air oven at 40°C. After complete solvent evaporation, the filtrates were stored in capped labeled bottles and kept in the refrigerator at 4°C until use (Bagavan *et al.*, 2009). 15 mg of each solvent residue were dissolved in 1ml of Dimethyl Sulfoxide (DMSO) as a solvent and were used as the test extracts for anthelmintic activity. The extraction rate (%) was calculated as follows (Eloff, 1999).

$$\text{Extraction rate (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of plant before extraction (g)}} \times 100$$

**Phytochemical screening;** The methanol extract of *Parthenium hysterophorus* subjected to phytochemical screening using a standard screening procedure to know secondary metabolites for the detection of the saponins, tannins, phenolics, alkaloids, steroids, flavonoids, glycosides and phlobatannins (Harborne, 1973) as shown in Table 1.

Table 1. Methods used in phytochemical screening of the study plant

Chemicals analyzed	Amount in ml	Methods used	Time	Result
Saponin	1.0ml	Frothing test	3 min	Frothing remains for 10-15 min
Tannins	2.0 ml	Ferric chloride test	10 min	Blue-black coloration
Phenolics	0.2 ml	Ferric chloride test	10 min	Bluish-green coloration
Alkaloids	1.0 ml	Mayer's test	5 min.	Brown or white precipitate
Steroids	1.0 ml	Liebermann Burchardt test	3 min	Ring of blue-green coloration
Flavonoids	3.0 ml	Shinoda test	3 min	Pink or red coloration
Glycosides	0.5 ml	Ferric chloride test	15 min	Green to black precipitate
Phlobatannins	0.2 ml	Hydrochloric acid test	5 min	Red precipitate

**Working Concentrations:** The same concentration (1000, 500, and 250 mg/ml) were used for adulticidal, larvaecidal and egg hatchability efficacy test. 0.5% DMSO and distilled water were used as negative control while albendazole used as positive control.

### In vitro Anthelmintic Activity

*In vitro* anthelmintic activity of the plant extract is evaluated against *H. contortus* using

- ✓ Adult motility assay (AMA)
- ✓ Egg hatch test (EHT)
- ✓ Larval inhibition test (LIT)

For EHT, procedure described (Coles *et al.*, 1992) is adopted while AMA methodology is designed according to (Singh *et al.*, 1985).

**Adult Motility Assay:** Live worms are collected by giving the longitudinal incision along the greater curvature of abomasum of freshly slaughtered sheep in the local Haramaya abattoir. The worms present in ingesta or attached to the surface of guts are picked manually using forceps. The worms are washed and finally suspended in a bottle containing cooled (4°C) phosphate buffer saline (PBS). Five worms were exposed in three replicates to each of the following treatments in separate petri dishes at room temperature (25-30°C):

- ✓ Group 1: Treated with different concentrations (1000, 500, 250 mg/ml) of extract
- ✓ Group 2: Treated with different concentration of albendazole {positive control (1000, 500, 250 mg/ml)}
- ✓ Group 3: Phosphate Buffered Saline (sham treatment)

The inhibition of motility and/or mortality of the worms kept in the above treatments were used as the criterion for anthelmintic activity. The motility is observed after 0, 1, 2, 3, 4, 6, 8, 12 and 24 hours intervals. Live and dead worms are recorded for each group. The percentage mortality was calculated by using a formula given elsewhere (Krishnaveni and Venkatalakshmi, 2014).

$$\text{Mortality (\%)} = \frac{\text{Number of dead parasite}}{\text{Total number of parasite}} \times 100$$

### Egg Hatch Test

Female *Haemonchus contortus* were crushed using pestle and mortar to release egg. Approximately 100 freshly collected eggs (1 ml egg suspension) are added per test tubes and mixed with the same volume of concentrations plant extract and albendazole. Egg suspension and phosphate buffer saline is administered to the control test tubes. The test tubes are incubated at 27°C for 48 hours. Un hatched eggs as well as first stage larvae in each well of the plate are counted. Three replicates are used for each concentration of extract.

**Larval inhibition test:** The egg of *Haemonchus contortus* was cultured in sterile sheep feces for three weeks then larvae (L3) were collected using Baerman technique. 1ml of the water containing larvae is placed in each test tube already containing different concentrations of the plant extract, albendazole and distilled water then observations was recorded after examination of test sample microscopically at 1hrs, 2hrs, 6hrs, 12hrs, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11days, followed by active and dead larvae counted for each time.

### Data Analysis

Statistical comparison was determined by one way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukey's test/HSD) to compare parameter within and between groups. The results were expressed as mean  $\pm$  standard error of mean, the difference between the means were considered significant at  $p < 0.05$ .

### Results

Physical characteristics features and percentage yield of the extracts are shown in table 2. Variation in the yield among different plant parts was observed but not significant. Percentage yield was higher for leaf extract and lower for bark. The plant assessed for phytochemical and the presence of different secondary metabolites was confirmed as indicated in table 3

*In vitro* adult mortality activity test revealed that adult *Haemonchus contortus* do have longer survival time after exposure to different concentration of albendazole. Its longest survival time was 24hrs which was the longer than plant extracts and the negative controls. Regarding the efficacy of different parts of *P.hysterophorus* leaf extracts show significant number of adult death within three hours and hence the most effective against adult *Haemonchus* at different dose however more effective at higher dose. In positive treated control groups the majority of larvae which receive a concentration of albenadozle 1000mg/ml die within 12 hours but low dose at concentration of 500 mg/ml and 250 mg/ml shows higher mortality after exposure to one day but very small number of larvae survive maximum of four day in 250 mg/ml treated groups. The negative control which was left untreated and treated with 5% DMSO, result indicates few number of larvae die at day two but significant number of larvae were die at day five, but very few numbers of larvae able to survive until day 11.

The result on egg hatchability indicates only three in root, two in bark and leaf, and one flower crude extract treated group shows hatched larvae, these indicates almost all eggs are not hatched. Similar results were seen both in positive and negative control.

Death of larvae before and at 6hrs exposure the following is the comparison scenario: Leaf extract shows the highest percentage of mortality which was 60% at 1 hour and 40% at 2hour for higher dose exposure 1000mg/ml, there is also higher mortality percentage for 500mg/ml and 250mg/ml which was 80% and 60% at 1 hour and 20% at 6hour respectively this indicates leaf stand the first in killing larvae than others. Higher dose of flower 1000mg/ml shows 60% death at 6hours while no death seen at lower doses. 60% of death seen at 1000mg/ml dose of root at 2hours while 20% seen at 6hour; for 500mg/ml 40% death at 6 hours. But no death seen at this time range from positive control group receiving different concentration of albendazole and negative control groups in distil water and 0.5% DMSO (Table 4).

Death of larvae (L3) after 6hours of exposure indicates 60% death in 12hours for larvae exposed to bark 500mg/ml while 80% death in 12 hours for larvae exposed to 1000mg/ml dose of albendazole. The longest time surviving larvae seen in bark 250mg/ml treated group which was 60% death in 6 day and 40% death at 11day. In negative control groups 40% death seen at day 2 and day 5 while 20% die at 11days (Table 4).

The table 5 discusses the comparison efficacy of different part of *Parthenium hysterophorus* to adult *Haemonchus contortus*. At 3hrs of exposure there was significant different in killing between plant parts, which were higher mortality in leaf and flower but low for bark and root treated groups ( $P < 0.05$ ). There was a significant different between different extract treatment groups at 4 to 8hours were bark extract treated shows highest adult death ( $P < 0.05$ ) which was relatively delay than leaf and flower but faster than root. No statistical

difference seen for different concentration group. In case of positive and negative control groups all death was seen after >12 hours. Generally leaf and flower show more effective for adult *Haemonchus contortus* than bark and root in treating since it show killing of adult parasite in short period than others.

Table 2. Physical characteristic features and percentage yield of plant extracts

<i>Parthenium hysterophorus</i> part	Weight of dry Powder (g)	Weight of extract (g)	Yield (%)	Extract Color	Extract Consistency	Solvent for Dilution
Leaf	150	17.88	11.92	Dark green	Semiliquid	Methanol
Flower	150	16.50	11	Yellow	Semi Oil	Methanol
Bark	150	15.09	10.06	Dark green	Semiliquid	Methanol
Root	150	15.97	10.65	Yellow	Semiliquid	Methanol

Table.3 Preliminary phytochemical screening of Methanolic extract of *Parthenium hysterophorus*

Phytochemical	Test	Result
Steroids	Libermann Burchardt test	+
Flavonoids	Shinoda test	-
Alkaloids	Mayer's test	+
Phenol	Ferric chloride test	+
Tannins	Ferric chloride test	+
Saponins	Frothing test	+
Glycosides	Ferric chloride test	+
Phlobatannins	Hydrochloric acid test	-

Table 4. Comparisons of larvacidal activity of *Parthenium hysterophorus* against *Haemonchus contortus* larvae (L3) at 6hrs, 1day and after 1 day exposure

Concentration	Mean Mortality % and SE											
	Before and/or at 6hrs				12hrs to 1 day				After one day			
	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root
1000 mg/ml	100 <sup>a</sup> ±0	66.7 <sup>a</sup> ±6.7	13.4 <sup>a</sup> ±13.4	53.4 <sup>a</sup> ±13.4	0 <sup>a</sup> ±0	20 <sup>b</sup> ±11.6	13.4 <sup>a</sup> ±13.4	26.7 <sup>a</sup> ±13.4	0 <sup>a</sup> ±0	13.4 <sup>a</sup> ±13.4	73.4 <sup>a</sup> ±13.4	20 <sup>a</sup> ±0
500 mg/ml	100 <sup>a</sup> ±0	33.4 <sup>a</sup> ±33.4	26.7 <sup>a</sup> ±26.7	40 <sup>a</sup> ±23.01	0 <sup>a</sup> ±0	66.7 <sup>ab</sup> ±33.4	0 <sup>a</sup> ±0	6.7 <sup>a</sup> ±6.7	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	73.4 <sup>a</sup> ±26.7	53.4 <sup>a</sup> ±6.7
250 mg/ml	73.4 <sup>ab</sup> ±17.6	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	13.4 <sup>a</sup> ±6.7	46.7 <sup>a</sup> ±6.7	20 <sup>a</sup> ±20	20 <sup>a</sup> ±11.6	0 <sup>a</sup> ±0	53.4 <sup>a</sup> ±6.7	80 <sup>a</sup> ±20	80 <sup>a</sup> ±11.6
PC	0±0				80±11.6				20±0			
NC	0±0				0±0				100±0			

PC: Positive control

NC: Negative control

a=leaf; b flower; c= bark and d= root

Values are expressed as mean of mortality parentage ± standard error (SE). Mortality parentage values with different letters in the same row for each time exposure are significantly different (P < 0.05)

Table 5. Comparisons of adulticidal activity of *Parthenium hysterophorus* against *haemonchus contortus* at ≤3hrs, 4 to 8hours and >12 hours exposure

Concentration	Mean Mortality % and SE											
	≤3hrs				4 to 8hours				>12 hours			
	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root
1000 mg/ml	83.3 <sup>a</sup> ±16.7	100 <sup>a</sup> ±0	50 <sup>a</sup> ±0	16.7 <sup>a</sup> ±16.7	16.7 <sup>ab</sup> ±16.7	0 <sup>a</sup> ±0	50 <sup>a</sup> ±25	58.4 <sup>a</sup> ±22	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	25 <sup>a</sup> ±25
500 mg/ml	83.3 <sup>a</sup> ±8.3	91.7 <sup>a</sup> ±8.4	25 <sup>a</sup> ±0	8.4 <sup>a</sup> ±8.4	16.7 <sup>a</sup> ±8.4	8.4 <sup>a</sup> ±8.4	58 <sup>b</sup> ±30	58.4 <sup>a</sup> ±8.4	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	16.7 <sup>a</sup> ±8.4	33.4 <sup>a</sup> ±8.4
250 mg/ml	25 <sup>a</sup> ±14.4	50 <sup>a</sup> ±28.9	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	66.7 <sup>a</sup> ±16.7	50 <sup>b</sup> ±28.9	75 <sup>a</sup> ±0	33.4 <sup>a</sup> ±8.4	8.4 <sup>a</sup> ±8.4	0 <sup>a</sup> ±0	0 <sup>a</sup> ±0	66.7 <sup>a</sup> ±8.4
PC	0±0				0±0				100±0			
NC	0±0				0±0				100±0			

PC: Positive control

NC: Negative control

a=leaf; b flower; c= bark and d= root

Values are expressed as mean of mortality parentage ± standard error (SE). Mortality parentage values with different letters in the same row which compares between different parts of plant for each time exposure are significantly different (P < 0.05).

## Discussion

The concentration of metabolites like alkaloids, tannins, and glycosides is highest in leaves followed by, fruit, root, and stem (Kapoor, 2012). The variation in activity of the extract type of the plant part might be due to difference in the proportion of the active components and also the activity of botanical compounds found from plant materials depends on the type of extractant and the method of extraction (Eloff, 1998).

Increasing the concentration of the plant extracts resulted in increased mortality of larvae and adult in dose dependent activity. The results of our finding supported by work of other scholar that shows *P.hysterophorus* leaf extracts had higher anthelmintic activity compared with flower, bark, and root (Min *et al.*, 2005). Egg hatching inhibition test, reveals nearly all eggs incubated did not hatch. Some studies showed that as *Parthenium hysterophorus* is a rich source of tannin, it reduce the hatching of faecal eggs (Belay *et al.*, 2013). The tannins could also bind with feed nutrients and possibly prevent bacterial growth in the faeces (larva feed on bacteria) and so limit the feed available for larval growth, or in some other way inhibit larvae growth and movement (Belay *et al.*, 2013).

## Conclusion and Recommendations

The result demonstrated that, methanol extracts of *Parthenium hysterophorus* shown promising *in vitro* anthelmintic activity against adult and larvae (L3) of *H. contortus*. Extracts from leaf and flower have showed relatively good effect. Based on the above finding the following recommendations are forwarded

- ✓ *In vivo* anthelmintic activity should be done in future especially using leaf and flower crude extracts.
- ✓ Other method of extraction should be done one root and barks since this are relatively low efficacy than leaf and flower.
- ✓ The low efficacy of Albendazole needs attention.
- ✓ Further studies are required to screen active ingredients and toxicological evaluation should also be needed to performed

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## Competing Interests

All authors agreed on the publication of this manuscript and declare that they have no competing interests.

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