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Herbal Extract as Alternative to Chemical Drugs Against *Haemonchus contortus*, in Ethiopia

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Abstract: Experimental study was done on anthelmintic effects of methanol extract of *Parthenium hysterophorus* leaf, stem, flower and roots against *Haemonchus contortus*. Three graded concentrations of *P. hysterophorus* crude extracts (100 mg/ml, 50 mg/ml and 25 mg/ml) were prepared for each leave plant parts in dimethyl sulfoxide then evaluated for *in vitro* anthelmintic effects against *Haemonchus contortus* using standard techniques of Adult Motility Assay (AMA); Larvae Inhibition Test and Egg Hatch Test (EHT). *Parthenium hysterophorus* leaf extract showed significant number of death (larvae L3) in short time followed by flower, in comparison to other parts and control groups ($P<0.05$). There was also significant number of death (adult) in groups treated with leaf and flower crude extracts with in short period of time as compared to other parts, even faster than the positive control ($P<0.05$). The crude extract showed dose and time dependent larvaecidal and adultcidal effects against tested, *H. contortus*. The best larvaecidal and adultcidal was demonstrated by leaf; 100% mean mortality percentage was seen within 2 hours for larvae and 3 hours for adult. But the effect of *P. hysterophorus* in egg hatchability was not visible. Therefor methanol extract of *P.hysterophorus* possessed anthelmintic effects especially the leaf and flower worked best against adult and larvae (L3). For appropriate recommendation and usage of the plant as herbal anthelmintic further studies on toxicity and *in vivo* test needs to be done.

Key words: *Haemonchus contortus* · *Parthenium hysterophorus* · Anthelmintic Activities

INTRODUCTION

Infection with *Haemonchus contortus* is among the most common and economically important disease that limit production of small ruminants in developing countries where mismanagement and poor control practices are prevalent [1]. *Haemonchus contortus* is a highly pathogenic parasite of small ruminants causing acute disease and high mortality [2]. The life cycle of *Haemonchus* is direct where adult male and female worms reside in the abomasum and reproduce sexually. The cycle begins when infective (L3) stage larvae are ingested by a sheep on pasture. Development to the adult stage takes about three weeks in the gut; then adult worms attach to abomasal mucosa and feed on their blood. The eggs produced during this stage secreted in the animal's faces, depending on local environment egg hatch and develop through the immature developing stages (L1 and L2) to infective stage (L3) in the pasture [3].

The general mechanism of nematode parasitic control is the use of anthelmintic drugs but currently because of several factors including the issue of drug resistance nematodes limits the use of these chemical drugs [4]. In addition public health concern of drug residue in food animals and also drugs are sometimes associated with adverse effects on host, which include hypersensitivity, immunosuppressant and allergic reactions, are also current issues regarding the use of anthelmintic drugs [5]. These push different scholars to search for new, safe and effective drugs by pharmacological screening of medicinal plants. In developing countries, traditional medicine is accessible and affordable treatment [6]. Study by McCorkle *et al.* [7] reported 80% of people in developing countries rely on phyto-medicine for primary healthcare both humans and animals.

The medicinal plants considered as rich resources of bioactive molecules which can be used in drug development and synthesis [8]. Ethno veterinary research,

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play an important role that help to evaluate effective, available and low cost anthelmintic of plant origin [9]. Plants have been used from ancient times to cure diseases of man and animals. Attempts are being made to identify new naturally occurring plants having anti-parasitic activity [10]. Most of the drugs today are obtained from natural sources or semi synthetic derivatives used in the traditional systems of medicine [11]. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant [12]. Antimicrobial and anthelmintic compounds of plant origin may be found in plant stems, roots, leaves, bark, flowers, or fruits [13].

Parthenium hysterophorus is invasive weed which is cited as the seventh most devastating and hazardous weed. It adapts various agro-climatic conditions and almost distributed to range of environmental conditions [14]. In Ethiopia, *Parthenium* has become a notorious weed since its discovery in the 1980's. It has been spreading from the eastern route of Ethiopia between 1974 and 1980 [15]. This noxious weed was known for its adverse effect on environment, biodiversity, agriculture and health of animals and human [16]. Though the plant is invasive weed and shows some toxic effects, it shows various medicinal values including anti-inflammatory, antimicrobial, anthelmintic, antifungal, anti-cancerous, pesticide, thrombolytic activities [17].

Parthenium hysterophorus has been used as traditional remedy for the treatment of infectious and non-infectious diseases. The phytochemical investigation of the plant by different scholars reported the presence of various chemical constituents which plays role in pharmacological effects: for example, alkaloids, proteins, saponins, tannins, carbohydrate, glycosides, terpenoids, steroids, volatile oils, amino acids, amino sugars, lignans, phenolic compounds, flavonoids, metallic elements, organic acids and terpenoids are known constituents of this plant [18-20]. Different mechanisms of action of these phytochemicals have been suggested. They may inhibit microorganisms; interfere with some metabolic processes or may modulate gene expression and signal transduction pathways [21-23].

Researches indicated phytochemicals like alkaloids, tannins and glycosides have been associated with anthelmintic activity [24]. Tannins are known to produce anthelmintic activity through binding to glycoprotein on the cuticle of the parasite. They hinder energy production in helminth parasites by uncoupling oxidative phosphorylation [25]. Various extracts of *P. hysterophorus* tested showed different degree of antimicrobial activities [26, 27]. But effect of *P. hysterophorus* against parasites is not well studied. Currently there is a continuous and urgent need to discover new drugs with diverse chemical structures and novel mechanisms of action for drug resistant nematodes. Therefore, this researcher was done with objective to evaluate the *in vitro* anthelmintic effect of *Parthenium hysterophorus* against *Haemonchus contortu*.

MATERIALS AND METHODS

Plant Extractions: The experimental study was conducted at Haramaya University on freshly harvested *Parthenium hysterophorus* from Haramaya University farms and authenticated at plant sciences department. One kg fresh *Parthenium* leaf, bark, flower and root were separated and washed for 2-3 times with running tap water and allowed for shade drying at room temperature for two weeks. The powdered was prepared and collected by passing through sieve then fine powder is used for extraction. Fine powder were soaked in methanol in the ration of one to five, dry powder to solvent and shaken for 24 h by automatic orbital shaker. The mixture was later sieved using a Whatman filter paper (No. 1: 125 mm) and the filtrate was concentrated in a vacuum rotary evaporator. The filtrates were stored in capped labeled bottles and kept in the refrigerator at 4°C until use [28]. Fifteen mg of each solvent residue were dissolved in 1 ml of Dimethyl Sulfoxide (DMSO) as a solvent and used as the test extracts for anthelmintic activity.

Phytochemical Screening: The crude methanol extract of *P.hysterophorus* was subjected to phytochemical screening using a standard screening procedure for the presence of secondary metabolites with pharmaceutical values including anthelmintic properties: saponins, tannins, phenolics, alkaloids, steroids, flavonoids, glycosides and phlobatannins using the method described by Harborne [29].

Working Concentrations: Three graded dose of crude extract for each plant part (100, 50 and 25 mg/ml) were used for adultcidal, larvaecidal and egg hatchability efficacy test. 0.5% DMSO used as negative control while albendazole as positive control.

Anthelmintic Assay: *In vitro* anthelmintic activity of the *P. hysterophorus* extract of leaf, stem, flower and root was evaluated against *H. contortus* using three standard test methods (Adult motility assay (AMA), Egg hatch test (EHT) and Larval inhibition test (LIT)) as described by Coles *et al.* [30] and Singh *et al.* [31].

Adult Motility Assay: Live worms were collected from freshly slaughtered sheep in the local Haramaya municipal abattoir. The worms present in ingesta or attached to the surface of guts were picked manually using forceps. The worms were 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 (100, 50, 25 mg/ml) of *P. hysterophorus* extract (leaf, stem, flower and root).
- Group 2: Treated with different concentration of albendazole {positive control (100 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, 3, 6, 12 and 24 h intervals. Live and dead worms were recorded for each group. The percentage mortality was calculated by using a formula given elsewhere [32]. Mortality (%) is the ration of number of dead parasite from the total number of parasite exposed to crude extract.

Egg Hatch Assay: Female *H. contortus* were spared from collected sample by gross observation of vulvar flap under microscope and then crushed using pestle and mortal to release eggs. One ml of egg suspension approximately containing 100 freshly collected eggs were added per test tubes and mixed with the same volume of concentrations of *P. hysterophorus* extract and albendazole; in addition untreated egg suspension used for control test tubes. The test tubes were incubated at 27°C for 48 h. Unhatched eggs as well as first stage larvae in each well of the plate were counted.

Larval inhibition Assay: Collected egg of *H. contortus* was cultured in sterile sheep feces for three weeks then larvae (L3) were harvested using Baerman technique. Larvae motility checked and approximately 1 ml of the water containing larvae was placed in each test tube containing different concentrations of the *P. hysterophorus* extract, albendazole and DMSO 0.5% then observations was recorded at 1 h 2 h, 6 h, 12 h, 1 day and also observation was continued to the end of the day that all larvae die out, followed by active and dead larvae counted for each time.

Data Analysis: One way analysis of variance (ANOVA) was used to draw the result with multiple comparison tests (Post Hoc/Tukey's test/HSD) to compare parameter within and between groups. The results were expressed as mean ± standard error of mean, the difference between the means were considered significant at $p<0.05$.

RESULTS

Mixed part of the crude extract was checked for the presence of secondary metabolites and the result disclosed the presence metabolites like saponins, tannins, phenolics, alkaloids, steroids and glycosides except flavonoids and phlobatannins (Table 1).

The overall degree of survival revealed that adult *H. contortus* showed longer survival time after exposure to albendazole. It was the longest survival time recorded during the experiment which was 24 hrs in comparison to *P. hysterophorus* extract (Figure 1). Among different parts of *P. hysterophorus* leaf extracts showed significant number of adult death in short time (3 hrs) and hence considered to be the most effective against adult *Haemonchus* at different dose however it was more effective at higher dose (100 mg/ml).

The result on egg hatchability test revealed that eggs exposed to different concentration of *P. hysterophorus* crude extract from all four parts (root, bark, leaf and flower) showed no effect on egg hatchability. Similar results were seen in positive control group and negative control where majority of eggs were hatched.

Leaf crude extract showed 100% mortality of larvae (L3) with in 2 hours at higher dose exposure (100 mg/ml). In addition, larvae exposed to 50mg/ml and 25mg/ml dose also showed higher percentage mortality of 100% and 80% at the range of 6hrs respectively (Figure 2). Regarding exposure of larvae to higher dose of flower extract (100 mg/ml), the result displayed 60% death at 6hrs while no death seen at lower doses (25 mg/ml). In case of larval group exposed to 100mg/ml of root extracts, 80% of death was seen at 6hrs; while 40% of larval death when exposed to 50mg/ml concentration.

The result of larvae mortality in bark extract group showed 60% and 80% mortality at 12 h for 50 mg/ml and 100 mg/ml dose. The longest larvae surviving time was seen at concentration of 25 mg/ml bark treated group with 60% of death at 6 day and 40% death at 11day. In negative control groups, 40% of death was seen at day 2 and day 5 while 20% of larvae died at 11days (Table 2).

Table 1: Phytochemical screening of methanol 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 2: Comparisons of larvacidal activity of *Parthenium hysterophorus* different part against *Haemonchus contortus* larvae (L3)

	Mean Mortality % and SE											
	≤ 6h				6h to one day				> one day			
Concentration	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root
100mg/ml	100^{a}±0	66.7^{b}±6.7	13^{c}±13	53.4^{d}±13	-	20^{b}±11.6	13.4^{b}±13	26.7^{b}±13	-	13.4^{b}±13	73.4^{c}±13.4	20^{b} ±0
50mg/ml	100^{a}±0	33.4^{b}±33	27^{c}± 26	40^{d}± 23	-	66.7^{b}±33	-	6.7^{d}±6.7	-	-	73.4^{c}±26.7	53.4^{c}±7
25mg/ml	73^{a}± 17	-	-	-	13^{a}±7	46.7^{a}±7	20^{a}±20	20^{a}±12	-	53.4^{c}±6.7	80^{c}±20	80^{c}±12
PC	-				80±.11.6				20 ±0			
NC	-				-				100±0			

PC: Positive control

NC: Negative control

-: no death recorded

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

Table 3: Comparisons of adultcidal activity of *Parthenium hysterophorus* different parts against *Haemonchus contortus*

	Mean Mortality % and SE											
	≤ 3hrs				4 to 8hours				>12 hours			
Concentration	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root	Leaf	Flower	Bark	Root
100 mg/ml	83^{a}±17	100^{a}±0	50^{b}±0	17^{c}±16	17^{a}±16	-	50^{b}±25	58^{b}±22	-	-	-	25^{d}± 25
50mg/ml	83^{a}±8	91.7^{a}±8	25^{b}±0	8.4^{c}±8	16.7^{a}±8.4	8.4^{a}±8	58^{b}±30	58.4^{b}±8	-	-	17^{c}±8	33^{d}±8.4
25mg/ml	25^{a}±14	50^{a}±29	-	-	66.7^{a}±17	50^{a}±29	75^{b}±0	33.4^{a}±8	8.4^{a}±8	-	-	66.7^{d}±8
PC	-				-				100± 0			
NC	-				-				100±0			

PC: Positive control

NC: Negative control

-: no death recorded

Values are expressed as mean of mortality percentage ± standard error (SE). Different letter in row show mean mortality difference between different treatment concentration is statistical significant (p value <0.05)

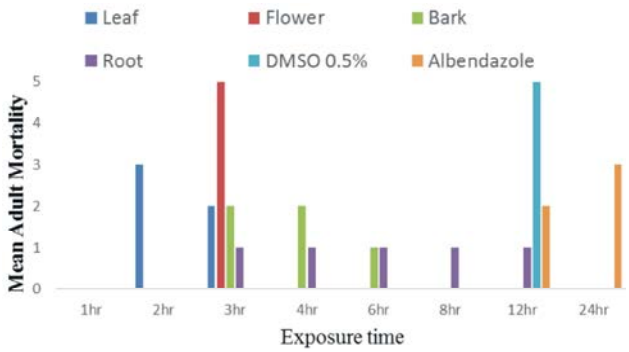

Fig. 1: Mean mortality of adult *H. contortus* after exposure to different parts of *Parthenium hysterophorus* at 100mg/ml

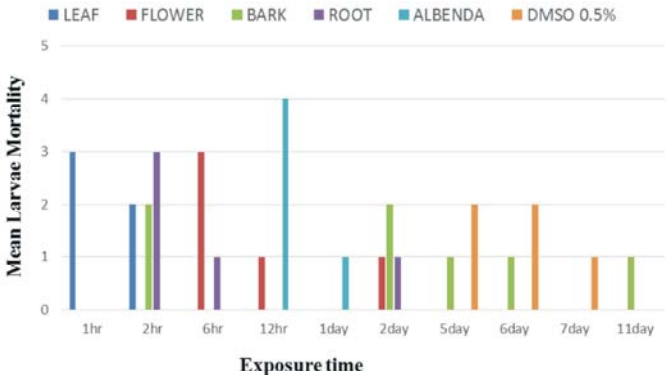

Fig. 2: Mean mortality of larvae after exposure different parts of *Parthenium hysterophorus* at 100 mg/ml

The result of comparative efficacy of different part of *P. hysterophorus* on adult *H. contortus* showed, observation at 3 hrs of exposure there was significant different in killing the adult *H. contortus* between plant parts, which were higher mortality in leaf and flower but low in bark and root treated groups ($P<0.05$). There was a significant different between different crude extract treatment groups at 4 to 8hours where bark extract treated group showed the highest adult death ($P<0.05$) that was relatively delayed than leaf and flower but faster than root. Though there was no statistical difference for different concentration group, relative reduction in the percentage mortality as dose decrease was recorded. In case of positive and negative control groups, all death was seen after >12 hours. Generally leaf and flower showed more effect against adult *H. contortus* than bark and root since it showed killing of adult parasite in short period than others (Table 3).

DISCUSSION

In vitro assays are commonly used to screen anti-parasitic properties of the plants and plant extracts with low costs and rapid turnover. In addition, this method measures the effect of anthelmintic activity directly on the processes of hatching, development and motility of parasites without interference of internal physiological functions of the host on pharmacodynamics and pharmacokinetic properties of drugs [33-36]. *H. contortus* proved to be model nematode because of its longer survival in phosphate buffer saline which allows more number of observations can be recorded on the motility of worms and larvae [37]. In addition, *Haemonchus* are recognized by their resistance for the several of anthelmintic drugs. These initiate researcher to evaluate the performance of different plant extract including Neem, Tamak, Corolla etc. against Haemonchosis [38].

P. hysterophorus is a rich source of different phytochemicals like alkaloids, tannins and glycosides which have been associated with anthelmintic activity [24]. Different research indicated the presence of variation in the concentration of secondary metabolites in different parts of plants; for example: - alkaloids, tannins and glycosides are the highest in leaves followed by fruit, root and stem [39]. Factors incriminated in the variation in pharmaceutical activity of the plant might be due to difference in the proportion of the active components in different plant parts, the stage of plants used the type of extractant and also the method of extraction [39].

Unlike to antibacterial and antifungal effects of *P. hysterophorus* few reports have addressed its anthelmintic activity. The results of the present study showed leaf, flower, bark and root exhibited *in vitro* anthelmintic activity against larvae & adult stage of *H. contortus* but no consistent result was seen in egg hatchability.

The present result on anthelmintic activity of *P. hysterophorus* showed dose dependent increase mortality percentage of larvae and adult of *H. contortus*. The results of our finding supported by work of other scholar that showed *P. hysterophorus* leaf extracts had higher anthelmintic activity compared with flower, bark and root [40]. Egg hatching inhibition test, revealed any of the crude extract didn't show significant effects in hatchability. The variation in activity of the extract type of the plant part might be due to difference in the proportion of the active components responsible for the tested anthelmintic activity resulting from the difference in solubility in methanol.

A report showed whole part of *P. hysterophorus* is good anthelmintic activity against *Pheretima posthuma* [41]. Some studies also reported *P. hysterophorus* is a rich source of tannins which reduces the hatching of faecal eggs [42]. The tannins could also bind with feed nutrients and possibly prevent bacterial growth in the faeces and so limit the feed available for larval growth, or in some other way inhibit larvae growth and movement [42]. Tannins may also react directly with adult worms by attaching to their "skin" and causing them distress, or indirectly by improving protein nutrition of the host and boosting the immune system [43].

CONCLUSION

The result of present study has proven, methanol extracts of *P. hysterophorus* showed promising *in vitro* anthelmintic activity against adult and larvae (L3) of *H. contortus*. Extracts from leaf and flower have showed relatively good effect. Further studies encompassing *in vivo* anthelmintic activity especially using leaf and flower crude extracts; screening of active ingredients and also toxicological evaluation should be instigated. In addition, the lower percentage morality by root and barks crude extract should also be checked by using different extraction methods.

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