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In-vitro Anthelmintic activity of *Smilax myosotiflora* Plant (locally known as Ubi Jaga) Extracts Against *Haemonchus contortus* Worms in Goats

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ABSTRACT This study was conducted to evaluate the *in-vitro* anthelmintic activity of *Smilax myosotiflora* plant (locally known as ubi jaga) extract against third-stage *Haemonchus contortus* larvae from goats. Two trials were conducted, whereby each trial followed similar protocols in which *Smilax* leaves were extracted using methanol solution. The larvae were than tested with different concentrations of the extract. *Smilax* plant extract was effective against worm larvae in goats. A 100% mortality had been achieved at 5 mg/ml of concentration.

(**Keywords**: *in-vitro*, *Smilax myosotiflora*, *Haemonchus contortus*, goats)

INTRODUCTION

Goats and sheep have numerous gastrointestinal parasites which can cause reduced growth rates, weight loss, unthriftiness, diarrhoea, decrease in feed utilization, and death. Several species contribute to parasitism in sheep, *Haemonchus contortus*, being the most devastating species, because of its blood-sucking behaviour.

There is extensive information available on the use of plants in traditional veterinary medicine, and researchers such as Hammond *et al.* (1997) have presented excellent reviews on the potential of using plant anthelmintics. Studies in Malaysia and Philippines reported the activity of *Azadirachta indica* (Neem plant) against nematode parasites in ruminants (Baldo 2001; Chandrawathani *el al*. 2002). Also, cattle provided with feed blocks containing different levels of dried leaves of *A. indica* had significantly lower faecal worm egg counts.

Smilax myosotiflora with the local common name of Ubi Jaga, is a herbaceous climber with slender smooth stem and branches. From recent studies, the extracts of *Smilax china* roots were reported to have high levels of radical scavenging activity to inhibit lipid peroxidation and enhance the effects of various antioxidant enzymes (Chen *et al*., 1999). *Smilax* rhizomes have various pharmacological activities (Ban *et al*. 2006), such as immunomodulatory (Jiang & Xu, 2003), antibacterial, antifungal, antioxidant and hepatoprotective (Chen *et al.* 1999).

However, very little is known on the use of *Smilax* plant on controlling endoparasites and its use as anthelmintics has not been tested. This present research was carried out to test the presence of anthelmintic activity, if any, of the *Smilax* plant extract against *H. contortus* nematodes from goats.

MATERIALS AND METHODS

Culturing of third-stage trichostrongylid worm larvae

Fresh goat faeces were collected *per rectum* from a farm in Penang and kept in sealed plastic bags and specimen bottles and taken back to the laboratory for cultures. Using a spatula, faecal samples were broken into pieces in a Petri dish partially filled with distilled water. The faecal mesh was then smeared onto one side of wet filter papers cut into 14 x 2 cm size, leaving about 4 cm of both ends of the paper free of any faecal smear.

Each piece of filter paper was then rolled and placed into a glass test tube filled with about 3 ml of distilled water. The test tube was then closed with a rubber stopper and incubated at 30^{o}C for 11 days.After incubation, the filter paper was removed from the test tube. The sides of the test tube were washed with distilled water so that any larvae that might be found on its sides would be washed down to the bottom of the test tube. Trichostrongylid larvae were sucked up using a pipette and used in the experiment.

Preparation of Smilax plant extract

The *S. myosotiflora* plant used in this research was obtained at the Herbal Garden of School of Biological Sciences in University Science of Malaysia. The main four parts of the plant i.e stems, tendrils, leaves and thorns were used for the extraction.The plant was cut and crushed to smaller pieces using a grinder. Extraction was carried out in a 100ml flask where the crushed

Smilax and 500ml of methanol were stirred for 1 hour at 60^{o}C. It was filtered through filter paper (Whatman No. 40) after leaving the methanolic solution to cool. The filtrate was then freeze-dried overnight.

Extract concentrations of the *Smilax* plant (1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml and 5mg/ml) were prepared by using an electrical weighing machine. Extractions of different concentrations were placed in separate Eppendorf tubes. Distilled water was added to the extractions in the Eppendorf tubes and shaken till the extractions were dissolved. Each Eppendorf tube was labelled according to its respective concentration. The anthelmintic activity was determined in 3 replicates for each concentration.

A 24-well microtitre plate was used when testing the efficacy of the extract against the trichostrongylid larvae of different concentrations in 3 replicates each time it was carried out. A total of 18 wells were used including the 3 replicates as experimental controls. The wells were labelled according to different concentrations used.

In-vitro testing of Smilax extract against trichostrongylid larvae

Ten trichostrongylid worms of similar size from the above culture were placed in each well. Prepared extractions of the *Smilax* plant were then being poured into the wells according to their respective concentrations.The mortality of the worms was recorded at intervals of 24, 48, 72 and 96 hr after treatment. The microtitre plate was shaken lightly for a while before observation was made. Three trials were carried out for each concentration and the mean value recorded.

Statistical Analysis

Statistical analysis were performed with the Statistical Programs fro the Social Science (SPSS) version 11.5. Data satisfied the assumptions of the general linear model and were not transformed. Statistical significance of data was assessed by analysis of variance (ANOVA). When ANOVA indicated there were significant effects ($p<0.05$), the Tukey test was used to compare the means.

RESULTS

Some mortality in larvae could be seen after being treated with *Smilax* extraction for 24 hr (Table 1). The percentage of mortality of treated larvae reached as high as 90% for 3 mg/ml, 4 mg/ml, and 5 mg/ml concentrations. However, there was 7 % mortality for the untreated larvae or control.After 48 hr, there were increases in mortality for extract concentrations of 1 mg/ml, 2 mg/ml, 5 mg/ml as well as the control except for 3 mg/ml and 4 mg/ml which still recorded 90% mortality. However, mortality of treated larvae for concentration of 5 mg/ml had reached 100% (Table 2). There was 13% mortality rate for control larvae.

Increases in mortality still occurred after 72 hr for every extract concentration including control, except for 4 mg/ml. The extract concentration of 3 mg/ml recorded 93% of mortality. It was higher than the mortality rate from 4 mg/ml (Table 3).Table 4 showed that there were increases on mortality percentages for all treated larvae while the mortality of the larvae from control did not change (Table 4). The concentration of 3 mg/ml recorded 97% mortality and was still higher than at concentration 4 mg/ml which recorded 93% mortality. The controls showed similar mortalities at both 72 hr and 96 hr of exposure to the plant extract.

After 120 hr, the mortality of control had reached 20% while both concentrations of 3 mg/ml and 4 mg/ml remained at the same mortality as after 96 hr which were 97% and 93% respectively (Table 5). Mortality at concentration of 2 mg/ml recorded 93% after 120 hr, which was similar at concentration 4 mg/ml. At 5 mg/ml concentration of extract *Smilax*, 100% mortality rate to larvae was recorded and proved to be the most effective.

Figures 1 to 5 showed the percentages of mean mortality of trichostrongylid larvae after being treated with the different concentrations of *Smilax* plant from all the results of three trials that had been recorded. The figures also showed the significant difference for the different concentrations treated on the larvae.

There was no significant difference between concentrations 3 mg/ml, 4 mg/ml, and 5 mg/ml (Figure 1). These three concentrations were significantly different with concentrations 1 mg/ml and 2 mg/ml, as well as the control while 1 mg/ml and 2 mg/ml concentrations also showed significant difference with control.

After 48 hr, there was significant difference between the mortality rate at concentrations 3 mg/ml and 5 mg/ml and no significant difference was seen between concentrations 2 mg/ml and, 3 mg/ml and 4 mg/ml which did not happen from the 24 hr period (Figure 2).

The same situation happened to the mortality rate from the 72 hr though the mortality rates of every concentration became higher including control (Figure 3).

Table 1: Mortality rate of trichostrongylid larvae treated with different concentrations of *Smilax* plant extract after 24 hr

Extract concentration (mg/ml)	Mortality of larvae after 24 hr						
	Replicates			Total	(%)	Mean	SE (±)
	1	2	3				
0	1	0	1	2	7	0.67	0.48
1	7	8	4	19	63	6.33	1.45
2	8	7	5	20	67	6.67	1.49
3	9	9	9	27	90	9.00	1.73
4	9	10	8	27	90	9.00	1.73
5	9	9	9	27	90	9.00	1.73

Table 2: Mortality rate of trichostrongylid larvae treated with different concentrations of *Smilax* plant extract after 48 hr

Extract concentration (mg/ml)	Mortality of larvae after 48 hr						
	Replicates			Total	(%)	Mean	SE (±)
	1	2	3				
0	1	1	2	4	13	1.33	0.67
1	7	8	6	21	70	7.00	1.53
2	10	8	6	24	80	8.00	1.63
3	9	9	9	27	90	9.00	1.73
4	9	10	8	27	90	9.00	1.73
5	10	10	10	30	100	10.00	1.82

Table 3: Mortality rate of trichostrongylid larvae treated with different concentrations of *Smilax* plant extract after 72 hr

Extract concentration (mg/ml)	Mortality of larvae after 72 hr						
	Replicates			Total	(%)	Mean	SE (±)
	1	2	3				
0	1	2	2	5	17	1.67	0.75
1	8	8	6	22	73	7.33	1.56
2	10	9	6	25	83	8.33	1.67
3	10	9	9	28	93	9.33	1.76
4	9	10	8	27	90	9.00	1.73
5	10	10	10	30	100	10.00	1.82

Table 4: Mortality rate of trichostrongylid larvae treated with different concentrations of *Smilax* plant extract after 96 hr

Extract concentration (mg/ml)	Mortality of larvae after 96 hr						
	Replicates			Total	(%)	Mean	SE (±)
	1	2	3				
0	1	2	2	5	17	1.67	0.75
1	9	10	6	25	83	8.33	1.67
2	10	9	8	27	90	9.00	1.73
3	10	10	9	29	97	9.67	1.79
4	9	10	9	28	93	9.33	1.76
5	10	10	10	30	100	10.00	1.82

Table 5: Mortality rate of trichostrongylid larvae treated with different concentrations of *Smilax* plant extract after 120 hr

Extract concentration (mg/ml)	Mortality of larvae after 120 hr						
	Replicates			Total	(%)	Mean	SE (±)
	1	2	3				
0	1	3	2	6	20	2.00	0.82
1	9	10	7	26	87	8.67	1.70
2	10	10	8	28	93	9.33	1.76
3	10	10	9	29	97	9.67	1.79
4	9	10	9	28	93	9.33	1.76
5	10	10	10	30	100	10.00	1.82

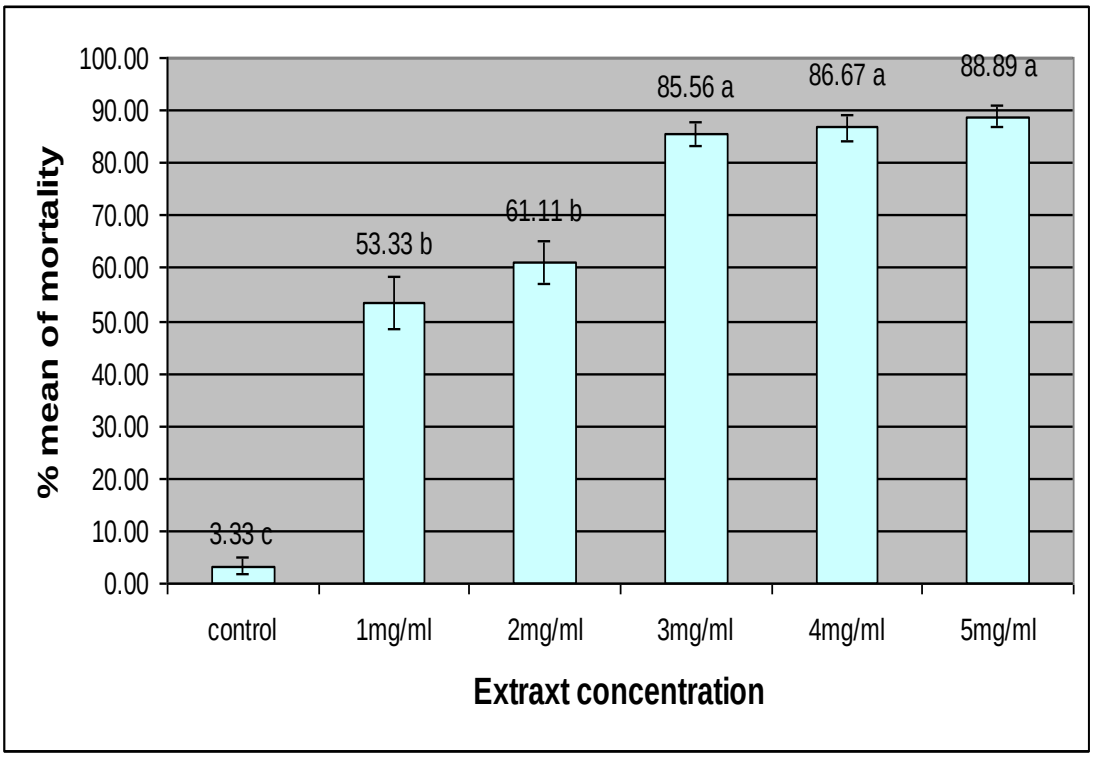
However, there were no significant differences observed from concentrations 3 mg/ml and 4 mg/ml, as well as concentrations 1 mg/ml and 2 mg/ml (Figures 1 & 2).The mortality rate for concentration 5 mg/ml showed no significant difference to both 3 mg/ml and 4 mg/ml though only 3 mg/ml and 4 mg/ml showed significant difference to concentration 2 mg/ml.

There were significant differences observed between control and other concentrations (Figure 2).After 120 hr, there were no significant difference observed between 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml concentrations (Figure 5). However, 2 mg/ml was significantly different from 1 mg/ml while concentrations of 3 mg/ml, 4 mg/ml, and 5 mg/ml showed significant differences to that of 1 mg/ml.

DISCUSSION

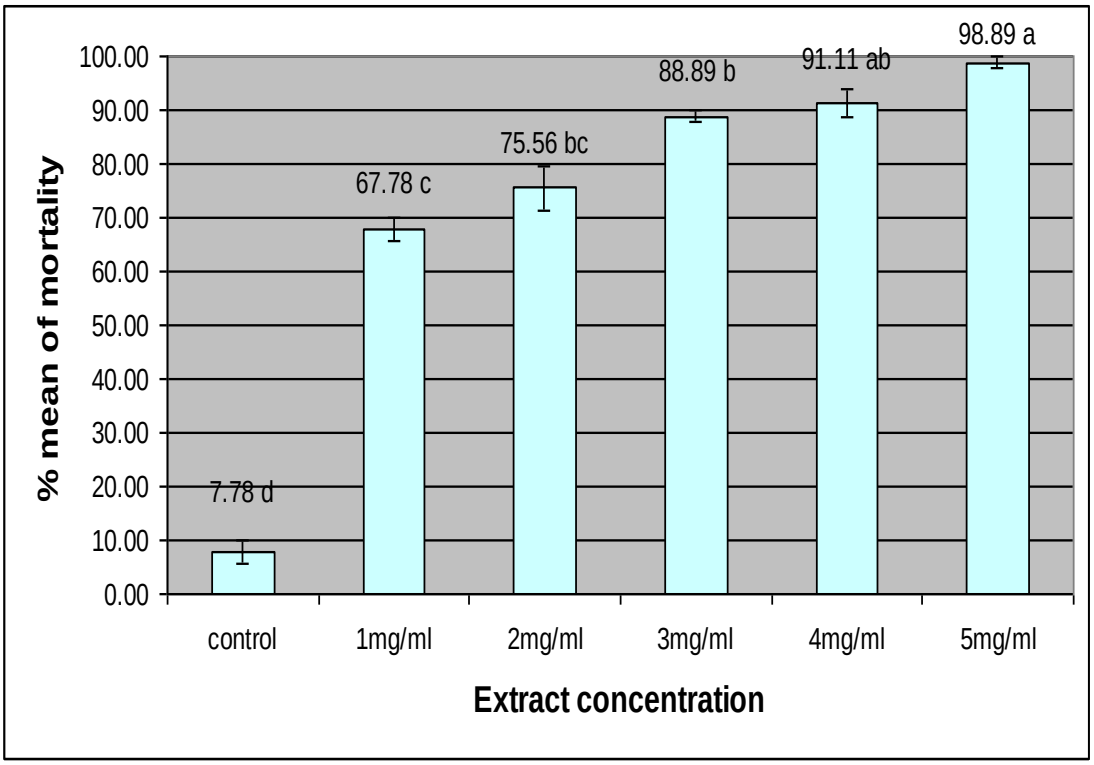
The present study indicated that *Smilax* plant extract was effective against trichostrongylid larvae of the goats. The percentage reduction of the larvae gave an indication of how effective the extraction of *Smilax* plant was at specifically being used as an antihelmintic.

A 100% of mortality rate had been achieved at 5 mg/ml of concentration. Although many traditional plants had been proved being active towards nematode parasites, using *Smilax* plant is still a new approach as an anthelmintic to nematode parasite control. There is little information available addressing the mechanism behind how the *Smilax* plant affects trichostrongylid larvae and other abomasal nematodes. However, *Smilax* plant proved to be effective against infective third stage trichostrongylid[3] larvae.



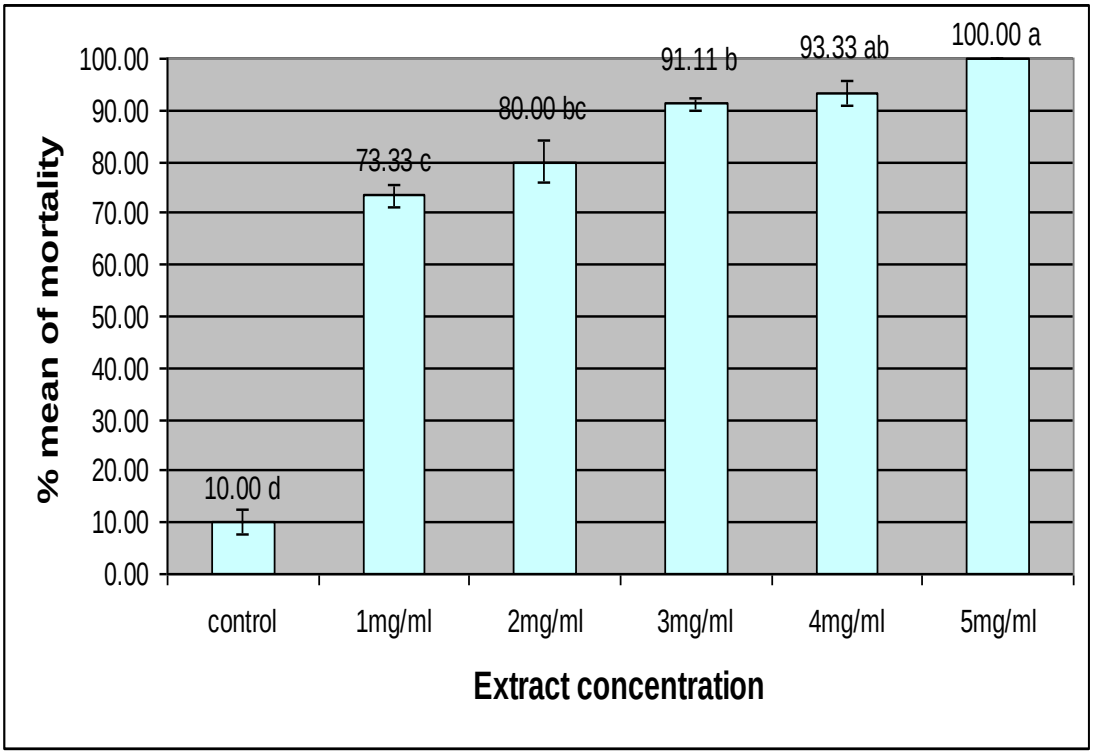
Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

Figure 1: Effect of exposure to extracts of *Smilax* plant on the mortality of trichostrongylid larvae during the 24 hr of treatment



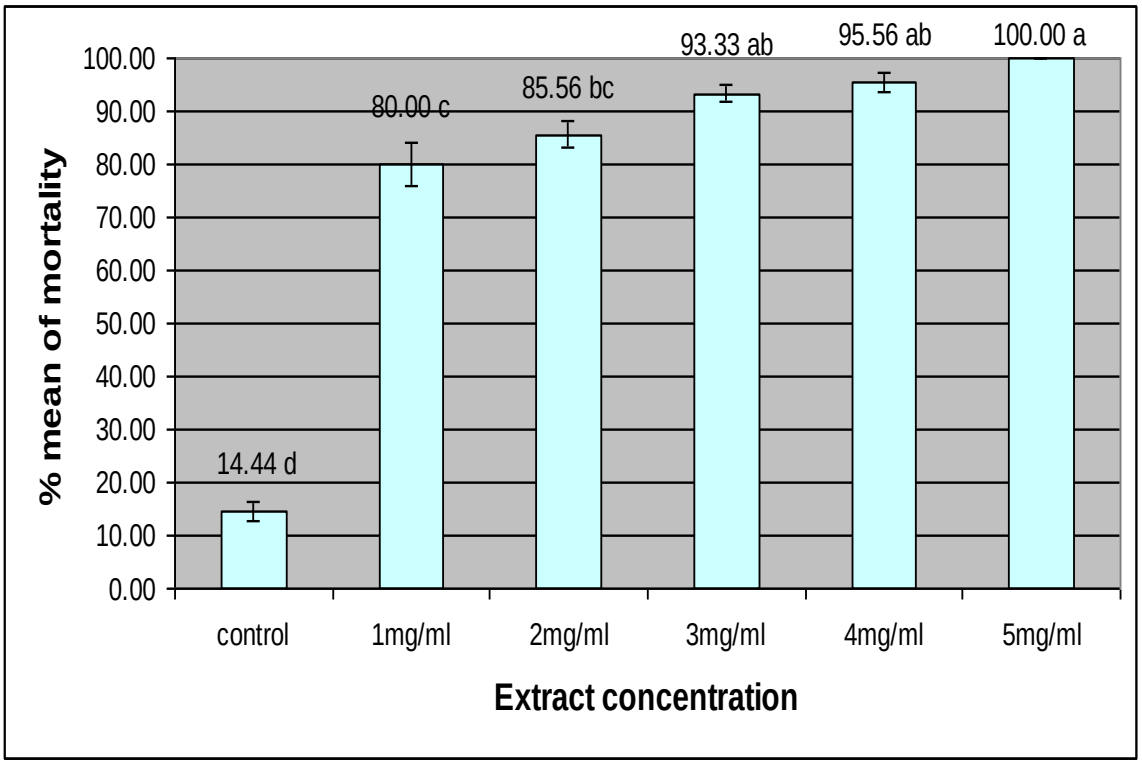
Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

Figure 2: Effect of exposure to extracts of *Smilax* plant on the mortality of trichostrongylid larvae during the 48 hr of treatment



Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

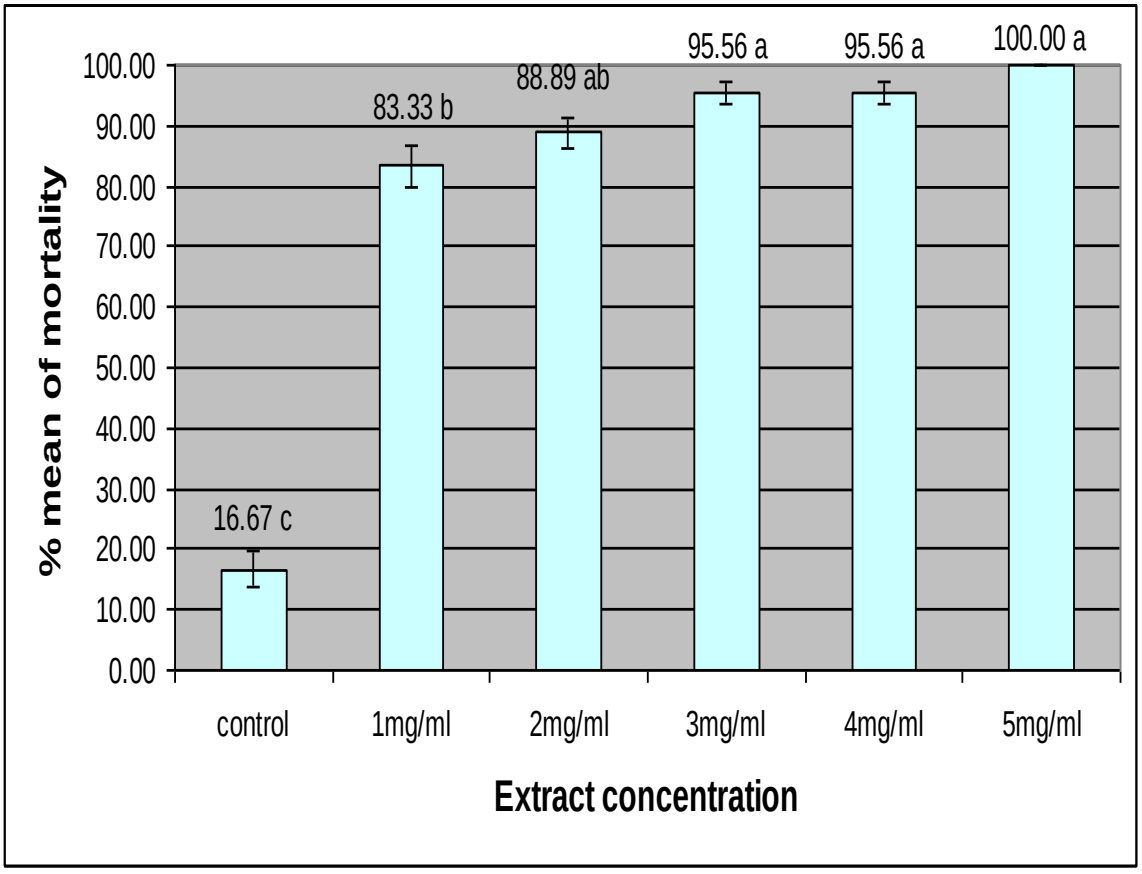
Figure 3: Effect of exposure to extracts of *Smilax* plant on the mortality of trichostrongylid larvae during the 72 hr of treatment



Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

Figure 4: Effect of exposure to extracts of *Smilax* plant on the mortality of trichostrongylid larvae

during the 96 hr of treatment



Means followed by a common letter do not differ significantly (Tukey's test, $p<0.05$).

Figure 5: Effect of exposure to extracts of *Smilax* plant on the mortality of trichostrongylid larvae during the 120 hr of treatment

Therefore, there are several assumptions to be made on the contributions of the *Smilax* plant that could possibly bring to control nematode parasites. When the goats and sheep were treated with *Smilax* plant, the number of larvae would be reduced.

Thus, the infection levels in the grazing host would consequently be reduced and causes fewer eggs being passed out in the faeces. Therefore, this lowered egg output, decreases the percentage of larvae on pasture available to infect the grazing animals, and so the overall free-living population is reduced. Treatment with *Smilax* plant extract showed a high mortality in the trial.

However, additional research needs to be conducted before *Smilax* plant could be safely and effectively used in an integrated parasite control program for goats and sheep. Besides, studies to determine the mode of action of the *Smilax* plant on the gastrointestinal parasites are needed to help maximizing the use of the plant as an anthelmintic.

However, the use of *Smilax* plant in conjunction with other control methods may be a useful tool for producers and help reduce reliance on the conventional use of anthelmintics for control.

CONCLUSIONS

Many traditional plants had been proved to be active against various species of nematode parasites. In the present study *Smilax* plant extract was shown to be effective against *Haemonchus contortus* in goats. In fact, 100% mortality had been achieved at 5 mg/ml of concentration.

Perhaps in the near future, the efficacy of this same plant could also be tested on other groups of nematode parasites to see its effectiveness.

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