Anthelmintic Activities of *Spondias mombin* Leaves and Fruits Extracts against Trichostrongylid Nematodes in Goats

Wahab A Rahman and Hiew Pay Pay
School of Food Science and Technology, Universiti Malaysia Terengganu
21030 Kuala Terengganu, Malaysia

ABSTRACT---- ***This research was carried out to determine the anthelmintic activity of Spondias mombin against trichostrongylid nematodes from goats and sheep. To achieve the purpose, Spondias fruits and leaves were used and their effect on mortality rates of trychostrongylid L_3 larvae were observed. Two different extract methods, the chloroform and 80% methanol extractions were used to extract the fruits and leaves. Results showed that the LC_{50} of S. mombin leaves extracts were lower for both chloroform and 80% methanol extractions than its fruits. The LC_{50} values for the leaves extract were 0.158mg/ml and 1.279mg/ml respectively for both methods. Whereas the LC_{50} of the S. mombin fruits extracts were 0.416mg/ml and 2.200mg/ml for chloroform and 80% methanol extracts respectively. This indicated that S. mombin leaf was more toxic compared to its fruits extract. The differences between concentrations were statistically significant ($p<0.05$). Anthelmintic activities of S. mombin increased with concentration.***

Keywords--- anthelmintic activity, *Spondias mombin*, trichostrongylid nematodes, sheep, goats, LC_{50}

1. INTRODUCTION

The most common and economically important disease in ruminants are parasitic nematodes (McLeod, 1995). Globally, parasitic diseases continue to be a major contribution to the loss of ruminant productivity. In Malaysia it is a particular problem for goats and sheep (Chandrawathani *et al.* 2002). Infected animals show reduction in outputs of animal product, by-products, manure and traction (Waller, 1999). Although gastrointestinal nematodes cause health problems, the impact of economic losses due to mortality is lower than losses in productivity (Waller, 2004). Thus, it is important to diagnose the clinical signs of parasitic infections such as anemia, bottle jaw, weight loss, diarrhea or scours before the situation develops into a worst condition.

The species of gastrointestinal nematodes infecting goats and sheep vary according to climate and season. In tropical and subtropical regions like Malaysia *Haemonchus contortus* is the most dominant in small ruminants, while *Trichostrongylus* spp. and *Ostertagia* spp. tend to be more common in temperate regions (Rahman 1997; Chandrawathani *et al* 2002).

There are many approaches to handling parasitic nematode problems. The most common method is the use of anthelmintics (McKenna and Watson 1987; Strong and Wall, 1990; Rahman, 1997). However, frequent and unsupervised used of anthelmintic have created anthelmintic resistance in small ruminants; once the parasite is resistant to an anthelmintic it will become resistant to the whole class of the anthelmintic and no longer affected by these anthelmintic (Waller, 1985; Sangster *et al* 1988; Taylor 1991). As a result, medicinal plants as alternative sources of anthelminthic drugs have been discovered (Chandrawathani *et al*, 2002a).

Spondias mombin grows in Nigeria, Brazil, and several other tropical countries in the world which is commonly used as traditional medicine (Elizabetshy, 1992). *Spondias mombin* is reported to possess antifungal, antimicrobial, antibacterial and antiviral properties (Ajao *et al*, 1985; Abo *et al*, 1999; Corthout *et al*, 1991; Rodrigues and Hasse, 2000. In *vitro* and in *vivo* studies had also been carried out to examine the direct anthelmintic effect of ethanolic and aqueous extract of *Spondias mombin* against gastrointestinal nematodes (Ademola *et al*. 2005). The present paper describes the efficacy of S*pondias mombin* anthelmintic activities against trichostrongylid nematodes in goats.

2. MATERIALS AND METHODS

1. *Spondias* leaf and fruit extracts preparation

Spondias leaves and fruits were washed thoroughly. The fruits with seeds were cut into small pieces and crushed using a blender. The fresh juice of fruit was then filtered out. The clean leaves were finely blended for extraction. The 80% methanol and chloroform extraction methods were used to extract the biological active component of the *Spondias*

plant. After grinding, both leaves and fruits were placed in 250ml beakers. Each beaker was added with either 80% methanol or chloroform until the chemicals covered the leaves or fruits. It was then placed in a heater to heat it up to 50° C for 1 h, after which the extracts were filtered and poured into petri dishes. The chloroform extraction of leaves and fruits were left in the fume hood to dry at room temperature overnight, while 80% methanol extraction of leaves and fruits were left in the dryer to dry overnight. Consequently four types of dry extracts were obtained (chloroform *S. mombin* leaves extract, chloroform *S. mombin* fruits extract, 80% methanol *S. mombin* leaves extract and 80% methanol *S. mombin* fruits extract). For 80% methanol extraction method, the highest concentration of 5mg/ml of extracts were prepared by dissolving the proper amount of dry extracts with a correct proportion amount of solvent. On the other hand, the highest concentration of 1mg/ml was prepared for the chloroform extraction of leaves and fruits. The dry extracts were weighed by using an electrical weighing machine (Model mark 205A with a capacity of 205.0000 g). Different solvents were used according to the extraction methods. Extracts prepared by 80% methanol extraction were dissolved using distilled water while the chloroform extraction of leaves and fruits were dissolved using 2% acetone. The different concentration extracts of 1mg/ml, 2mg/ml, 3mg/ml and 4mg/ml for the 80% methanol extracts were prepared by diluting the 5mg/ml 80% methanol extract with distilled water. On the other hand, different concentration chloroform extracts of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml were prepared from diluting the 1mg/ml chloroform extract with distilled water.

(ii) Culturing of trichostrongylid larvae

Fecal samples were obtained from goats reared on several properties in the country, where the goats had been reared solely for their meat. Feces were collected, mixed with vermiculite in glass bottles, and covered with aluminum foil. Cultures were maintained at 24° C for 14 days and then subjected to the Baermann technique for harvesting of infective larvae. Larvae were identified and stored at 10° C until use. More than 60% of the larvae collected were those of *Haemonchus contortus*. Two 8-week old goats reared worm free were infected with 10,000 mixed larvae by intra-ruminal infection.

At necropsy, 3 weeks after infection, the abomasum was removed and worms recovered. Adult *H. contortus* were separated. Two further worm-free goats were each infected with 3,000 adult *H. contortus* through a stomach tube. Feces were collected, cultured and the larvae harvested and stored as before.

(iii) Testing anthelmintic activity of *Spondias* extracts

Twenty four (24) well micro-titer plates were used to test the efficacy of anthelmintic activity of *Spondias* extracts at different concentrations. The wells were labeled according to the concentration used. Ten similar size strong larvae were picked up by pipette into each well then the prepared extracts were poured into the well according to concentration. The same step was repeated for each following well for different concentrations. For each concentration four (4) replicates were carried out. Distilled water was used as a control for the 80% methanol extraction of *Spondias* fruit and leaf extracts and 2% acetone was used as a control for the chloroform extraction of *Spondias* fruit and leaf extracts. The mortality rate was observed and recorded at intervals of 24, 48, 72, 96 and 120 h after treatment. The micro-titer plate was shaken slightly before examination. For each type of extract five (5) trials were carried out.

(iv) Statistical Analysis

Data were analysed with the "Statistical Program for the Social Sciences" (SPSS) version 17. The LC_{50} value was determined and statistical significance of data were assessed by the analysis of variance (Two way ANOVA) prior to the comparison of mean with the Tukey test.

3. RESULTS

(i) Cumulative mortality of larvae at different concentrations

Four types of extracts, chloroform fruits extract, chloroform leaves extract, 80% methanol fruits extract, 80% methanol leaves extract all showed anthelmintic activities against trichostrongylid larvae. The mortality rate of trichostrongylid larvae increased over time. It was also concentration dependent. The higher the concentration the higher the mortality. A two way ANOVA showed that the difference between chloroform extraction of fruits and leaves was significant. The Tukey test showed that concentrations for each extract were significantly different.

Table 1: Percentage of average of cumulative mortality of chloroform fruit extract at different concentration

Hours	Percentage of Average of Cumulative Mortality (%) Chloroform Fruits extract concentration (mg/ml)					
	0	0.2	0.4	0.6	0.8	1
24	0	10	28	38	48	58
48	0	10	33	40	48	58
72	3	23	40	48	50	63
96	3	30	43	50	50	68
120	5	40	43	53	58	68

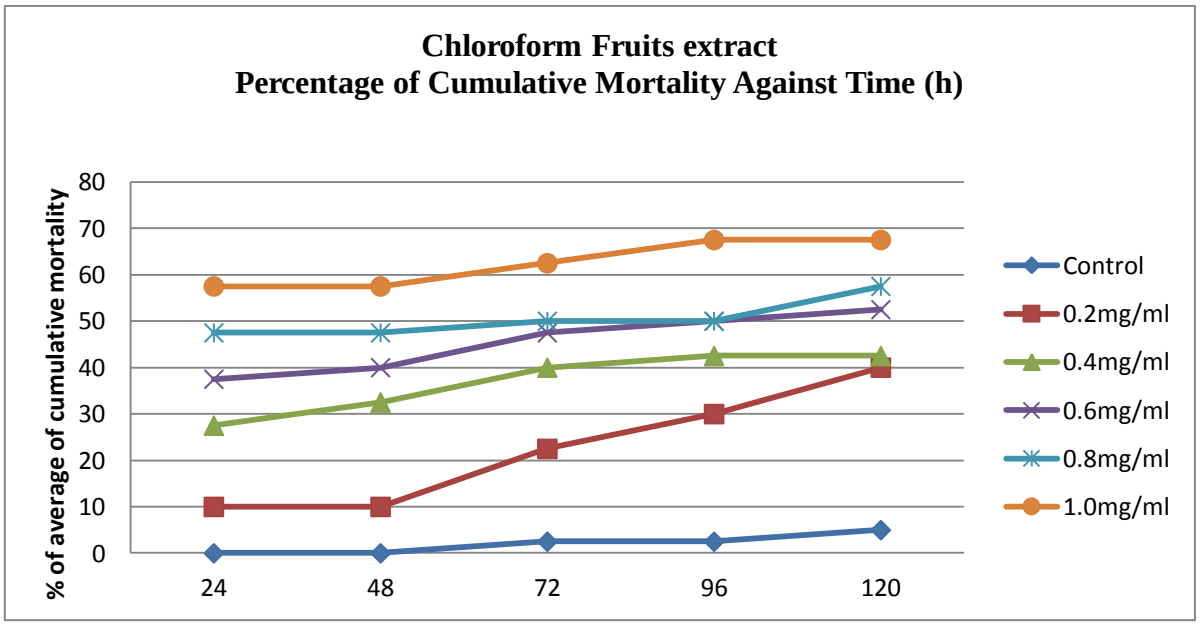

Fig. 1: Percentage of average of cumulative mortality of chloroform fruits extract against time (h)

At the lowest concentration of 0.2mg/ml, the chloroform extraction of fruits showed 10% mortality of larvae 24 h post treatment. The mortality rate remained unchanged for the next 24 h. The mortality rate then continued to increase over time and recorded the highest mortality rate at the interval of 120 h after treatment. For the concentration of 0.4mg/ml, the lowest mortality was at the interval of 24 h post treatment at 28%. It then increased steadily to 43% after 120 h. The fruit extract carrying the concentration of 0.6 mg/ml showed increasing mortality over time where the highest mortality achieved was 53%. On the other hand, the fruit extract with a concentration of 0.8 mg/ml gave 48% mortality of larvae at the intervals of 24 h and 48 h post treatment, 50% of mortality was achieved at the intervals of 72 h and 96 h, and the highest mortality rate of 58% was achieved at the interval of 120 h. For the highest concentration of extracts, the mortality rate was 58% after 48 h. It then increased to 63% at the interval of 72 h and continued to increase to 68% after 96 and 120 h (Table 1 & Fig. 1).

Table 2: Percentage of average of cumulative mortality of chloroform leaves extract at different concentration

Hours	Percentage of Average of Cumulative Mortality (%) Chloroform leaves extract concentration (mg/ml)					
	0	0.2	0.4	0.6	0.8	1
24	0	35	43	43	43	65
48	3	45	50	53	55	75
72	3	48	55	55	58	75
96	5	58	63	65	65	80
120	5	58	63	68	73	85

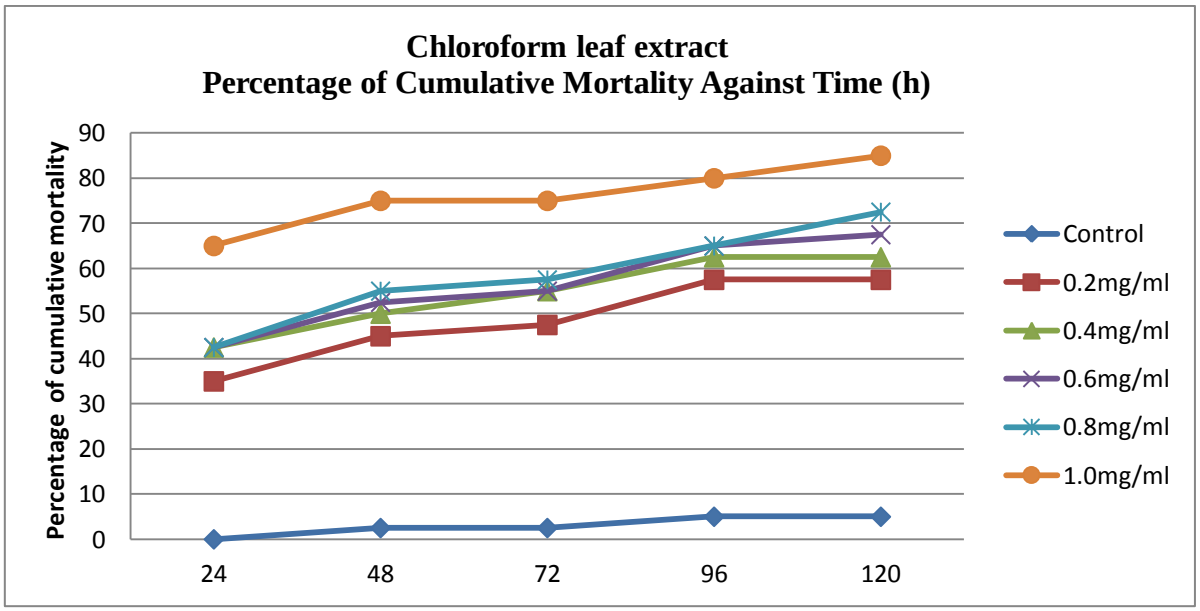

Fig. 2: Percentage of average of cumulative mortality of chloroform leaves extract against time (h)

Overall, chloroform extraction of leaves showed a higher mortality rate compared to chloroform extraction of fruits. At the lowest concentration of 0.2 mg/ml, the leaves extract chalked 35% of mortality of tested larvae. The mortality rate continued to increase over time and reached 58% of mortality at intervals of 96 and 120 h post treatment. The leaf extract with the concentration of 0.4 and 0.6 mg/ml showed a slight difference in mortality rate. After 24 h of treatment, both recorded 43% of larval mortality. At the interval of 48 h, leaf extract with a concentration of 0.6 mg/ml showed slightly higher mortality rate compared to the leaf extract of 0.4 mg/ml concentration. The 0.6 and 0.4 mg/ml extracts recorded mortality rates of 53% and 50% respectively. After 72 h, both extracts recorded 55% larval mortality. The mortality rate continued to increase over time and achieved 63% for 0.4 mg/ml leaf extract after 96 h. This rate remained the same at 120 h post treatment, whereas the leaf extract with a concentration of 0.6 mg/ml recorded 65% and 68% mortalities at the intervals of 96 and 120 h respectively. For the concentration of 1 mg/ml leaf extract, the percentages of mortality were 65%, 75%, 75%, 80% 85% at intervals of 24, 48, 72, 96 and 120 h respectively (Table 2 & Fig. 2).

Table 3: Percentage of average of cumulative mortality of 80% methanol fruits extract at different concentration

	Percentage of Average of Cumulative Mortality					
	80% methanol fruit extract					
	Concentration (mg/ml)					
Hours	0	1	2	3	4	5
24	0	13	15	15	20	33
48	0	20	23	23	30	55
72	3	23	30	28	33	65
96	3	28	38	38	43	78
120	5	35	48	50	55	83

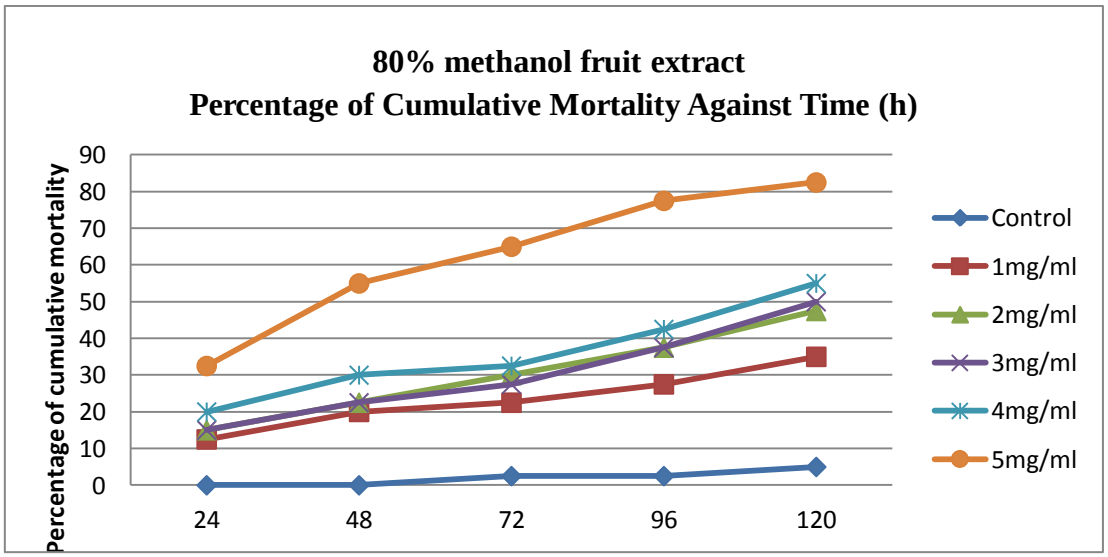

Fig. 3: Percentage of average of cumulative mortality of 80% methanol fruits extract against time (h)

From Fig. 3, the 80% methanol fruit extract with a concentration of 1 mg/ml recorded the lowest mortality rate. After 24 h of treatment, the mortality of larvae was 13% and continued to increase over time. At the interval of 120 h, 35% of larvae were dead. The mortality rate of both 2 and 3mg/ml concentration fruit extracts were almost the same. A mortality rate of 15% was recorded after 24 h of treatment which increased to 23% after 48 h of treatment. Both concentrations of fruit extract showed a slow increase in mortality in the next 3 intervals and shares a common rate of 38% at the 96 h interval. At 120 h post treatment, the 2 mg/ml concentration of fruit extract chalked 48% mortality while the 3 mg/ml of fruit extract resulted in 50% mortality. For the fruit extract with a 4 mg/ml concentration, 20% of tested larvae were found dead after 24 h. The mortality rate continued to increase steadily. After 120 h, more than half of the larvae were found dead. The mortality rate for the 5 mg/ml concentration fruit extract was the highest among the five different concentrations. After 24 h, the mortality was 33% which then increased by 22% to 55% at an interval of 48 h. The mortality rate then continued to rise and recorded a high of 83% mortality at the interval of 120 h (Table 3 & Fig. 3).

Table 4: Percentage of average of cumulative mortality of 80% methanol leaves extract at different concentration

Hours	Percentage of Average of Cumulative Mortality 80% methanol leaf extract Concentration (mg/ml)					
	0	1	2	3	4	5
24	0	28	38	33	40	50
48	0	30	38	40	48	63
72	3	33	45	48	53	78
96	5	48	50	53	70	80
120	5	50	55	60	75	85

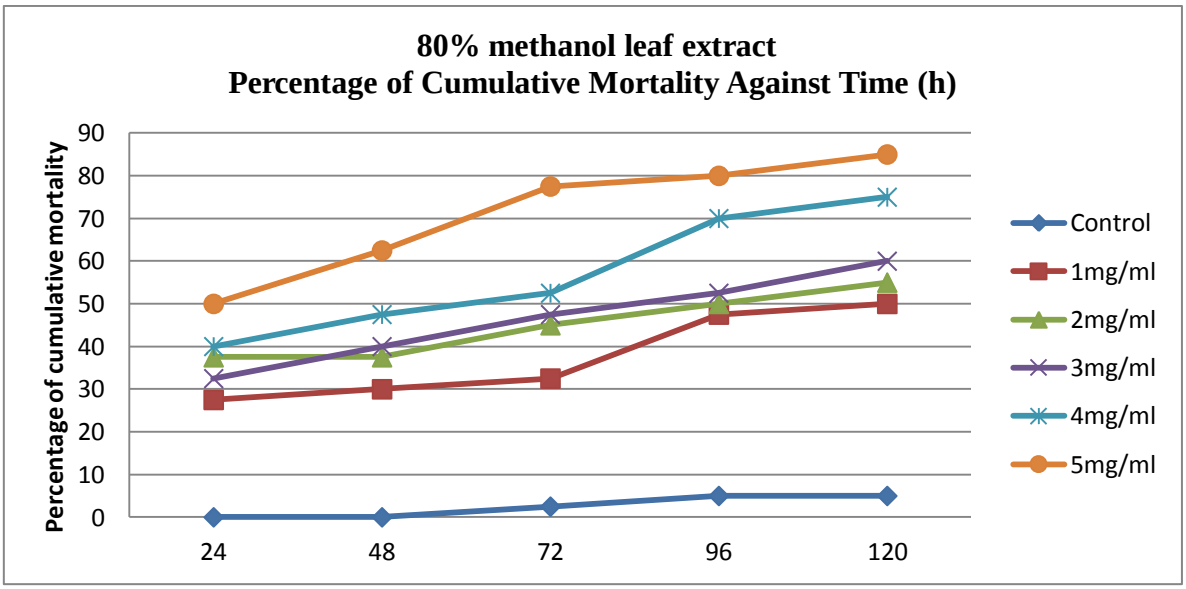

Fig. 4: Percentage of average of cumulative mortality of 80% methanol leaves extract against time (h)

At 24 h post treatment, observations showed 28% of larvae mortality for the 1 mg/ml 80% methanol leaf extract. Percentage of mortality increased over time and half of the tested larvae were found dead after 120 h of treatment. The leaf extract with a concentration of 2 mg/ml gave 38% mortality at both the 24 and 48 h interval. After 120 hours, it recorded 55% mortality, whereas the leaf extract with a concentration of 3 mg/ml gave a slightly higher mortality rate as compared to the 2 mg/ml extract except for the interval of 24 h, where it recorded a mortality rate of 33%. The mortality rate then increased over time and the highest mortality rate reached 60% at 120 h post treatment. The leaf extract of 4 mg/ml concentration showed a steady increase of mortality at the first three observation intervals, which were 40, 48, and 53% at intervals of 24, 48 and 72 h respectively. The mortality rate then increased from 17% to 70% after 96 h. At the interval of 120 h, the mortality rate increased a further 5% to reach 75% mortality. The leaf extract of 5 mg/ml concentration recorded 50% of mortality after 24 h. Mortality rate then continued to increase at every subsequent observation. At the interval of 120 h, 85% of tested larvae were found dead (Table 4 & Fig. 4).

(ii) LC50 of *Spondias mombin* fruits and leaf extracts

Table 5: LC_{50} of *Spondias mombin* extract against trichostrongylid larvae

Part of the plant / Extraction method	LC_{50} (mg/ml)	
	S. mombin leaves	*S. mombin* fruits
80% Methanol extraction	1.279	2.200
Chloroform extraction	0.158	0.416

LC_{50} is the lethal concentration that killed 50% of test population. Probit analysis showed that *Spondias mombin* leaf extract showed a lower LC_{50} value compared to its fruit extracts. The LC_{50} of 80% methanol extraction and chloroform extraction of *S. mombin* leaves were 1.279 and 0.158 mg/ml respectively, whereas the LC_{50} of 80% methanol extraction of *S. mombin* fruits were 2.200 and 0.416 mg/ml for chloroform fruit extract. This indicated that *S. mombin* leaves had a higher toxicity compared to its fruits against trichostrongylid larvae (Table 5).

4. DISCUSSION

The results indicated that both *S. mombim* fruits and leaves contained anthelmintic properties which are capable of killing the L_3 larvae. Activities of larvae decreased with the increasing extract concentrations. Larvae L_3 treated with the highest concentration showed highest mortality. Previous studies have also shown that *Spondias* plant contain anthelmintic, antiviral, antimicrobial and properties (Ajao *et al*, 1985; Corthout *et al* 1991; Abo *et al*, 1999; Rodrigues and Hasse, 2000).

The *S. mombin* leaves extracts were found to have lower LC_{50} values, which are 1.279 mg/ml for 80% methanol extraction of leaf extract and 0.158 mg/ml for chloroform extraction of leaf extract. On the other hand, the LC_{50} of the 80% methanol extraction of *S. mombin* fruit extract were 2.200 and 0.416 mg/ml for chloroform fruit extract. This meant that the *S. mombim* leaves possess higher anthelmintic properties and is more toxic compared to its fruits. Other researches also showed that *S. mombin* leaves possess great medical values. The leaves were used in abortions (Offiah and Anyanwu, 1989), anti- diarrhea (Irvine, 1961), anti-microbial (Abo *et al*, 1999) and anti-viral (Corthout *et al*, 1991). The leaves contain a lot of vitamin C (Keshinro, 1985). The dry powder of leaves can be applied on inflammations and wounds to speed up the healing (Rodrignes and Hesse, 2000; Rodrigne and Samuels, 1999; Corthout *et* al, 1994; Ajao and Shonukan, 1985), while the decoction of young leaves is a remedy for diarrhea and dysentery.

Other similar research on *S. mombin* anthelmintic activities had been conducted (Ademola *et al*, 2005) where in *vitro* and in *vivo* studies of ethanol and aqueous extracts of *S. mombin* had been carried out to determine the efficacy as anthelmintic against gastrointestinal nematodes in sheep. A larval development assay (LDA) was used to study the in *vitro* effect of extracts on strongyle larvae. Another in *vivo* study was conducted to evaluate the therapeutic efficacy of extracts by administrated orally at dose rate of 125, 250, and 500 mg/kg for naturally infected sheep. The presence of *S. mombin* extract in *in vitro* culture of larvae reduced the survival of infective stage or L_3 larvae. The LC_{50} for ethanol extract of *S. mombin* was 0.456 mg/ml, while the LC_{50} of aqueous extract of *S. mombin* was 0.907 mg/ml. On the other hand, *in vivo* study indicated that *S. mombin* extract was effective in reducing fecal eggs of *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp, and *Trichuris* spp. on day 12.

A plant becomes a medicinal plant only when its biological activities has been ethnobotanically reported or scientifically established (Elujoba, 1997). In 1979, the World Health Organization (WHO) emphasizes on the importance of scientific research into herbal medicine. Since then many developing countries would try to enlist traditional medicinal plants as possible addition to the WHO's "essential drugs" list once the value of the plants have been clinically proven (Ayoka *et al*., 2008). Medicinal plants are popularly used in ruminant farms in the Philippines (Mateo, 1996). Several plants have been tested for their efficacy as anthelmintic for goats and sheep in the Philippines. Crude extracts of *Mimosa pudica* and *Tinosphora rumphii* were highly effective against *Haemonchus* larvae (Mateo 1996). They were successful in reducing the egg count and worm number in *vitro.* Whereas fresh leaves of neem, *Azadirachta indica* are provided to animals to reduce worms. Current studies in Vietnam on effects of plants on *Haemonchus* larvae in vitro showed promising results with extracts of legumes, such as *Leucaena, leucocephala, Acacia mangium* and *Calliandra sp.* (ACIAR, 2004).

In this research, mortality of larvae were observed under microscope. The higher concentrations of extract gave darker color, thus making it more difficult to observe the larvae. The highest concentration of the extract used was dependent on the visibility of the larvae in the extract. The chloroform extracts were darker compared to the 80% methanol extracts. In this case, lower concentrations such as 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml for the chloroform fruit and leaf extracts were used instead of the higher concentrations where observations were impossible due to the lack of visibility. On the other hand, the 80% methanol fruit and leaf extracts were more visible. As a result, higher concentrations such as 1, 2, 3, 4 and 5mg/ml were used.

In conclusion, based on the efficacy and cost effectiveness, the use of *Spondias* plants as an anthelmintic should be encouraged among goat farms especially in the rural areas to to reduce worm number.

5. REFERENCES

Abo K. A., Ogunleye V. O. and Ashidi J. S. (1999). Antimicrobial potential of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea* . Phytotherapy Research 13: 494-497.

Ademola I. O., Fagbemi B. O. and Idowu S. O. (2005). Anthelmintic activity of extract of *Spondias mombin* against gastrointestinal nematodes of sheep; studies in *vitro* and *in vivo*. Tropical Animal Health and Production 37: 223-235.

ACIAR .(2004). Worm control of small ruminants in Tropical Asia. Australia: Scribbly Gum Publications Pty Ltd. pp 22-23.

Ajao A. O. and Shonukan O. (1985). Antibacterial effect of aqueous and alcohol extracts of *Spondias mombin* and *Alchomea cordifolia*: Two local antimicrobial remedies. International Journal of Crude Drug Research 23: 67-72.

Ayoka A.O, Akomolafe R.O, Akinsomisoye O.S & Ukponmwan O.E. (2008). Medicinal and economic important of *Spondias mombin*. African Journal of Biomedical Research 11 :129 -136.

Chandrawathani, P., Brelin, D., Nor Fasiha, S., Adnan, M., Jamnah, O, Sani, R.A., Hoglund, J., Waller, P.J. (2002a). Evaluation of the neem tree (*Azadirachta indica*) as a herbal anthelmintic for nematode parasite control in small ruminants in Malaysia. Tropical Biomedicine, 19: 41-48.

Chandrawathani, P.,Waller,P.J.,Adnan, M. and Hoglund, J. (2002b). Evolution of high level, multiple anthelmintic resistance on a sheep farm in Malaysia. Tropical Animal Health and Production 34: 50-58.

Corthout J., Pieters L. A., Claeys M., Vanden Berghe D. A. and Viletinck A. J. (1991). Antiviral; Ellagitannins from *Spodias mombin* . Phytochemistry 30: 1190.

Corthout J., Pieters L. A., Claeys M., Vanden Berghe D. A. and Viletinck A. J. (1994). Antibacterial and molluscicidal phenolic acid from *Spondias mombin.* Planta Medica 60: 460-463.

Elizabetshy E., Figueiredo W. and Oliveira G. (1992). Spices and medicinal plants. Journal of Herbs 1: 125-162.

Elujoba A. A. (1997). Phytotherapy the challenges of our time. Nigerian Journal of Natural Products and Medicine 2: 34-36.

Irvine F. R. (1961): Anti-inflammatory activity of selected Nigerian medicinal plants. Nigerian Journal of Natural Products and Nuts of Ghana. Oxford, London.pp. 167.

Keshinro O. O. (1985) The unconventional sources of ascorbic acid in the tropics. Nutrition Report International 31: 381-387.

Mateo C.D. 1996. Herbal medicine in animal health care. Animal Husbandry and Agricultural Journal 30: 54-61.

McKenna, P.B. and Watson, T.G. (1987). The comparative efficacy of four broad spectrum anthelminthic against some experimentally induced trichostrongylid infections in sheep and goats. N.Z. Vet. J., 35: 192-195.

McLeod, R.S. (1995). Cost of major parasites to the Australian livestock industries. International Journal Parasitology 25: 1363-1367.

Offiah V. N. and Anyanwu I. I. (1989). Abortifacient activity of an aqueous extract of *Spondias mombin* leaves. Journal of Ethnopharmacology 26: 317-320.

Rahman, W.A. (1997). Role of ivermectin and its formulations in the control of trichostrongylid nematodes on smallhorder goat farms of Malaysia. Small Ruminant Research, 25: 83-87.

Rodrigues K. F. and Hasse M. (2000). Antimacrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin* . Journal of Basic Microbiology 40: 261-267.

Rodrigues K. F. and Samuels G. J. (1999). Fungal endophytes of *Spondias mombin* leaves in Brazil. Journal of Basic Microbiology 39: 15-18.

Sangster, N.C., Riley, F.L., and Collins, G.H. (1988). Investigation of the mechanism of levamisole resistance in trichostrongylid nematodes of sheep. International Journal of Parasitology., 18: 813-818.

Strong, L., and Wall, R. (1990). The chemical control of livestock parasite: Problems and alternative. Parasitology Today 6: 291-295.

Taylor, M.A. (1991). Resistance of parasites to antiparasitic drugs. In: J.C. Boray, P.J. martin and R.T Roush (Editors), Round Table Conferecne at the VIIth Conferecne, Rahway, NJ, 89 pp.

Waller, P.J. (1985). Resistance to anthelmintics and the implications for animal production. In: N Anderson and P.J. Waller (Editors), Resistance in Nematodes to Anthelmintic Drugs. CSIRO, Division of Animal Health, Australian Wool Corporation, Glebe, NSW, Australia, pp 1-11.

Waller,P. J. (1999). International approaches to the concept of integrated control of nematode parasites of livestock. International Journal of Parasitology 29: 155-164.

Waller, P. J. (2004). Management and control of nematode parasites of small ruminants in the face of total anthelmintic failure. Tropical Biomedicine 21:7-13.