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Acaricidal Effect Of Spondias Mombin Leaf Extract On Rhipicephalus (Boophilus) Microplus Female Tick

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ABSTRACT

The leaf extract of *Spondias mombin* has demonstrated anthelmintic activity against gastrointestinal nematodes in sheep and adult *Haemonchus placei*. The active ingredients are molecules that are structurally related to Haem and are hypothesized to function by biological antagonism of haem uptake. Considering that several ectoparasites of livestock are blood sucking, we hypothesized that their survival could be dependent on haem-uptake. The experiments were performed using the adult immersion test (AIT), larval immersion test (LIT), and egg hatchability test (EHT) in 1.25% Triton X-100.The study on AIT was done by numbering the female laying eggs, placing them on petri dishes and incubating them at 27–28ºC and 70–80% relative humidity. After 14 days, the eggs were collected, weighed, and observed. Each treatment was incubated in replicate with standard drug solutions of cypermethrin using seven different dilutions. The AIT - response data fitted a sigmoidal equation well (R^2 0.92, Sy.x = 7.90) with an EC50 value of 2.30mg/ml at 95 % confidence interval, while Cypermethrin gave an R^2 value of 0.88, Sy.x = 11.00, with an EC50 value of 0.58mg/ml at 95 % confidence interval. In the LIT, *Spondias mombin* leaf extract gave an R^2 value of 0.73, Sy.x = 13.00 with an EC50 value of 4.0mg/ml at 95 % confidence interval, than cypermethrin (7.40 mg/mL) with an R^2 value of 1.0, Sy.x = 0.78 at 95 % confidence interval. In EHT, toxicity to egg hatching was found at 1.25% Triton X-100, as 6% of the eggs hatched to larvae. The findings from this study provides experimental evidence supporting the "haem-uptake antagonism" hypothesis. This establishes a fundamentally different way to kill parasites compared to common synthetic pesticides (like cypermethrin, whose site of action is the nervous systems of the insect).

Keywords: *Spondias mombin, Haemonchus placei*, Cypermethrin, Ticks, livestock.

Introduction

Spondias mombin plant is a tropical tree with a small, yellow pulp fruit and native to tropical rain forest, commonly known as “Iyeye” in Yoruba, it is commonly known as Hog plum in English (Morton, 1987); S*pondias mombin* belongs to the family, Anacardiaceae; order, Sapindales; class Magnoliopsida and division, Magnoliophyta. It is a hermaphrodite tree that flowers between March and April. It has been found useful as a shade tree, for

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medicinal use, for food, for fuel, for timber, and for fodder (Ayoka *et al*., 2008).

The leaf of *Spondias mombin* L. (*Anacardiacea*) was previously shown to contain antiviral ellagitannins and caffeoyl esters as well as antibacterial and molluscicidal phenolic acids (corthout *et al*., 1994) The leaves have demonstrated to have anthelmintic (Ademola *et al*., 2005) activity against gastrointestinal nematodes (eggs and larvae) in sheep and adult *Haemonchus placei* in cattle. (Ogedengbe *et al*., 2019).

In another study carried out by Ademola *et al*. (2005) to evaluate the activities of *in vitro* and *in vivo* aqueous and ethanolic *Spondias mombin* leaf extracts against ovine gastrointestinal nematodes, a larval development assay was used to investigate the *in vitro* effect of the extract on larvae. In the *in vivo* study, the therapeutic efficacy of *Spondias mombin* leaf extract was evaluated in sheep that were naturally infected with gastrointestinal nematodes. The *in vitro* studies showed by the LC50 that the ethanolic extract was approximately twice as potent as the aqueous extract (0.456mg/ml vs 0.907 mg/ml). Interestingly, this activity profile is corroborated by a study carried out by Ogedengbe *et al*. (2012) in which various extracts were screened for anthelmintic activity, and the acetone extract was found to be approximately twice as potent as the aqueous extract.

The active ingredients of *Spondias mombin* leaf extract are molecules that are structurally related to haem and are hypothesized to function by biological antagonism of haem uptake by the parasite. Haem is a coordination complex consisting of an iron coordinated to a porphyrin acting as a tetradentate ligand and to one or two axial ligands.

The iron porphyrin compounds occupy a unique position among the oxidation-reduction systems of biological importance because, on combining with nitrogenous compounds, they form complexes possessing manifold properties, all of them connected with the function of respiration. Heam is most commonly recognised as a component of haemoglobin, the red pigment in blood. Parasites possess haemoglobin that plays a major biological role, which is essential for their survival. Parasites are unable to synthesize haem on their own. Research has shown that in most eukaryotes, haem is produced from a series of intermediates through a defined evolution pathway which ends with the insertion of ferrous iron in proto protoporphyrin IX ring. This led to the hypothesis that targeting the haem transport pathway in parasites could be a control measure for ectoparasites. The importance of heam in the metabolism of parasitic organisms justifies using porphyrin, their precursors, and derivatives as

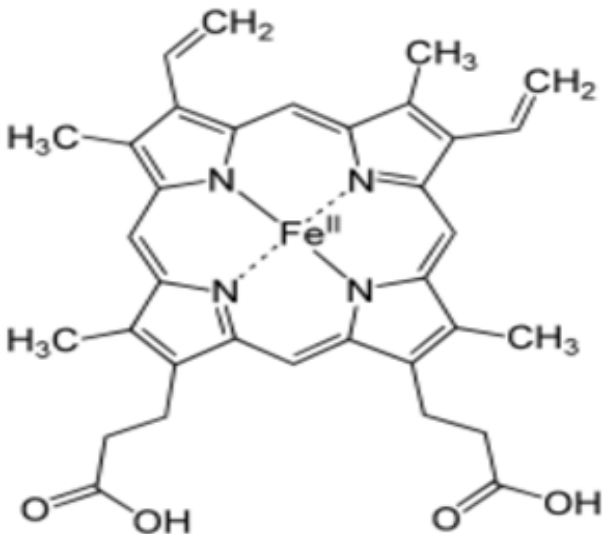

Figure 1. Structure of Haem

Figure 2. Porphyrin Ring

potential anti-parasitic.

Methodology

Plant Collection and Identification

Fresh leaves of *Spondias mombin* L. were collected from the University of Ibadan, around the Staff school premises, Ibadan, Nigeria. The Voucher specimen was authenticated at the Forest Research Institute of Nigeria (FRIN) herbarium (FHI), Ibadan, Nigeria, with a voucher specimen number FHI 112494.

Preparation of the Extract used

The air-dried plant was powdered using the laboratory blender. One hundred and 20 grams (120g) of the pulverized plants were defatted with ten liters, seven liters, and five liters of Hexane consecutively, with a three-day interval, using the cold maceration method of extraction. The defatted plant was extracted with seven litres, five litres, and four litres of methanol consecutively, with a three-day interval between each extraction using the cold maceration method of extraction.

The obtained extract was concentrated using a Buchi Rotavapor at 40 °C. Further air drying of the concentrated extract was carried out at room temperature to obtain a solid crude extract. The weight and percentage yield of the crude extract were determined, and qualitative phytochemical analysis was carried out on the extract. TLC Fractionation was also carried out on the Acetone extract to monitor the distribution of the Secondary Metabolite.

Biological Activity of the Crude Plant Extract

Acaricidal assay was carried out on the crude extract. The solution used in the adult immersion test (AIT), larval immersion test (LIT), and egg hatchability test (EHT) was prepared using 1.25% Triton X-100.

Preparation of 1.25% Triton X-100 solutions

The solutions of 1.25% Triton X-100 were prepared by pipetting 1.25ml of Triton X-100 into a 100 mL volumetric flask and making up to the mark with distilled water.

Collection of *RhipicephaluS (BoophiluS) microplus* female ticks.

The ticks used for the bioassay were collected at the Akinyele Local Government Kara market, Ibadan, Oyo State, Nigeria.

Adult Immersion Test (AIT)

Groups of 12 (~3 g) *Rhipicephalus (Boophilus) microplus* females were weighed and immersed for 5 min in the test solution of dilutions 30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL, 1.875 mg/mL, 0.9375 mg/mL and 0.46875 mg/mL, in a 50 mL Becker flask which was gently agitated. These were carried out in three replicates each at room temperature.

The surfactant solution (1.25% Triton X-100) and distilled water were used as the controls.
Ticks were recovered from the solutions, dried, and randomly placed in each Petri dish (5.5 cm diameter, 1.5 cm high).

The Petri dishes were incubated at 27–28ºC, 70–80% relative humidity. After 14 days, the number of females laying eggs was recorded, and the eggs were collected, weighed, and observed.

The eggs were placed in petri dishes, incubated under the same conditions. After 14 days, the tubes were observed until most of the eggs started to hatch, and the hatching rates of the different treatments were visually estimated and compared to that of the control. Cypermethrin 100g/L was used for the positive control drug, while TritonX-100 and distilled water were used as the negative controls. The Percentage inhibition of egg laying was calculated.

$$index\ of\ egg\ laying\ (IE) = \frac{weight\ of\ eggs\ laid\ (g)}{weight\ of\ females\ (g)}$$

$$\%\ inhibition\ of\ egg\ laying\ = \frac{IE\ control\ group - IE\ treated\ group}{IE\ control\ group} \times 100$$

Larval immersion Test (LIT)

The Larval immersion test was performed with *Rhipicephalus (Boophilus) microplus* by placing approximately 200 embryonated eggs (0.01 g) on a Whatman filter paper. The filter papers were then placed in 7 petri dishes, one for each concentration.

The petri dishes were incubated at 27–28 ºC and 70–80% relative humidity, until the eggs started hatching, After days of incubation, the filter paper containing the larvae ready for testing was immersed for 5 min in 10mL of the test solutions (30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL, 1.875 mg/mL, 0.9375 mg/mL and 0.46875 mg/mL). This process was repeated for the positive control drug, using Cypermethrin 100g/L.

TritonX-100 and distilled water were used as the controls. The filter paper was placed in a Petri dish (5.5 cm diameter, 1.5 cm high), and the lid of the Petri dish was opened for about 1h to allow the solvents to evaporate. It was then incubated at 27–28 ºC and 70–80% relative humidity for 48 hours. Larvae (alive and dead) will be counted to assess the percent mortality.

The percentage survival of larvae was calculated.

$$\% \, Larval \; survival = \frac{LS \; control \; group - LS \; treated \; group}{LS \; control \; group} \times 100$$

Egg Hatchability Test (EHT)

Approximately, 200 *Rhipicephalus (Boophilus) microplus* embryonated eggs (0.01 g) were weighed and placed in a Whatman filter paper, the filter papers were placed in 7 petri dishes one for each Concentrations and immersed for 5 min in 10mL of the test solutions (30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL, 1.875 mg/mL, 0.9375 mg/mL and 0.46875 mg/mL). This process was repeated for the positive control drug, using Cypermethrin 100g/L. TritonX-100 and distilled water was used as the controls, the filter paper was placed in a Petri dish (5.5 cm diameter, 1.5 cm high), the lid of the petri dish was opened for about 1h to allow the solvents to evaporate, it was then incubated at 27–28 ºC and 70–80% relative humidity until the eggs starts hatching. TritonX-100 and distilled water were used as the control.

The percentage of egg hatching was calculated.

$$\% \, Egg \; hatching = \frac{HE \; control \; group - HE \; treated \; group}{HE \; control \; group} \times 100$$

Data Analysis

The acaricidal studies, dose-response data were analysed using sigmoidal curve fitting or dose-response relationship, while the lethal concentration to eliminate 50% (LC50) of adult ticks for the extract that caused more than 50% mortality at 95% confidence interval (CI) were determined by probit analysis using SPSS 23.

Results and Discussion

Quantitative and Percentage Yield of Crude Extracts

The bulk extraction of 1.2 grams of powdered leaves of *spondias mombin,* with Acetone prior to defatting it with n-hexane using cold maceration yielded 0.033g of the crude extract, which translates into a percentage yield of 2.75%. This percentage yield is consistent with yields reported for other bioactive plant extracts obtained via similar maceration techniques (Gurjar *et al*., 2012).

Qualitative Ecto-parasitic Activity of Acetone Extract

The plant extract, at 30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL, 1.875 mg/mL, 0.9375 mg/mL and 0.46875 mg/mL concentrations, were tested against cypermethrin, the reference drug (at 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/

mL, 0.625 mg/mL,0.3125 mg/mL). Activity was recorded as mean ± standard error of the mean in the analysis. The acetone extract showed dose-dependent acaricidal activity against *Rhipicephalus (Boophilus) microplus* across all tested concentrations in the adult immersion test and larval immersion test with reference to cypermethrin.

Adult Immersion Test

The activities of the *Spondias mombin* leaf extract were investigated, and the EC50 was estimated by sigmoidal curve-fitting analysis. The Adult Immersion Test (AIT) - response data fitted a sigmoidal equation well (R^2 0.92, Sy.x = 7.90) with an EC_{50} value of 2.30mg/ml at 95 % confidence interval for the inhibition of egg laying. While this demonstrates potent intrinsic activity, the synthetic pyrethroid cypermethrin was more potent, with an EC_{50} of 0.58 mg/mL (R^2 = 0.88, Sy.x = 11.00) at 95 % confidence interval. The efficacy of the *S. mombin* extract (EC_{50} = 2.30 mg/mL) compares favorably with other plants investigated for tick control. For instance, ethanolic extracts of *Annona squamosa* seeds and *Azadirachta indica* leaves have been reported with EC_{50} values of 2.77 mg/mL and 3.45 mg/mL, respectively, against *R. microplus* females (de Souza *et al*., 2021). The activity of *S. mombin* is notably more potent than that reported for *Mentha piperita* essential oil (EC_{50} = 18.7 mg/mL) in similar AITs (Kumar *et al*., 2021). The high R^2 value for the *S. mombin* dose-response curve indicates a reliable and predictable pharmacological effect, characteristic of a defined mechanism of action..

Effect On Egg Hatchability

Toxicity to egg hatching was found at 1.25% Triton X-100, as 6% of the eggs hatched to larvae, it was observed that triton X-100 has inhibiting effects on the eggs' hatchability. This solvent interference precludes a definitive assessment of the extract's specific ovicidal activity in this assay. Similar challenges with solvent toxicity in tick egg bioassays have been reported, underscoring the need for careful selection of inert carriers in phytochemical studies (Fernández-Salas *et al.,* 2011).

Larval Immersion Test

In the LIT, the *Spondias mombin* leaf extract gave an R^2 value of 0.73, Sy.x = 13.00 with an EC50 value of 4.0mg/ml at 95 % confidence interval, than cypermethrin (7.40 mg/mL) with an R^2 value of 1.0, Sy.x = 0.78 at 95 % confidence interval. The lower

	Spondias mombin	Cypermethrin
Sigmoidal dose-response (variable slope)		
Best-fit values		
BOTTOM	0	0
TOP	100	100
LOGEC50	0.36	0.079
HILLSLOPE	1.2	1.2
EC50	2.3	1.2
Goodness of Fit	Spondias mombin	Cypermethrin
Degrees of Freedom	2	2
R^2	0.92	0.88

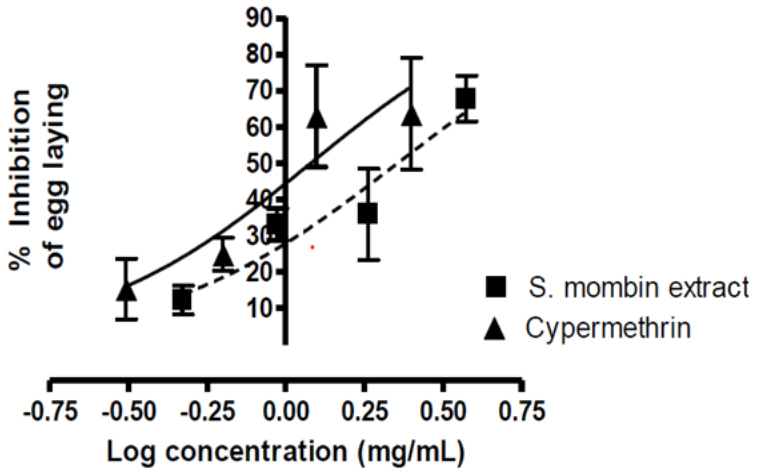

Figure 2. Sigmoidal curve for the percentage Inhibition of Egg Laying

potency of cypermethrin against larvae compared to adults is a known phenomenon and may be attributed to differences in cuticle permeability or detoxification enzyme activity in early life stages (Viturella *et al*., 2017).

The larvicidal potency of *S. mombin* (EC_{50} = 4.0 mg/mL) is within the active range reported for other plant extracts. For example, a hexane extract of *Calea serrata* showed an LC_{50} of 5.7 mg/mL against *R. microplus* larvae (Ribeiro *et al*., 2007). However, it is less potent than some highly active essential oils, such as that from *Syzygium aromaticum* (clove), which has shown LC_{50} values below 1 mg/mL (Ferreira *et al*., 2010).

	Spondias mombin	Cypermethrin
Sigmoidal dose-response (variable slope)		
Best-fit values		
BOTTOM	0	0
TOP	100	100
LOGEC50	0.4415	-0.02808
HILLSLOPE	0.6751	3.206
EC50	2.764	0.9374
Goodness of Fit	*Spondias mombin*	Cypermethrin
Degrees of Freedom	5	0
R^2	0.9443	1

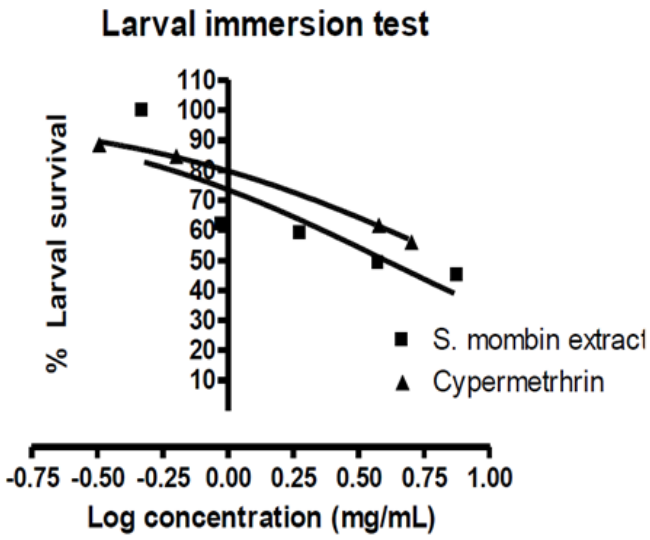

Figure 3. Sigmoidal curve for the percentage larva survival

Table 1. Qualitative phytochemical Screening of the acetone extract

	Parameters	Sample
1	Saponin (Froth's Test)	+
2	Alkaloid (Hager's Test)	+
3	Flavonoid (Lead acetate Test)	+
4	Tannin (Braymer's Test)	+
5	Phenol	+
6	Steroid	+
7	Terpenoid	+
8	Cardiac Glycosides	+
9	Glycosides	-
10	Quinone	+
11	Anthraquinone	-

+ signifies the presence of the phytochemical in the acetone extract
-ve signifies the absence of the phytochemical in the acetone extract

Qualitative phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, phenols, steroids, terpenoids, cardiac glycosides, and quinones in the acetone extract (Table 1). The acaricidal activity of plant extracts is frequently linked to these secondary metabolites. . Studies have shown that the acaricidal activity of plant extracts is linked to their composition (the presence of saponin, alkaloid, glycosides, and phenol) as secondary metabolites. These chemicals have the capacity to initiate an acaricidal mechanism of action in *in vitro* and *in vivo* activities, causing mortality against ticks (Kumar *et al*., 2011).

Indeed, the acaricidal activity of the ethanolic extract of *Tagetes gracilis* has been attributed to the presence of flavonoids (Abdala *et al*., 2003). Likewise, the flavonoids could be responsible for the acaricidal activity of the ethanolic extract of *Tagetes maxima* (Parejo et al., 2004). Godara *et al*. (2014) also reported that the alkaloids are implicated in the acaricide activity observed by the ethanolic extract of *Atropa belladonna*. Coumarins are known for their repellent action against flies and ticks (Emilie, 2011). Finally, it has been shown that tannins are responsible for the acaricidal activity observed with extracts of *Acacia pennatula*, *Piscidia piscipula*, *Leucaena leucocephala,* and *Lysiloma latisiliquum* (Fernandes-Salas *et al*., 2011). The acaricide activity followed by the different extracts could therefore be linked to the presence of these chemical constituents. This activity was more pronounced with hydro-alcoholic extracts than hexanic ones. This could be explained by the fact that it is much more linked to polar than nonpolar compounds.

Some extracts did not cause direct mortality on the *Rhipicephalus (Boophilus) Microplus* female tick despite the presence of secondary metabolites. The phytochemical compositions of *Spondias mombin* leaf extract are supported by these findings reported to be linked to its acaricidal activities.

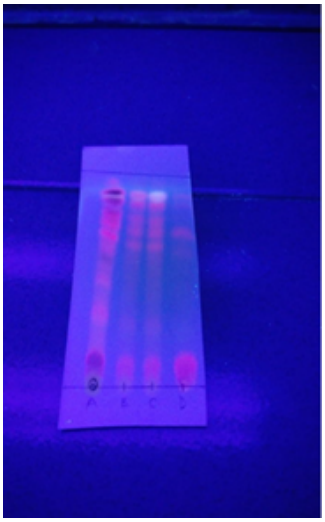

Figure 4. TLC chromatogram of the crude acetone extract under 365nm developed with a mobile phase of hexane-ethyl acetate in the ratio of 6:4 to monitor the distribution of the secondary metabolite.

The TLC chromatogram in Figure 4 above revealed a complex mixture of secondary metabolites, suggesting that the overall acaricidal effect is likely synergistic rather than due to a single compound.

Conclusion and Recommendation

The leaf extract of *Spondias mombin* is less effective than Cypermethrin in inhibiting egg laying by adult ticks but more effective than Cypermethrin in preventing larval survival. Its activity is competitive with other well-studied botanicals and may involve a beneficial combination of broad-spectrum phytochemical actions (e.g., tannins, flavonoids) and a potentially novel haem-targeting mechanism. The interference of Triton X-100 in the EHT highlights an important methodological consideration for future work. *Spondias mombin* leaf extract could serve as a potential acaricide for the management of tick infection. It is recommended that further studies should explore the activities of *Spondias mombin* on the different fractions of the acetone extracts.

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