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Original Research Article

Anthelmintic, haematological and antioxidant potential of *Spondias mombin* L. in young dogs

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ABSTRACT

Associated side effects and anthelmintic resistance to synthetic anthelmintics limit their use. This study evaluated the anthelmintic, haematological and antioxidant activities of *Spondias mombin* (SM) in young dogs. In addition, phytochemical analyses and *in vitro* antioxidant assays of SM's fractions were done using standard methods. Ten Nigerian indigenous dog breed (aged 2-6 months), were randomly allocated into two groups (A and B) and pre-treated with 200 µg/kg ivermectin subcutaneously. Two weeks after, each dog was infected with 400 L_3/mL *Ancylostoma caninum* suspension orally and topically. On day 31 post infection, Group A was administered 5 mg/kg pyrantel pamoate (PP) once, while Group B was administered 500 mg/kg aqueous SM leaf extract for two days. Their physical condition, faecal egg count, body weights (BW), haematological, antioxidant and serum biochemistry were evaluated before and two weeks post treatment (PT). Data were presented as mean ± SEM, analyzed using student t-test and Chi-square, and p-value ≤ 0.05 was considered significant. Melanic faeces voided by dogs in both groups returned to normal colour and consistency PT, with SM aqueous extract comparing well with PP. There was slight decrease in WBC (8.74±4.85 to 8.37±1.78x 10^3/µL), improvement in PCV (30.60±6.54 to 33.20±4.87%), serum biochemistry and antioxidant values for Group B, though not statistically significant (p=0.2). Total cholesterol and phospholipids were significantly reduced PT in Group B (p = 0.05). Nine phytochemicals were identified in SM fractions and the plant demonstrated high free radical scavenging activity. Crude aqueous extract of *Spondias mombin* leaf is an effective anthelminthic, which can be developed further in the management of canine helminthosis.

Keywords: Dogs, fractions, helminthosis, Nigeria, *Spondias mombin*

INTRODUCTION

Dogs have a close association with humans providing companionship, security, source of income and guide to the disabled (Brooks *et al.*, 2018). The relationship, though offering significant benefits, also presents potential public health risk (Sowemimo, 2009). This is because natural transmission of parasites and other infections from dogs to humans may occur, directly or indirectly due to human behavioural and environmental factors (Sowemimo & Ayanniyi, 2017).

Helminthosis (worm infection), is a disease of animals and humans, in which a part of the body is infected with parasitic worms (helminths) (Narasimha *et al.*, 2018). Various species of helminths have been reported in dogs from different regions of Nigeria, with *Toxocara canis* (roundworm), *Ancylostoma* species (hookworm), *Trichuris vulpis* (whipworm), *Dipylidium caninum* (flea tapeworm), *Taenia* and *Strongyloides* species being the most common (Kamani *et al.*, 2021). In addition, some helminths cause zoonotic diseases such as toxocariasis (visceral larva migrans), ancylostomosis (cutaneous larva migrans), hydatid disease and emerging/re-emerging infections such as cryptosporidiosis and giardiasis (Esonu *et al.*, 2019).

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Anaemia is an important clinical sign of helminthosis (especially *Ancylostoma* infection) due to intestinal haemorrhages (Uppal *et al.,* 2017). Diarrhoea is common after heavy infection and faeces may be mixed with blood and mucus. Other clinical signs include lethargy, anorexia, vomiting, enlarged abdomen, dehydration and poor growth. This depends on the age of animal, the worm burden, location in the body and developmental stage of the worms (Sowemimo & Ayanniyi, 2017). As with most parasitism, clinical signs are exacerbated by malnutrition, stress or concurrent disease, though, infection may be asymptomatic (Reinemeyer, 2016).

Various classes of anthelmintics including the Benzimidazoles (mebendazole, febendazole), Piperazines (piperazine citrate), Heterocyclics (praziquantel), Macrocyclic lactones (avermectin), Vinyl pyrimidines (pyrantel pamoate), Amides (niclosamide) and Imidazothiazoles (levamisole) have been used over the years (Zajíčková *et al.,* 2020). Despite this, helminthosis remains one of the most common disease condition diagnosed in companion animal practice in Nigeria, as in many parts of the developing world (Deplazes *et al.,* 2011). The cumulative high cost of repeated use of these drugs, associated side effects such as hypersensitivity reactions, and emergence of anthelmintic resistance limit their use. The option of herbal anthelmintic provides an important and viable alternative to prevent, treat or alleviate clinical signs of helminthosis. It may also be explored in managing anthelmintic resistance (Tariq, 2018).

Spondias mombin L. (Family: Anacardiaceae), called Hog plum in English, *Ewẹ́ ìyeyè* in Yoruba, *Ijikara* in Igbo, *Tsadermaser* in Hausa, *Chabbuli* in Fulani and *Nsukakara* in Efik, is adapted to Nigeria, Brazil and other tropical countries (Adenubi *et al.,* 2019). The plant is commonly used in folk medicine in Nigeria for the treatment of gastrointestinal diseases associated with diarrhoea. The leaves, bark and seed are used for cough, sore throat, malaria, as stomachic, and to aid child birth. The fruit is used to cure dizziness, fibroid, and eaten as food (Adenubi *et al.,* 2019; Sameh *et al.,* 2018). The *in vitro* and *in vivo* anthelmintic efficacies of the crude extracts of *Spondias mombin (S. mombin)* leaf have been reported (Ademola *et al.,* 2005). Other pharmacological properties of the plant include anti-inflammatory, antioxidant, antiviral, antibacterial, mosquito adulticidal, and anti-ulcer (Maria *et al.,* 2021).

Despite the traditional use of crude extracts of *S. mombin* as anthelmintic, the clinical potentials of the plant in small animal medicine have not been fully explored. This study hypothesized that crude aqueous extract of *S. mombin* leaves will compare favourably with conventional anthelmintics (in this wise pyrantel pamoate) in the management of canine helminthosis (*Ancylostoma caninum* infection) in dogs. The anthelmintic activity, haematological and antioxidant potentials of crude extract of *S. mombin* leaves in dogs, as well as, its phytochemical constituents were evaluated..

MATERIALS AND METHODS

ETHICAL APPROVAL

Approval was obtained from the College Research and Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB/COLVET/CREC/2020/07/07). All protocols experimentally adopted and carried out were in accordance with international principles and protocols for laboratory animal usage.

COLLECTION AND PREPARATION OF PLANT MATERIAL

The leaves (visually green ones) of *S. mombin* were collected from the wild within Abeokuta metropolis. The plant species was identified, authenticated, and deposited at the Nigeria Natural Medicine Development Agency, Lagos State where a voucher specimen number (MPNH/2021/01011) was given. The plant leaves were washed with clean water and air dried for two weeks. It was then blended into fine powder using a kitchen blender (Kenwood, UK) and kept in a glass container with a cover lid. Plant extraction was done according to the method of Eloff and McGaw (Eloff & McGaw, 2006). Aqueous extract was used for the *in vivo* anthelmintic and antioxidant studies, and this was done by soaking 500 g of powdered leaf sample in 5 litres of distilled water for 48 hours, agitated gently at intervals. The extract was filtered using a sterilized muslin cloth and Whatmann No. 1 filter paper. The filtrate was evaporated to dryness in a water bath at 60^{o}C.

Methanol extraction and further fractionation were done for the *in vitro* antioxidant assays and phytochemical analyses. About 500g of powdered leaf sample was transferred into a glass container and 5 litres of absolute methanol added, stirred and allowed to stand for 72 hours. Thereafter, filtration was done and the filtrate was concentrated with a rotary evaporator (Heidolph laborota, Germany, model 517-01002-002) set at 40^{0}C, and was further concentrated using a vacuum oven set at 40^{0}C with a pressure of 700mmHg. Forty grams of the methanol extract was poured into a clean 500mL glass beaker, and 100mL of methanol, made into solution and pre-absorbed with 150g of silica gel. The dried pre-absorbed extract was packed into a Vacuum Liquid Chromatography (VLC) set up with 200g of silica gel. Hexane, chloroform, ethylacetate, methanol and distilled water were added consecutively, after a clear solution of the preceding solvent was obtained. The percentage yield of the extracts and fractions (hexane, chloroform, ethylacetate,

methanol and aqueous) were calculated, and they were stored at 4^{o}C until use.

The yield of S. mombin was calculated using the formula described below as:

% yield (w/w) = weight of dry extract x 100/weight of dry plant material

ANTHELMINTIC STUDY

EXPERIMENTAL ANIMALS

Ten, apparently healthy, intact, male Nigerian indigenous breed of dogs, aged two to six months old, were purchased from local markets in Abeokuta. They were identified with neck tags and body markings and kept in two groups of five dogs per concrete kennel (390 by 270 cm). The animals were fed twice daily (morning and evening) with home-made food and water was served *ad libitum*. The dogs were kept under close observation for two weeks. During this period, they were treated with ivermectin (1% Ivanor®, China) at a dose of 200 µg/kg subcutaneously to clear off any gastrointestinal or ectoparasites.

EXPERIMENTAL PROCEDURE

At the end of the acclimatization period, fresh faecal samples from the dogs were collected. Each faecal sample was first examined grossly for larvae or adult worms before transferring some quantity into properly labeled specimen bottles. These samples were examined microscopically for helminths by centrifugal floatation technique (CAPC, 2015). This was to ascertain that the puppies were free of gastrointestinal helminths.

Infective *A. caninum* larvae were previously harvested from faecal cultures of a naturally infected donor dog. This was used to prepare larval inocula by diluting larval suspensions with distilled water to obtain the required concentration (400 L_3) in 1 mL of suspension. Oral administration was done using an improvised stomach tube while topical administration was done by dispensing the suspension in between the footpad using a 5 ml syringe. Each dog was held by a handler for approximately 30 minutes to allow the *A. caninum* larvae penetrate the skin (percutaneous) and to prevent vomiting. All dogs were experimentally infected orally and topically with 400 L_3 of *A. caninum* (Idika & Nwosu, 2017). Thereafter, they were randomly assigned to two groups (Groups A and B) of five dogs each.

Administration of the test anthelminthic sample was done on day 31, after helminthosis was established [by faecal egg count (FEC) and clinical signs: dry, rough and falling hair coat, diarrhoea (melena, bloody and greenish), anorexia and weakness]. To the dogs in Group A, pyrantel pamoate (Combantrin®, Canada) at 5 mg/kg was administered orally once while Group B were administered 500 mg/kg aqueous extract of *S. mombin* for two days [effective dose reported by Ademola *et al.* (2005)]. Vital parameters (temperature and pulse rate) and physical examination with emphasis on the colour of the visible mucous membranes, consistency and colour of the faeces, and body condition were monitored. The body condition score (BCS) of 1 = very thin; 2 = underweight; 3 = ideal weight; 4 = overweight; and 5 = obese described by Buzahdt (Buzhardt, 2021) was used. In addition, the FEC, body weights, haematology, serum chemistry and antioxidant parameters were evaluated before treatment and two weeks post treatment (PT).

HAEMATOLOGY, SERUM CHEMISTRY AND ANTIOXIDANT PROFILE

About 5 mls of blood was collected from each dog via the cephalic vein into Ethylenediaminetetraacetic acid (EDTA) and plain bottles for haematology and serum biochemistry respectively. Blood samples were estimated for packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts using standard procedures (Dacie, 2006). Mean Corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular and Haemoglobin concentration (MCHC) were also calculated.

Serum glucose, protein (total protein, albumin, globulin) and lipid profile [total lipid, total cholesterol, high-density lipoprotein (HDL) and triglycerides] as well as oxidant/antioxidant status [superoxide dismutase (SOD), catalase (CAT) glutathione-S-transferase (GST) & malondialdehyde (MDA) level] of the puppies before, and PT were determined spectrophotometrically using the commercial kit (Randox Ltd, UK) (Javed & Usmani, 2015). Phospholipids, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were also calculated.

IN VITRO ANTIOXIDANT ASSAYS

2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) SCAVENGING ACTIVITY ASSAY

The method described by Mensor *et al*. (2001) was used. Five concentrations of test samples (200, 400, 600, 800 and 1000µg/mL) were prepared with methanol. Then, 1mL of each concentration was transferred into a test tube and 1mL of 0.3 mM DPPH solution was added. After 30 minutes, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated.

FERRIC ION REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Ferric ions reducing power was measured according to the method of Oyaizu (1986). Different concentrations (20, 40, 60, 80 and 100µg/ml) of the test samples were mixed with 2.5mls of 20 mM phosphate buffer and 2.5 mls of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 30minutes. Afterwards, 2.5 mls of 10% trichloroacetic acid and 0.5mL of 0.1% ferric chloride were added and left to stand for 10 minutes. Ascorbic acid was used as positive reference standard and absorbance was measured at 700 nm. All assays were run in triplicate and averaged.

PHOSPHOMOLYBDENUM ASSAY

Varying concentrations of test anthelminthic sample (200, 400, 600, 800 and 1000µg/ml) were put in test tubes containing 3 mls of distilled water and 1 ml of Molybdate reagent (1ml each of 0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate in 50 mls of distilled water). These tubes were incubated at 95^0C for 90 minutes and then kept at room temperature for 20-30 minutes. The absorbance of the reaction mixture was thereafter measured at 695 nm (Prieto *et al.*, 1999). Mean values from three independent samples were calculated for each sample and ascorbic acid used as positive reference standard.

PHYTOCHEMICAL SCREENING (QUALITATIVE AND QUANTITATIVE ANALYSES)

Qualitative and quantitative analyses for the alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, glycosides, arthraquinones and steroids present in *S. mombin* were carried out using standard procedures as described by Sofowora (Sofowora, 2008).

DATA ANALYSIS

Data generated was presented as mean ± standard error of mean and analyzed using SPSS 15.0 for Windows. Differences in body weights, haematological and serum biochemistry indices were analyzed by student t-test while BCS and mucous membrane were analyzed using Chi-square test; $p \leq 0.05$ was considered as statistical significance. For the *in vitro* antioxidant assays, the 50% inhibitory concentration (IC_{50}) of the test anthelminthic samples was determined using linear regression with $p \leq 0.05$ been considered as significant.

RESULTS

PERCENTAGE YIELD OF CRUDE EXTRACTS AND FRACTIONS OF *SPONDIAS MOMBIN* LEAVES

The percentage yield of the crude extracts and fractions of *S. mombin* leaves are given in Table I. The methanol fraction had the highest yield (58.58%), followed by the hexane fraction (14.18%) while the aqueous fraction was the least (8.52%).

ANTHELMINTIC ACTIVITY OF AQUEOUS EXTRACT OF SPONDIAS MOMBIN LEAVES IN DOGS

During and up unto day 31 post infection, faecal examination did not reveal any *A. caninum* eggs for both groups. One to two larvae were observed in dogs in group B (S. mombin) (Figure I). There were however, classical signs of helminthosis [temperature and pulse rate within normal range (38.5-39.3^{0}C; 90-120 beats/min), dry, rough and falling hair coat, diarrhoea (faeces which ranged from brown, bloody, greenish to yellowish and black in colour), anorexia and weakness] evident. After treatment, faeces returned to normal colour and consistency in Group B, comparing well with the standard drug (pyrantel pamoate) (Table II). The body weight and BCS in both groups before and PT was not significant (p=0.4) (Table III).

EFFECT OF AQUEOUS LEAF EXTRACT OF SPONDIAS MOMBIN ON HAEMATOLOGY OF DOGS EXPERIMENTALLY INFECTED WITH ANCYLOSTOMA CANINUM

Post treatment, there was a decrease in PCV for Group A (32.40±5.73 to 30.40±5.98%). However, the Group B had an improvement in their PCV (30.60±6.54 to 33.20±4.87%), though both were not statistically significant (p=0.8). There was an increase in the WBC count in Group A (6.06±3.35 to 8.17±1.14x 10^3/µL) PT as opposed to the decrease in WBC using S. mombin (8.74±4.85 to 8.37±1.78x 10^3/µL). There was no statistical significant difference (p=0.306) in all the haematological indices between the two groups before and PT (Table IV).

Table I: The percentage yield of the crude extracts and fractions of Spondias mombin leaves

Crude extract	***% content (w/w)***
Aqueous	*71.2*
Methanol	*8.40*
	Fractions
	Hexane *14.18*
	Chloroform *9.20*
	Ethylacetate *9.67*
	Methanol *58.58*
	Aqueous *8.52*

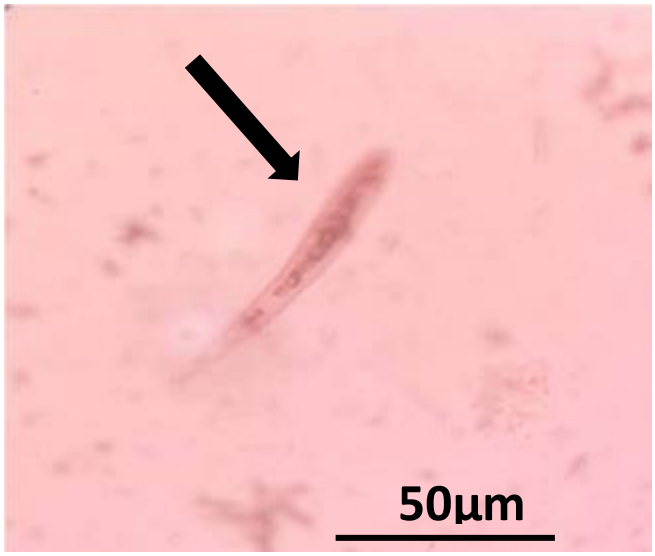

Figure I: Ancylostoma larva identified (thick arrow) in faecal sample before treatment (group B) (× 400)

Table II: The effect of aqueous leaf extract of *Spondias mombin* on faecal colour and consistency of dogs experimentally infected with *Ancylostoma caninum*

		COLOUR					CONSISTENCY			
Group	**Treatment**	**Bloody**	**Black**	**Greenish**	**Yellow**	**Brown**	**Soft and pasty**	**Diarrhoeic**	**Formed**	**Hard**
A- Pyrantel pamoate (5 mg/kg)	Before	1	1	0	3	0	4	1	0	0
	Post	0	0	0	0	5	0	0	5	0
B- *S. mombin* (500 mg/kg)	Before	2	0	1	2	0	2	2	1	0
	Post	0	1	0	0	4	0	0	5	0

Table III: The effect of aqueous leaf extract of *Spondias mombin vis-à-vis* PP on faecal colour and consistency of dogs experimentally infected with *Ancylostoma caninum*

Group	**Treatment**	**Average Body weight (kg)**	**Average eight difference (kg)**	**Body condition score**				**Mucous membrane**		
				Very thin	**Underweight**	**Ideal weight**	**Overweight**	**Jaundiced**	**Pale**	**Pink**
A- Pyrantel pamoate (5 mg/kg)	Before	2.98±0.98		0	3	2	0	0	0	5
	Post	4.01±1.39	1.03±0.23	0	0	4	1	0	0	5
B- *S. mombin* (500 mg/kg)	Before	2.80±0.70		1	2	2	0	1	0	4
	Post	3.39±1.00	0.59±0.18	1	0	4	0	0	0	5

EFFECT OF AQUEOUS LEAF EXTRACT OF *SPONDIAS MOMBIN* ON HAEMATOLOGY OF DOGS EXPERIMENTALLY INFECTED WITH *ANCYLOSTOMA CANINUM*

Post treatment, there was a decrease in PCV for Group A (32.40±5.73 to 30.40±5.98%). However, the Group B had an improvement in their PCV (30.60±6.54 to 33.20±4.87%), though both were not statistically significant (p=0.8). There was an increase in the WBC count in Group A (6.06±3.35 to 8.17±1.14x $10^3/\mu L$) PT as opposed to the decrease in WBC using *S. mombin* (8.74±4.85 to 8.37±1.78x $10^3/\mu L$). There was no statistical significant difference (p=0.306) in all the haematological indices between the two groups before and PT (Table IV).

EFFECT OF AQUEOUS LEAF EXTRACT OF *SPONDIAS MOMBIN* ON SERUM BIOCHEMISTRY IN DOGS EXPERIMENTALLY INFECTED WITH *ANCYLOSTOMA CANINUM*

The total serum protein, albumin and globulin values were within normal range, before and PT and no statistically significant difference (p=0.2) in both groups A and B (Table V). Total cholesterol, VLDL and phospholipids were significantly reduced (p=0.05) PT in the extract treated Group B (Table VI). There were no statistically significant changes (p=0.4) in the antioxidative enzymes in animals treated with the standard drug and *S. mombin* (Table VII).

***IN VITRO* ANTIOXIDANT ACTIVITY OF FRACTIONS OF *SPONDIAS MOMBIN* LEAVES**

All the five concentrations assayed (200, 400, 600, 800 and 1000µg/ml) had radical scavenging activity with the methanol fraction showing more than 80% activity at the highest concentration in the DPPH assay (Figure II). On the other hand, the aqueous fraction showed the highest activity using the FRAP determination (Figure III). The total antioxidant capacity was greater than 1 in all the fractions reaching up to 3 in the methanol fraction (Figure IV).

PHYTOCHEMICAL ANALYSES OF FIVE FRACTIONS OF *SPONDIAS MOMBIN* LEAVES

Nine phytochemicals were present in varying proportions in the five fractions, with alkaloids being present in all fractions while anthraquinones were only present in the hexane fraction (Table VIII). Flavonoids, tannins, terpenoids and phenols were absent in the hexane fraction and alkaloids had the highest concentration in the ethylacetate fraction (Table IX).

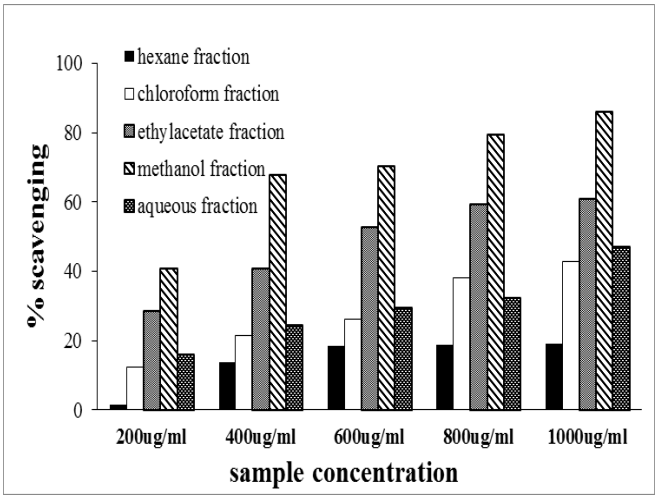

Figure II: Radical scavenging activity of fractions of *Spondias mombin* leaves using DPPH assay

Table IV: The effect of aqueous leaf extract of *Spondias mombin* on the haematology of dogs experimentally infected with *Ancylostoma caninum*

Parameters	Treatment	A- Pyrantel pamoate 5 mg/kg	B-*S. mombin* 500 mg/kg	*Reference range
Packed cell volume (%)	Before	32.40±5.73	30.60±6.54	35.0-57.0
	Post	30.40±5.98[a]	33.20±4.87[a]	
Haemoglobin concentration (g/dl)	Before	14.40±0.67	14.38±1.29	11.9-18.9
	Post	14.40±0.67[a]	13.80±0.67[a]	
White blood cell count (x 10^3/µl)	Before	6.06±3.35	8.74±4.85	5.0-14.1
	Post	8.17±1.14[a]	8.37±1.78[a]	
Red blood cell count (x 10^6/µl)	Before	7.06±1.75	6.66±2.80	4.95-7.87
	Post	5.85±2.27[a]	6.65±2.81[a]	
Mean corpuscular volume (fl)	Before	43.25±6.34	48.78±13.31	66.0-77.0
	Post	43.42±5.34[a]	50.18±11.34[a]	
Mean corpuscular haemglobin (pg)	Before	17.33±3.15	22.34±9.73	21.0-26.2
	Post	18.66±1.28[a]	23.52±8.44[a]	
Mean corpuscular haemoglobin concentration (g/dl)	Before	4.00±0.17	4.46±1.02	32.0-36.3 (mg/dl)
	Post	4.32±0.30[a]	4.60±0.56[a]	

*msdvetmanual.com; amean values are not statistically different

Table V: The effect of aqueous leaf extract of *Spondias mombin vis-à-vis* PP on the serum protein profile of dogs experimentally infected with *Ancylostoma caninum*

Group	Treatment	Mean Total protein (g/dl)	Mean Albumin (g/dl)	Mean Total protein (g/dl)
A- Pyrantel pamoate (5 mg/kg)	**Before**	6.41±0.26	4.22±0.15	2.37±0.23
	Post	7.05±0.26[a]	3.88±0.15[a]	3.21±0.23[a]
	Difference	0.64±0.26	-0.34±0.31	0.84±0.25
B- *S. mombin* (500 mg/kg)	**Before**	6.09±0.26	4.08±0.15	2.05±0.23
	Post	7.36±0.26[a]	4.02±0.15[a]	3.34±0.23[a]
	Difference	1.28±0.37	-0.06±0.09	1.29±0.43
	***Reference range**	5.4-7.5	2.3-3.1	2.7-4.4

*msdvetmanual.com; [a]mean values are not statistically significantly different

Table VII: The effect of aqueous leaf extract of Spondias mombin vis-à-vis PP on serum glucose and antioxidative enzymes level of dogs experimentally infected with Ancylostoma caninum

Group	Treatment	Mean Glucose	Mean CAT	Mean SOD	Mean MDA	Mean GST
		mg/dl	µ/l	µ/l	µ/l	µ/l
A- Pyrantel pamoate (5 mg/kg)	Before	61.15±2.52	0.03±0.01	1.26±0.26	0.77±0.20	0.01±0.01
	Post	62.89±2.52[a]	0.04±0.01[a]	2.56±0.26[a]	1.55±0.20[a]	0.02±0.01[a]
	Difference	1.75±1.36	0.01±0.00	1.30±0.53	0.78±0.22	0.01±0.01
B- *S. mombin* (500 mg/kg)	Before	61.79±2.52	0.03±0.01	1.26±0.26	1.16±0.20	0.01±0.01
	Post	62.28±2.52[a]	0.04±0.01[a]	2.15±0.26[a]	1.81±0.20[a]	0.01±0.01[a]
	Difference	0.49±4.96	0.01±0.01	0.88±0.28	0.64±0.17	0.00±0.00
	*Reference range	76-119				

*msdvetmanual.com; aMean values not statistically different; CAT-Catalase; SOD-Superoxide dismutase; MDA-Malondialdehyde; GST-Glutathione-S-transferase

Table VIII: Phytochemical constituents of the fractions of *Spondias mombin* leaves (Qualitative)

Phytochemical constituent	Fraction				
	Hexane	Chloroform	Ethylacetate	Methanol	Aqueous
Alkaloids	+ve	+ve	++ve	+ve	+ve
Flavonoids	-ve	+ve	++ve	++ve	++ve
Terpenoids	+ve	+ve	+ve	++ve	-ve
Saponins	-ve	+ve	++ve	++ve	++ve
Phenols	-ve	+ve	+ve	++ve	+ve
Tannins	-ve	+ve	+ve	++ve	+ve
Glycosides	-ve	-ve	+ve	+ve	+ve
Anthraquinones	++ve	-ve	-ve	-ve	-ve
Steroids	++ve	++ve	+ve	-ve	-ve

-ve: absent; +ve: present; ++ve: strongly present

DISCUSSION

Plant extracts are composed of structurally diverse molecules, which range from highly lipophilic (non-polar) constituents that are partitioned into n-hexane, and to very polar ones into water. It has been reported that the most potent anthelmintic principles in the leaf of *S. mombin* are polar compounds, so aqueous extract was used for the anthelmintic study. In addition, aqueous extract was reported as the safest among three tested extracts (n-hexane, ethylacetate and aqueous) for anthelmintic activity (Mondal *et al.*, 2020). De *et al.* (2016) reported that single dose administration of pyrantel pamoate at 5mg/kg per os, was effective for clinical treatment of spontaneous ancylostomosis in dogs and was chosen as the positive control for this study.

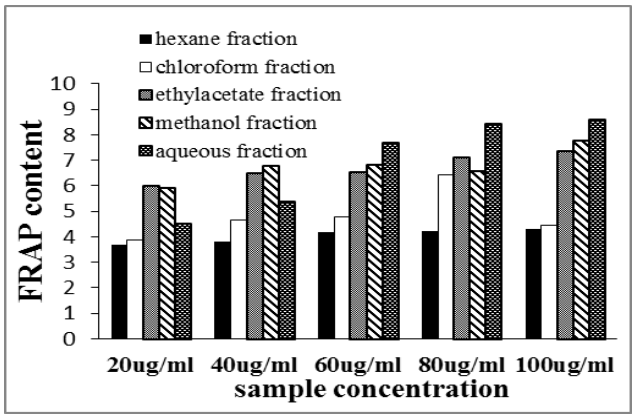

Figure III: Ferric ion reducing antioxidant power (FRAP) content for the different fractions of *Spondias mombin* leaves at ascorbic acid equivalence

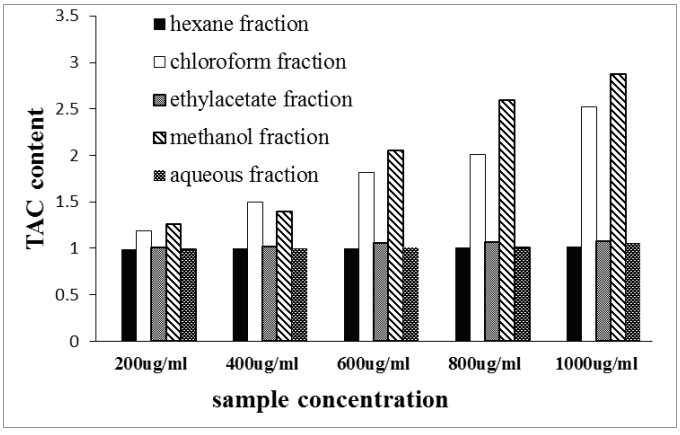

Figure IV: Concentration of total antioxidant capacity (TAC) for the different fractions of *Spondias mombin* leaves relative to the standard

In this study, the anthelmintic activity of the aqueous extract of *S. mombin* leaves in dogs could not be quantified as no *A. caninum* egg was seen in the faeces of both groups despite the pre-patent period of 24-31 days. This is longer than the pre-patent period of 14-15 days for ancylostomosis as reported by Idika & Nwosu (Idika & Nwosu, 2017). In contrast, Taweethavonsawat *et al.* (2010) reported that patent infection developed in 14 days post infection as all dogs were shedding eggs but were not showing clinical signs of ancylostomosis. This may imply that FEC alone may not be a good indicator for measuring the worm burden and severity of ancylostomosis in Nigerian indigenous breed of dogs. Both owned and free-roaming dogs are frequently infected with ancylostomosis, but sometimes dogs could be infected without apparent evidence of the parasites' presence as seen in this study. It is possible that the slow dissipation of ivermectin administered subcutaneously two weeks prior to infection could have impacted on the larvae and as such, they could not attain sexual maturity to lay eggs. In addition, the dogs could have possibly developed a form of resistance due to prior exposure to this helminth through trans-mammary transmission and the body systems find a way of expelling the worm even before it attains sexual maturity (Hossain & Bhuiyan, 2016). The effect of the extract could however be seen in the improved haemato-biochemical parameters, BCS of the dogs, appetite, consistency and colour of faeces and skin coat, comparing well with the standard drug. This portends the anthelmintic effects of the plant *in vivo*, as previously reported in sheep (Ademola *et al.*, 2005, Jola & Escobar, 2019). This may be attributed to its alkaloid and flavonoid content, which have been shown in extracts from other plants to possess anthelmintic activities (McAllister *et al.*, 2011).

Haematological changes were seen in the dogs because of the haematophagus nature of the parasite, leading to slight reduction in PCV (Reinemeyer, 2016). After treatment, there was slight improvement in PCV in the *S. mombin* treatment group when compared with the standard (pyrantel pamoate). There was a slight decrease in the WBC values in *S. mombin* group post treatment. This could be due to the anti-inflammatory/antioxidative potentials of some phytochemicals in *S. mombin* leaves, which agrees with other studies (Cabral *et al.*, 2016). Presence of these

Table IX: Phytochemical constituents of the fractions of *Spondias mombin* leaves (Quantitative)

Fraction	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoids	Phenols
			(%w/w)			
Hexane	1.85	0	0	0	0.75	0
Chloroform	0.75	1.52	0.08	0.91	0.3	0.75
Ethylacetate	10.85	2.94	0.95	0.92	099.7	0.85
Methanol	5.1	3.18	1.4	0.94	1	1.08
Aqueous	4.4	4.42	1.45	0.93	0.25	0.68

phytochemicals would aid animal recovery from pathologies associated with the presence of the parasite.

Naturally, body cells protect themselves against free radical damage through antioxidant defence compounds such as ascorbic acid, tocopherol and glutathione and via enzymes such as SOD and CAT (Ali *et al.,* 2020). They minimize the effect of free radicals on biomolecules by scavenging them. These protective mechanisms may be disrupted by parasitic infections such as helminthosis, and production of free radicals overwhelms the biological antioxidant systems (Ali *et al.,* 2020). An external source of antioxidants offers a promising way of preventing deleterious effects. It has also been reported that phytochemicals such as flavonoids and polyphenols present in *S. mombin*, as in many other Nigerian indigenous plants, are promising. Though, reports on the *in vivo* and *in vitro* antioxidant activity of *S. mombin* have been published (Mensor *et al.*, 2001; Cabral *et al.,* 2016; Sameh *et al.,* 2018), to the best of our knowledge, this is the first report of its antioxidant activity in dogs.

Reduction in serum globulins usually serves as key pointers to inflammation, immunologic conditions, and poor nutrition, which were evident with ancylostomosis before treatment. Reduction in globulin levels could also point to liver or kidney disease. Animals were hypoglycaemic and could be responsible for signs such as lethargy associated with the infection. Post treatment, the total protein, albumin and globulin values were within normal range.

Semi-polar compounds such as methanol act as intermediate solvents, which bring about miscibility of polar and non-polar liquids, hence its use in this study to evaluate the phytochemistry of *S. mombin*. Nine phytochemicals were present in varying proportions in the five fractions of *S. mombin* leaves, with alkaloids and flavonoids being the most abundant. Previous reports on the phytochemical constituents of *S. mombin* showed high alkaloid content in the leaves, seed, fruit and essential oil of the plant grown in Southwest and Southeast Nigeria (Igwe *et al.*, 2010) and Brazil (Cabral *et al.,* 2016). This study may however, represent the first study elucidating the different phytochemicals in fractions of the plant. High levels of tannins, flavonoids, and alkaloids were seen in plants with anthelmintic activity in Columbia (Jola & Escobar, 2019). Flavonoids are free radical scavengers; super antioxidants and potent water-soluble compounds, which prevent oxidative cell damage (Jola & Escobar, 2019). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their biological properties. Phaeophorbide-a isolated from *S. mombin* leaf extract has been reported to possess good anthelmintic activity (Ogedengbe-Olowofoyeku *et al.,* 2021).

CONCLUSION

Spondias mombin leaf crude extract is an effective anthelminthic and could find application in the ongoing effort to develop alternative anthelmintic therapy for managing canine helminthosis. This study provides direction for the task of isolating new chemical entities with anthelmintic properties from the plant. Further work is ongoing to evaluate alcoholic extracts and isolated compounds of the plant in naturally infected dogs.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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