Ethanol Extract of Spondias mombin L. Leaves Exhibits Antidiarrhoeal Activity by Stimulation of Na+–K+ ATPase, Inhibition of Prostaglandins or Suppression of Nitric Oxide

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Research Article

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Abstract

This study was to justify the acclaimed antidiarrhoeal activity of ethanol extract of *Spondias mombin* leaves (EESML) and to suggest probable mechanism of action.

EESML was screened for its secondary metabolites. The diarrhoeal models involved randomized Wistar rats in 5 groups of 6 animals each. Animals in groups A&B (negative and positive control) were treated with normal saline and loperamide respectively while those in groups C, D and E received 100, 200 and 400 mg/kg body weight of EESML respectively.

EESML contained saponins, alkaloids, flavonoids, tannins, steroids, phenolics and glycosides. EESML lengthened the onset time of diarrhoea and as well caused reductions in the number, fresh weight and total number of wet feaces; and increase in the inhibition of defecations. EESML increased the intestinal activity of Na^+–K^+ ATPase; the concentrations of intestinal Na^+, K^+, Cl^-, total protein and glucose but decreased the concentration of nitric oxide of the diarrhoeal rats. The intestinal fluid concentrations of Na^+, K^+, Cl^- were dose dependently increased by EESML. EESML also increased the length of the small intestine.

EESML possess antidiarrhoeal activity owing to the secondary metabolites, ability to enhance Na^+–K^+ATPase activity and electrolytes as well as suppression of nitric oxide.

1.0 Introduction

Diarrhoea, characterized by the frequent passage of liquid feaces, increased motility of the gastrointestinal tract, increased secretion and decreased absorption of fluid, as well as loss of water and electrolytes, is one of the leading causes of death in the world. It comes behind pneumonia as the leading cause of death among children both of which accounts for an estimated 40 % child death across the globe annually [1]. Diarrhoea causes an estimated 5 million deaths in children less than 5 years of age per year equivalent to 1 childhood death per minute [1, 2].

The continuous search of antidiarrhoeal principles from natural plant products from a number of medicinal herbs/plants that have been implicated in folklore medicine is therefore not a misplaced priority, and one of such invaluable plants is *Spondias mombin*. *Spondias mombin* L. (Anacardiaceae) is an evergreen tree distributed mostly in tropical areas like Nigeria, Ivory Coast, Cameroon, Brazil, Mexico and Peru. The plant is known as *Hog plum* in English. The native name in Nigeria is *Ichikara* in Igbo, *Tsardarmasar* in Hausa and *Akika etikan* or *Iyeye* in Yoruba [3]. Various parts of the plant including the leaves, fruits, stem bark, seed, pulp, root and flower have ethnomedicinal uses for the management or treatment of ailments [4]. The leaves and bark of *S. mombin* contain tannins, saponins, flavonoids, sterols, quinones, and antioxidant chemicals [7] and there has been few claims on both *in vitro* and *in vivo* efficacy [5–15].

The antidiarrhoeal study on the plant is still scanty and without information on the probable mechanism of action. This study was therefore aimed to report the constituent secondary metabolites of *S. mombin* leaves and screen the extract on various models for its antidiarrhoeal activity and suggest the mechanism of action.

2.0 Materials And Method

2.1 Plant Material

Plant material of the *Spondias mombin* voucher specimen (voucher number: UILH/001/1147) was obtained from Isaba Ekiti, Nigeria. The dried samples were then pulverized into fine powder with an electric blender (Trident Ltd, China]. Powdered plant material was stored in an airtight jar and further defatted with ethanol (25°C) and filtered using Whatman No. 1 filter paper. The ethanol solvent was recovered by evaporation using a rotary evaporator (Shanghai, China) that yielded a semisolid mass further lyophilized (New Brunswick) at 25°C for 24 hours. The obtained dry powder was reconstituted in physiological saline to give the doses of 100, 200 and 400 mg/kg body weight used in this study.

2.2 Phytochemical Screening

The phytochemical screening tests of the ethanol extract of *S. mombin* were performed for the detection of phytoconstituents steroids, anthraquinones, cardenolides and dienolides, phlobatannins [16]; alkaloid [17]; cardiac glycoside [18]; saponins [19], phenolics and flavonoids [20]; tannins and terpenes [21].

2.3 Animals

Wistar rats (130.7 ± 5.14 g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria). The rats were kept in well-ventilated house conditions (Temperature: 28–30°C), photo-period (12-hr light, 12-hr dark; humidity: 45–55%). Animals were fed with pellets (Premier Feeds, Ibadan) and water *ad libitum* except when fasted. The animals were handled and provided care in accordance with the ethical guidelines on the Care and Use of Laboratory Animals as highlighted by the National Research Council, USA [22].

2.4 Acute Oral Toxicity Test

OECD method for acute oral toxicity was adopted. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for a period of two days and then for a period of 14 days [23].

2.5 Castor Oil-Induced Diarrhoea in Rats

The method described by Doherty [24] but with some modifications was adopted in this model. Healthy Wistar rats fasted overnight for 18 hours were induced into diarrhoea following oral administering 1 mL of castor oil. Animals with diarrhoea after one hour of castor oil administration were completely randomized into five groups of six animals each. Rats of group A (negative control) received 1 mL of

normal saline orally while rats of group B (positive control) received 3 mg/kg of the standard drug, loperamide (orally). Animals in the groups C, D, and E (test groups) were orally administered equal volume of the extract corresponding to 100, 200 and 400 mg/kg body weight of the extract respectively. The animals were placed (individually) in metabolic cages on white clean preweighed Whatman filter paper, which was changed hourly. The severity of diarrhoea was assessed each hour for 6 hours during which the time of onset of diarrhoea, the total number of faeces, diarrhoeal faeces, total weight of faeces, and percentage inhibition of diarrhoeal defecation in each group were computed. The weight of the faeces was obtained from the difference in the preweighed Whatman filter paper and fresh weight of the stool. The faeces dry weight was derived by drying out the fresh faeces in an oven (Uniscope Laboratory Oven, SM9053, Surgifriend Medicals, England) at 100º C till a constant weight was obtained. Water content of the faeces was also obtained as the difference in the fresh weight of the faeces and dry weight of the faeces. After the 6-hour exposure period, the animals were sacrificed to prepare small intestine supernatants.

2.6 Magnesium Sulfate-Induced Diarrhoea in Rats

The modified method described by Doherty [24] in Sect. 2.5 was adopted in this model except that magnesium Sulfate was used here.

2.7 Castor Oil-Induced Enteropooling in Rats

The procedure described by Robert et al. [25] was adopted for the castor oil-induced enteropooling study. Wistar rats fasted for 18 hours overnight were completely randomized into five groups of six animals each (Table 3). Animals in all the groups were orally administered 1mL of castor oil. One hour later, Animals in Group A which served as the negative control received 1 mL of normal saline while those in groups B (positive control), C, D and E (test groups) were orally administered same volume corresponding to 3 mg/kg of loperamide (standard drug), 100, 200, and 400 mg/kg body weight of the extract respectively by oral treatment.

After 2 hours of these treatments, the rats were sacrificed according to the method described by Akanji and Yakubu [26].
The ends/edges of the pylorus and caecum of the small intestine were tied with thread. The small intestine was dissected, weighed and its content gouged into a measuring cup. The percentage of inhibition of the intestinal content was computed from the volumes and the masses. The differences between full and empty intestines were computed weighing the intestines again. The intestinal fluid was also analyzed for Na^+, K^+ and Cl^- concentration.

Table 3
Effect of Ethanol Extract of *S. mombin* Leaves in Magnesium Sulphate-Induced Diarrhoea in Rats

Parameters/Dose	**Normal saline**	**Loperamide**	**Ethanol extract of *S. mombin* leaves (mg/kg body weight)**		
		3 mg/kg body weight	**100**	**200**	**400**
Onset time (minutes)	55.98 ± 1.04^{a}	120.55 ± 1.15^{b}	74.65 ± 1.07^{c}	120.59 ± 1.19^{b}	> longest time the experiment lasted
Total number of feaces	6.65 ± 1.02^{a}	2.07 ± 0.05^{b}	3.01 ± 0.07^{d}	2.68 ± 0.06^{c}	1.99 ± 0.01^{c}
Number of wet feaces	4.44 ± 0.03^{b}	0.68 ± 0.03^{b}	1.58 ± 0.02^{c}	0.63 ± 0.04^{b}	Nil
Fresh weight of feaces (g)	3.98 ± 0.07^{a}	0.55 ± 0.01^{b}	0.90 ± 0.02^{d}	0.67 ± 0.95^{c}	Nil
Water content of feaces (mL)	1.59 ± 0.01^{a}	0.33 ± 0.04^{b}	0.99 ± 0.08^{d}	0.47 ± 0.11^{c}	Nil
Inhibition of defecation (%)	0	85	64	86	100
Intestinal Na^{+}–K^{+} ATPase activity (µmol Pi/mg protein/hour)	997.40 ± 4.09^{a}	1240.50 ± 4.93^{b}	1451.45 ± 5.29^{c}	1760.71 ± 5.16^{d}	1980.44 ± 4.19^{e}
Intestinal Nitric oxide (µmol/L)	148.61 ± 1.66^{a}	80.80 ± 0.91^{b}	82.99 ± 0.42^{b}	62.56 ± 0.29^{c}	42.50 ± 0.68^{d}
Intestinal Protein (mg/ml)	18.55 ± 0.16^{a}	26.18 ± 0.13^{b}	23.20 ± 0.20^{c}	25.50 ± 0.11^{bc}	26.16 ± 0.20^{b}
Intestinal Glucose (mmol/l)	14.10 ± 0.10^{a}	16.24 ± 0.16^{b}	16.77 ± 0.15^{c}	16.90 ± 0.11^{d}	16.99 ± 0.20^{d}
Intestinal Na^{+} (mEq/l)	155.40 ± 1.97^{a}	168.76 ± 2.06^{b}	160.10 ± 1.36^{c}	165.27 ± 1.64^{d}	168.99 ± 2.04^{b}
Intestinal K^{+} (mEq/l)	1.99 ± 0.00^{a}	2.78 ± 0.06^{b}	3.78 ± 0.03^{c}	3.89 ± 0.06^{c}	4.05 ± 0.05^{d}
Intestinal Cl^{-} (mEq/l)	40.55 ± 0.98^{a}	45.76 ± 1.09^{b}	43.67 ± 1.06^{c}	45.50 ± 0.66^{b}	45.99 ± 1.01^{b}

Values are mean of 6 replicates ± SEM, values with different superscripts across the rows for each parameter are significantly different at $P < 0.05$.

2.8 Magnesium Sulphate-Induced Enteropooling in Rats

The modified procedure described by Robert et al. [25] in Sect. 2.7 was replicated expect that magnesium sulphate was used in this case.

2.9 Gastrointestinal Motility Using Charcoal

The method described by Robert et al. [25] but with some modifications was adopted. Wistar rats were fasted overnight for 18 hours prior to the commencement of the experiment. The rats were completely randomized into five groups of six animals each. They were administered 1 mL of castor oil orally. One hour later, animals in the group A which served as the negative control received 1 mL of normal saline orally while those in group B were used as positive control and were orally administered 1 mL, corresponding to 5 mg/kg body weight of atropine sulphate. Animals in the groups C, D, and E were orally administered equal volume of the extract corresponding to 100, 200 and 400 mg/kg body weight. After 30 minutes of treatment, all the animals were again administered orally with 1 mL of charcoal meal (10% charcoal suspension in 5% agarose agar, prepared by thorough mixture of 10 g of charcoal powder and 5 g of agarose agar 100 mL distilled water). The animals were then sacrificed after 45 minutes of charcoal administration, using the diethyl ether as anesthesia as previously described by Akanji and Yakubu [26].

2.10 Determination of Biochemical Concentrations/Activity

The procedure described by Akanji and Yakubu [26] was adopted for the preparation of small intestine supernatants. The concentration/activity of total protein; glucose; Na+-K + ATPase; nitric oxide; chloride, sodium and potassium ions, were determined by the method described by Gornal *et al.* [27]; Barham and Trinder [28]; Bewaji et al. [29]; Wo et al. [30]; Tietz [31] respectively.

2.11 pH of Intestinal Fluid Secretion

The pH of the intestinal fluid collected was read using a pH cooperative paper (strip), ranged 5–9. This strip is capable of different colour change within this pH range which can be compared to a standard colour for a given pH value as supplied with the kit by the manufacturer [32]. The pH paper was dipped into the sample and the colour that developed was checked against known standards [32].

2.12 Statistical analysis

Data were expressed as the mean of six determinations ± Standard Error of Mean (SEM). The normally distributed data were analysed using Duncan Multiple Range Test and One-Way Analysis of Variance was used to compare each test value with the control. The differences were considered statistically significant at $pcript > 0.05$ (confidence level = 95%). All the analyses were done with SPSS version 20.0 software (SPSS Inc, Chicago, IL, USA).

3. Results

3.1 Secondary Metabolite Constituents in *Spondias mombin* Leaves

Alkaloids, saponins, tannins, anthraquinones, phenolic, flavonoids, triterpenes and steroids were detected in the leaves (Table 1).

Table 1
Secondary Metabolite Constituents in Ethanol Extract of Spondias mombin **Leaves**

Secondary metabolites	Observation	Concentration (mg/ml)
Saponins	Stable, persistent froth with distilled water	4.80 ± 0.35
Alkaloids	Cream colour with Mayer's reagent; Reddish-brown with Wagner's reagent	3.40 ± 0.10
Total Flavonoids	Dark yellow precipitate with NH_3	2.80 ± 0.36
Tannins	Greenish-brown precipitate	1.47 ± 0.06
Steroids	Presence of violet to blue or green colour with H_2SO_4	0.92 ± 0.09
Total Phenolics	Greenish precipitate with $FeCl_3$	1.73 ± 0.19
Cardiac Glycosides	Brown violet ring at the interface	0.01 ± 0.00
Anthraquinones	Absence of bright pink colour with NH_3	Not detected
Cardenolides and dienolides	Absence of turbid brown ring at the interface	Not detected
Chalcone	Absence of red coloration with concentrated sulfuric acid	Not detected
Phlobatnnins	Absence of reddish precipitate with HCl	Not detected
Terpenoids	Absence of reddish-brown colour with H_2SO_4	Not detected
Values are means of three determinations ± SEM		

3.2 Acute Toxicity Study

Oral administration of Ethanol extract of *Spondias mombin* leaves produced no visible signs of toxicity in the animals receiving up to a dose 2000 mg/kg body weight the test extract was not lethal to rats even at 2000 mg/kg dose.

3.3 Antidiarrhoeal Activity of the Ethanol Extract of *S. mombin* Leaves in Castor Oil-Induced Rats

The ethanol extract of *S. mombin* leaves significantly ($P < 0.05$) prolonged the onset time of diarrhoea. The total number of feaces as well as the number of wet feaces, fresh weight and water content of feaces were significantly ($p < 0.05$) decreased. There was a 67% and 80% inhibition of defecation recorded at 100 and 200 mg/kg body weight of the extract (Table 2). The percentage inhibition of defecation (86%) as well as the fecal parameters of rats treated with 400 mg/kg body weight of the extract compared favourably with those of the rats administered 3 mg/kg body weight of loperamide hydrochloride (Table 2). In addition, the extract significantly ($P < 0.05$) increased the intestinal activity of $Na^+–K^+$ATPase as well as the concentrations of intestinal Na^+, K^+, Cl^-, total protein and glucose but significantly ($P < 0.05$) decreased the concentration of nitric oxide of the diarrhoeal rats in a dose manner when compared with the normal saline treated diarrhoeal rats (Table 2). The 400 mg/kg body weight of the extract treated animals gave the profound activity when compared with the other treatment groups including that of the standard drug, loperamide (Table 2).

Table 2
Effect of Ethanol Extract of *S. mombin* Leaves in Castor Oil-Induced Diarrhoea in Rats

Parameters/Dose	Normal saline	Loperamide	Ethanol extract of *S. mombin* leaves (mg/kg body weight)		
	3 mg/kg body weight	100	200	400	
Onset time (minutes)	58.15 ± 1.05^{a}	121.78 ± 1.80^{b}	75.68 ± 1.17^{c}	92.19 ± 1.19^{d}	128.77 ± 1.82^{b}
Total number of feaces	5.81 ± 0.15^{a}	2.01 ± 0.12^{b}	3.91 ± 0.17^{c}	2.88 ± 0.16^{d}	2.09 ± 1.10^{b}
Number of wet feaces	5.12 ± 0.13^{b}	0.76 ± 0.03^{b}	1.68 ± 0.02^{c}	0.98 ± 0.04^{d}	0.71 ± 0.02^{b}
Fresh weight of feaces (g)	3.59 ± 0.11^{a}	0.51 ± 0.07^{b}	2.11 ± 0.10^{c}	0.90 ± 0.05^{d}	0.58 ± 0.05^{b}
Water content of feaces (mL)	1.88 ± 0.01^{a}	0.41 ± 0.04^{b}	1.01 ± 0.08^{c}	0.67 ± 0.11^{d}	0.45 ± 0.03^{b}
Inhibition of defecation (%)	0	85	67	80	86
Intestinal Na^{+}–K^{+} ATPase activity (µmol Pi/mg protein/hour)	778.20 ± 4.99^{a}	1114.51 ± 4.43^{b}	1301.40 ± 5.19^{c}	1596.78 ± 5.36^{d}	1720.04 ± 5.39^{e}
Intestinal Nitric oxide (µmol/L)	151.47 ± 1.91^{a}	82.78 ± 0.91^{b}	84.01 ± 0.42^{c}	67.20 ± 0.29^{d}	48.99 ± 0.11^{e}
Intestinal Protein (mg/ml)	20.60 ± 0.11^{a}	27.98 ± 0.19^{b}	25.25 ± 0.16^{c}	26.99 ± 0.13^{cd}	28.76 ± 0.15^{d}
Intestinal Glucose (mmol/l)	14.80 ± 0.98^{a}	18.19 ± 0.16^{b}	16.77 ± 0.44^{c}	17.01 ± 0.23^{d}	18.20 ± 0.54^{b}
Intestinal Na^{+} (mEq/l)	150.4 ± 1.34^{a}	165.78 ± 1.16^{b}	159.60 ± 1.69^{c}	162.77 ± 1.99^{d}	165.90 ± 1.58^{b}
Intestinal K^{+} (mEq/l)	1.89 ± 0.06^{a}	2.59 ± 0.09^{b}	3.02 ± 0.10^{c}	3.25 ± 0.16^{d}	3.55 ± 0.11^{e}
Intestinal Cl^{-} (mEq/l)	42.50 ± 0.36^{a}	45.12 ± 0.22^{b}	44.16 ± 0.16^{bc}	45.01 ± 0.26^{b}	45.77 ± 1.66^{b}

Values are mean of 6 replicates ± SEM, values with different superscripts across the rows for each parameter are significantly different at $P < 0.05$.

3.4 Antidiarrhoeal Activity of the Ethanol Extract of *S. mombin* Leaves in Magnesium Sulphate-Induced Rats

Ethanol extract of *S. mombin* leaves significantly ($P < 0.05$) prolonged the onset time of diarrhoea and as well caused a significant ($P < 0.05$) reduction in number of diarrhoeal feaces by 64 %, 86 %, and 100 % at a dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively (Table 3). Standard drug loperamide cause significant ($P < 0.05$) reduction in diarrhoeal feaces by 85% at a dose of 3 mg/kg body weight. Furthermore, the extract significantly ($P < 0.05$) increased the intestinal activity of $Na^+–K^+$ATPase as well as the concentrations of intestinal Na^+, K^+, $Cl^–$, total protein and glucose but significantly ($P < 0.05$) decreased the concentration of nitric oxide of the diarrhoeal rats in a dose manner when compared with the normal saline treated diarrhoeal rats (Table 3). The activity of the extract on these parameters was highest in the 400 mg/kg body weight of the extract treated animals when compared with the other treatment groups including that of the standard drug, loperamide (Table 3).

3.5 Antienteropooling Activity of the Ethanol Extract of *S. mombin* Leaves in Castor Oil-Induced Rats

The mass and volume of intestinal fluid were significantly ($P < 0.05$) decreased whereas the inhibitions of intestinal fluid accumulation significantly ($P < 0.05$) increased by all the doses of the ethanol extract of *S. mombin* leaves when compared with the normal saline treated rats (Table 4). The actions of the extract were most profound with the 400 mg/kg body weight of the extract treated animals when compared with the loperamide treated animals. In addition, the extract resulted to a 50%, 54%, and 65% inhibitions of intestinal fluid accumulation by the 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively while loperamide gave a 56% inhibitions when compared with the normal saline treated rats (Table 4). Furthermore, the intestinal fluid concentration of Na^+, K^+, and $Cl^–$ were equally increased dose dependently (Table 4).

Table 4
Antienteropooling Activity of the Ethanol Extract of *S. mombin* Leaves in Castor Oil-Induced Rats

Parameters/Dose	Normal saline	Loperamide	Ethanol extract of *S. mombin* leaves (mg/kg body weight)		
		3 mg/kg body weight	100	200	400
Mass of intestinal contents (g)	3.01 ± 0.03^{a}	1.33 ± 0.04^{b}	1.52 ± 0.07^{d}	1.39 ± 0.04^{b}	1.05 ± 0.02^{c}
Accumulated intraluminal fluid (ml)	3.10 ± 0.05^{a}	1.35 ± 0.05^{b}	1.50 ± 0.08^{d}	1.34 ± 0.06^{b}	1.10 ± 0.00^{c}
Inhibition of intestinal accumulation (%)	0	56	50	54	65
Intestinal fluid Na^{+} (mEq/l)	142.72 ± 1.07^{a}	159.25 ± 1.02^{b}	149.68 ± 1.02^{c}	155.61 ± 1.03^{bc}	156.77 ± 1.09^{b}
Intestinal fluid K^{+} (mEq/l)	1.92 ± 0.01^{a}	3.01 ± 0.07^{b}	2.99 ± 0.08^{d}	3.21 ± 0.03^{bc}	3.33 ± 0.11^{c}
Intestinal fluid Cl^{-} (mEq/l)	40.76 ± 0.59^{a}	46.78 ± 0.33^{b}	42.19 ± 0.19^{d}	45.66 ± 0.30^{bc}	47.80 ± 0.10^{b}
Intestinal fluid pH	4.0 ± 0.16^{a}	6.5 ± 0.10^{b}	4.8 ± 0.12^{d}	5.9 ± 0.19^{c}	6.4 ± 0.11^{b}

Values are mean of 6 replicates ± SEM, values with different superscripts across the rows for each parameter are significantly different at $P < 0.05$.

3.6 Antienteropooling Activity of the Ethanol Extract of *S. mombin* Leaves in Magnesium Sulphate-Induced Rats

The ethanol extract of *S. mombin* leaves significantly ($P < 0.05$) decreased the mass and volume of the intestinal fluid dose dependently when compared with the normal saline treated animals (Table 5). The most profound reduction by the 400 mg/kg body weight that compared favourably well with the standard drug, loperamide. Moreover, inhibitions of intestinal fluid accumulation significantly ($P < 0.05$) increased at 55%, 59%, and 62% by the 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight respectively by the extract as well as 61% by loperamide when compared with the normal saline treated rats (Table 5). Furthermore, the pH of the intestinal was decreased while the intestinal fluid concentration of Na^{+}, K^{+}, and Cl^{-} were increased dose dependently (Table 5).

Table 5
Antienteropooling Activity of the Ethanol Extract of *S. mombin* Leaves in Magnesium Sulphate-Induced Rats

Parameters/Dose	Normal saline	Atropine sulphate	Ethanol extract of *S. mombin* leaves (mg/kg body weight)		
		3 mg/kg body weight	100	200	400
Mass of intestinal contents (g)	3.33 ± 0.05^{a}	1.30 ± 0.04^{b}	1.50 ± 0.03^{d}	1.38 ± 0.09^{c}	1.25 ± 0.02^{b}
Accumulated intraluminal fluid (ml)	3.50 ± 0.05^{a}	1.30 ± 0.05^{b}	1.43 ± 0.07^{d}	1.29 ± 0.06^{b}	1.20 ± 0.07^{c}
Inhibition of intestinal accumulation (%)	0	61	55	59	62
Intestinal fluid Na^{+} (mEq/l)	140.56 ± 1.07^{a}	158.26 ± 1.02^{b}	149.45 ± 1.02^{d}	153.78 ± 1.03^{c}	156.18 ± 1.09^{b}
Intestinal fluid K^{+} (mEq/l)	2.02 ± 0.04^{a}	3.50 ± 0.07^{b}	3.21 ± 0.08^{c}	3.51 ± 0.13^{b}	3.63 ± 0.10^{b}
Intestinal fluid Cl^{-} (mEq/l)	39.26 ± 1.29^{a}	45.10 ± 1.43^{b}	42.50 ± 1.20^{c}	45.68 ± 1.06^{b}	47.50 ± 1.19^{b}
Intestinal fluid pH	4.1 ± 0.11^{a}	6.5 ± 0.16^{b}	4.7 ± 0.14^{d}	5.8 ± 0.19^{c}	6.4 ± 0.15^{b}

Values are mean of 6 replicates ± SEM, values with different superscripts across the rows for each parameter are significantly different at $P < 0.05$.

3.7 Antimotility Effect of the Ethanol Extract of *S. mombin* Leaves

The ethanol extract *S. mombin* leaves produced significant ($P < 0.05$) increase in the lengths of the small intestine by all the doses of the extract but the distance travelled by the charcoal meal was significantly ($P < 0.05$) decreased when compared to both the normal saline and atropine sulphate treated rat (Table 6). The percentage inhibitions of peristalsis were 7%, 26% and 42% by the respective dose of 100, 200 and 400 mg/kg body weight of the extract. The 400 mg/kg body weight compared favourably with the 50% by reference drug treated rats (Table 6).

Table 6
Antimotility Effect of the Ethanol Extract of *S. mombin* Leaves

Parameters/Dose	Normal saline	Atropine sulphate	Ethanol extract of *S. mombin* leaves (mg/kg body weight)		
	3 mg/kg body weight	100	200	400	
Length of Small Intestine (cm^3)	85.95 ± 1.15[a]	92.78 ± 1.20[b]	89.25 ± 1.19[c]	91.56 ± 1.99[d]	92.50 ± 1.20[b]
Distance covered by charcoal meal (cm^3)	70.10 ± 1.09[a]	42.10 ± 1.00[b]	65.20 ± 1.05[c]	52.18 ± 1.06[d]	40.51 ± 1.00[b]
Peristaltic index	81.55	45.37	73.05	56.98	43.79
Inhibition of peristalsis (%)	0	50	7	26	42

Values are mean of 6 replicates ± SEM, values with different superscripts across the rows for each parameter are significantly different at $P < 0.05$.

4. Discussion

The high mortality rate of Diarrhoeal has informed the continuous search of natural products from plants for antidiarrhoeal principles/compounds that can enhance increased mucosal absorption or decreased secretion and as well facilitate increase in resistance to flow via segmental contraction, decreased propulsion and peristalsis.

In the present study, dose-dependent inhibition of the rate of defecation as well as inhibition and prolonged/lenghtened onset time of diarrhoea, decreased faecal parameters (frequency of stool, total number of feaces, water content fresh weight, and number of wet feaces) suggest the profound antidiarrhoeal property of ethanol extract of *S. mombin* leaves. This might be by inhibiting prostaglandin that induces diarrhoea via enhanced contraction of the smooth muscle, vasodilation, and secretion of mucus in the small intestine [33,34]. This can be corroborated from the reversal in nitric oxide content known to have a protective effect (antiinflammation) on the gastrointestinal tract and preventing reabsorption [34,35]. This reversal suggests the antiinflammation as one of the probable mechanism with which the extract acted as an antidiarrhoeal agent. The marked increase in concentration of Na+, K+, Cl- and glucose by the extract suggest that the extract has protective effect on the intestinal mucosa against irritation and inflammation by castor oil by inhibiting release of prostaglandins to stabilize secretion of water and these electrolytes [36] as well as their motility or transport [37]. Furthermore, the reversal and resultant increase in the intestinal activity of Na^+–K^+ ATPase by the ethanol extract of *S. mombin* leaves additionally substantiate the antidiarrhoeal activity of the plants. It is also possible that the mechanism the plant extract exhibited its antidiarrhoeal agent was by this action because it is known that castor oil, via ricinoleic acid compromise electrolytes transport and reduces absorption of Na^+ and K^+ as a

consequence of either decreasing or inhibiting the activity of Na^+–K^+ ATPase in the small intestine and colon [38]. Ethanol extract of *S. mombin* leaves could also have demonstrated its antidiarrhoeal activity probably by inhibition of prostanglandin synthesis and production of platelet activating factors by the extract.

Again, the dose-dependent inhibition of the defecation rate as well as inhibition and elongated onset time of diarrhoea, decreased faecal parameters (total number of feaces, water content, frequency of stool, fresh weight, and number of wet feaces) suggest the profound antidiarrhoeal property of ethanol extract of *S. mombin* leaves. The antidiarrhoeal property could be due to increased absorption of water and electrolytes as evidenced in the significant increase in concentration of Na^+, K^+ and Cl^- as well as the activity of Na^+-K^+ ATPase. This further substantiate the probable mechanism of action of the antidiarrhoeal property of the extract by increasing the activity of Na^+-K^+ ATPase through its *de novo* synthesis or might have an influence on the NO/prostaglandin pathway [39]. This may be the reason that defecation and the activity of Na^+-K^+ ATPase were relatively higher than the known reference drug, laporamide, one of the most efficacious and widely employed antidiarrhoeal drug that slows down or halt transit in the small intestine, reduce colonic rate of flow, and consequently increase colonic water absorption [40].

Reduction in both weight and volume of intestinal content by ethanol extract of *S. mombin* leaves suggests its antienteropooling activity in castor oil-induced experimental. These effects can be said to direct effects of inhibiting induced intestinal accumulation of fluid by reducing electrolytes secretion and water in the small intestine. This suggest that the extract enhanced the reabsorption of electrolytes and water from intestinal lumen [41]. This can be corroborated by the increase in the Na^+–K^+ ATPase activity in the present study by the extract and may as well describe the enhanced reabsorption of the electrolytes and water [42].

Furthermore, the dose dependent reduction in masses and volumes of intestinal fluid in magnesium sulphate induced enteropooling in this study suggest that the ethanol extract of *S. mombin* leaves facilitated the reabsorption of electrolytes and water and consequently subdued the stimulated fluid accumulated in the intestine. The reabsorption of electrolytes and water by the extract might probably be a resultant effect of the increased activity of Na^+– K^+ ATPase in the present study [43].

Also in the present study, the suppression of the propulsive movement or gastrointestinal transit of charcoal meal suggest the capability of the extract in reducing the frequency of stools and further support the prolongation in the time required for the absorption of water and electrolytes in diarrhoeal conditions. This can be direct consequence of a decrease in peristaltic activity as evidenced by the peristaltic index computed, and ultimately reduction in the gastrointestinal motility. The inhibited gastrointestinal motility in the diarrhoeal condition by the extract of *S. mombin* leaves can be said to be through anticholinergic effect because anticholinergic agents are known to inhibit gastrointestinal hypermotility [41].

The antidiarrhoeal activity of ethanol extract of *Spondias mombin* leaves may therefore be attributed to the presence of some of these secondary metabolites especially flavonoids, alkaloids, saponins and phenolics that are present in the extract. It is possible they might have acted singly or in combination through any of these mechanisms [41,42]. These provide a scientific basis for the potential use of the extract in diarrhoeal conditions.

In conclusion, the overall result in this study substantiated the antidiarrhoeal activity of the ethanol extract of *Spondias mombin* leaves and further thus justified its traditional use in diarrhoea treatment. The antidiarrhoeal activity might have been conferred by the flavonoids, saponins, alkaloids and phenolics may have acted by stimulating the activity of the Na^+–K^+ ATPase, inhibition of prostaglandins or suppression of nitric oxide.

Declarations

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Ethical Approval

The study was conducted following guidelines on the care and use of laboratory animals and was approved by the University of Ilorin Ethical Review Committee with the reference letter UERC/ASN/2015/206

Consent to Participate

Not Applicable

Consent to Publish

Not Applicable

Authors' Contributions

Conception and Design: OB Ogunro.

Acquisition of Data: OB Ogunro.

Analysis and Interpretation of Data: OB Ogunro and EB Ofeniroro.

Drafting the Manuscript: OB Ogunro.

Revising for Intellectual Content: OB Ogunro and EB Ofeniroro.

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Competing Interest

The authors declare no conflict of interest

Availability of Data and Materials

The authors confirm that the data supporting the findings of this study are available within the article

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