

Ahmed, M.U., R.O. Arise, and I.Y. Sudi, Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extracts of *Annona senegalensis*. International Journal of Life Sciences and Biotechnology, 2020. 3(1): p. 1-17. DOI: 10.38001/ijlsb.656112

## Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extracts of *Annona senegalensis*

Maryam Usman Ahmed<sup>1\*</sup> , Rotimi Olusanya Arise<sup>2</sup> , Ismaila Yada Sudi<sup>1</sup> 

### ABSTRACT

Diarrhoea is a leading cause of deaths amongst children below five years. *Annona senegalensis* plant is used traditionally to treat diarrhoea. This study aims at evaluating the antidiarrhoeal potency of the aqueous root and stem bark extracts of *Annona senegalensis*. Antimicrobial, antioxidant and antidiarrhoeal activity of aqueous extracts of the root bark (ARB), stem bark (ASB) and mixture of stem and root barks (AM) of *Annona senegalensis* were assessed. Stool inhibition, enteropooling, inhibition of gastro-intestinal motility and electrolyte secretion potential of the extracts were assessed in castor oil - induced diarrhoeic rats following administration of 100, 200 and 400 mg/kg body weight ASB, ARB and AM. The aqueous stem bark (ASB) had the greatest total antioxidant capacity, H<sub>2</sub>O<sub>2</sub> scavenging activity and the ferric reducing assay power. The zone of inhibition of AM against *Escherichia coli*, *Bacillus aureus* and *Shigella dysenteriae* was greater than 6 mm. All the extracts inhibited stooling by a percentage ranging from 17 – 24.7 but was significantly lower ( $p < 0.05$ ) than the standard drug (30.0%). Inhibition of gastrointestinal tract transit was significantly increased by all the extracts but low dose (100 mg/kg b. wt. of ARB and ASB) significantly increased it the most. The weight and volume of intestinal fluid was significantly decreased by 100 mg/kg b. wt. ARB. The mixture (AM) exhibited synergistic antimicrobial effect. The aqueous stem bark exhibited good antioxidant property, antisecretory and pro-absorptive property while the root exhibited good antienteropooling activity. Isolation of bioactive compounds in the extracts should be carried out.

### ARTICLE HISTORY

**Received**

06 December 2019

**Accepted**

29 January 2020

### KEYWORDS

*Annona senegalensis*, antidiarrhoeal, enteropooling, gastrointestinal transit, intestinal fluid

## Introduction

Diarrhoea is an increase in the frequency, volume and water content of stool [1]. It is a leading cause of death amongst children below five years old, accounting for 9% of all deaths among children [2]. Globally, an estimate of 1.7 billion cases of diarrhoea diseases is recorded annually [3]. Diarrhoea can be classified as either infectious or non-infectious [4]. Causative agents in infectious diarrhoea include bacteria such as *Escherichia coli*,

<sup>1</sup> Department of Biochemistry, Faculty of Sciences, Adamawa State University, Mubi, Adamawa State, Nigeria.

<sup>2</sup> Department of Biochemistry, Faculty of life Sciences, University of Ilorin, Ilorin, Kwara State. Nigeria.

\*Correspondence authors: [mariam\\_usman@yahoo.com](mailto:mariam_usman@yahoo.com), +2348169787132

*Shigella species*, *Bacillus aureus*; virus e.g *rotavirus* and parasites e.g *Trichinella spiralis* [5, 6]. Diarrhoea caused by enteric infections is a major factor in morbidity and mortality [7]. It is widespread in developing countries due to lack of proper hygiene, good portable water and proper or inadequate sanitation facilities. Non-infectious diarrhoea may be as a result of reaction to some drugs, food allergy, toxins and acute inflammation [8], abnormality in gastrointestinal tract function and neurohumoral mechanisms [9]. It may also occur as a result of other diseases such as irritable bowel syndrome (IBS), ulcerative colitis, intestinal inflammation [1] and HIV/AIDS [10]. Infection, inflammation and intestinal irritation leads to excessive generation of reactive oxygen species which results in oxidative stress. Oxidative stress has been reported to be one of the causes of diarrhoea [11].

Plant extract provides opportunity for new drug discoveries [12]. Plants with antidiarrhoeal activity act by either inhibiting secretion or reducing gastrointestinal tract motility [13]. *Annona senegalensis* popularly known as African custard apple (English) is known as “gwandar daaji” in Hausa, uburu-ocha in Igbo and abo in Yoruba languages. It is referred to as ‘arere’ in Ilorin. It is found widely distributed in Central and West Africa. The plant has several medicinal uses. The Hausas in the northern region of Nigeria use the boiled root bark for the treatment of intestinal disorders while the Senegalese chew the stem for stomach ache [14]. Local livestock farmers in Nigeria use the plant as an antihelminthic [15], [16]. The leaves and stem barks are used for the treatment of cancer of the skin and leukaemia [17]. The plant’s smooth muscle relaxant [18], antibacterial [19], anti-inflammatory [20], antimalarial [21], anticonvulsant, sedative and central nervous depressant [22] properties have been reported. Its use as a medicinal plant may be attributed to the presence of phytochemicals such as tannins [23], flavonoids [24], saponins [25], alkaloids [26], glycosides, steroids [27], volatile oil [28] and anthocyanins [29]. The plant also contains minerals such as calcium, zinc, potassium, iron, magnesium, lead, copper, manganese, as well as ascorbic acid and amino acids [30]. Nineteen monoterpenes and sesquiterpenes have been isolated in essential oils extracted from the fruits and leaves of *A. senegalensis* from Nigeria [31]. The stem and root barks are used traditionally to treat gastrointestinal troubles and diarrhoea [32]. The methanolic stem bark extract of *A.*

*senegalensis* has been reported to attenuate spontaneous contractions in isolated rabbit ileum and inhibit gastrointestinal transit time in normal (diarrhoea not induced) mice [33]. The antisecretory and pro-absorptive properties of the stem bark extracts of this plant have not been studied in diarrhoeal animals. In addition, the traditional medicine practitioners in Adamawa State, Nigeria use a mixture of the root and stem of *Annona senegalensis* as a remedy for diarrhoea and this synergistic effect has not been scientifically proven. Synergistic interaction between different plant and different plant parts may increase efficiency, reduce undesirable effects and increase the bioavailability or stability of the free agents [34]. This study therefore, investigated the antidiarrhoeal activity of the stem and root bark extract in castor oil-induced diarrhoeal rats. The synergistic effect of the combined plant part on antimicrobial, antioxidant and *in vivo* antidiarrhoeal activity was also investigated.

## **Material and Methods**

### **Collection and preparation of plant**

Fresh stem bark and root of *Annona senegalensis* were collected in July, 2018 and authenticated at the Dept. of Plant Biology, University of Ilorin, Kwara State with a voucher number UILH/001/449. It was separately washed and air dried under shade (room temperature) and then pulverized separately using mortar and pestle into powder. The powdered samples were stored in airtight containers and kept at room temperature until required.

### **Extraction procedure**

The powdered samples (100 g each) were soaked separately in 1 L of distilled water for 48 h at 35°C with vigorous shaking at 3 h intervals. The mixture of root and stem bark was made in ratio 1:1. The crude extracts were filtered using Whatman No. 1 filter paper. Each of the filtrates was evaporated to dryness at 40°C under reduced pressure using rotary evaporator and water bath. The dried substance was stored in airtight bottles until required. The crude extracts were kept in a desiccator.

### **Percentage yield**

Percentage yield was calculated as: weight of extract/ weight of dried powdered sample × 100

### **Experimental animals**

Adult albino rats weighing between 130 – 150 g were purchased from the Breeding unit of Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. They were housed in well ventilated cages, with free access to drinking water and given standard laboratory diet (Bendel feeds, Ilorin). The rats were handled according to the guidelines for the protection and handling of laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the ethical committee of the Department of Biochemistry, University of Ilorin. Animals were allowed to acclimatize with the laboratory environment for one week before the experiment commenced.

### **Phytochemical screening**

Qualitative phytochemical screening was carried out as described by Sofowora [35], Trease and Evans [36] and Harbone [37]

### **Acute toxicity test**

The method described by Lorke [38] was used to determine the LD<sub>50</sub>.

### **In vitro antioxidant property**

The method described by Benzie and Strain [39], Lingnert *et al.* [40], Ruch *et al.* [41], Kikuzaki and Nakatani [42], Ottolangi [43] was used to determine the ferric reducing power, DPPH IC<sub>50</sub>, H<sub>2</sub>O<sub>2</sub> scavenging activity, ferric thiocyanate assay and thiobarbituric acid assay respectively.

### **Determination of antimicrobial activity of the plant extracts**

The antimicrobial activity was done using the method of Olila *et al.* [44]. Zones of inhibition greater or equal to 6 mm diameter were regarded as having antimicrobial activity.

### **Stool inhibition**

A total of 55 albino rats were randomly divided into eleven groups (I - XI) of five animals each. All rats were fasted for 12 hours and thereafter, received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric cannula to induce diarrhoea. Thirty minutes after castor oil administration, rats of group I (control) received 1.0 mL of 0.9% NaCl in distilled

water (normal saline), group II received 3 mg/kg loperamide (standard drug), groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark extract, groups VI - VIII received 100, 200 and 400 mg/kg b.wt. of aqueous stem bark extract, while group IX - XI received 100, 200 and 400 mg/kg b.wt of a mixture of root and stem bark aqueous extract p.o. respectively. The animals were placed separately in metabolic cages over white clean whatman filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 hours. The total number of diarrhoea faeces of the control group was considered 100%.

% inhibition =  $(\text{Control} - \text{Test}) \times 100 / \text{Control}$ .

### **Measurement of gastrointestinal transit time using charcoal**

Fifty-five (55) adult rats were fasted for 12 hours and randomly divided into eleven groups of five animals each. Castor oil (1 mL) was administered orally to the animals. One hour later, rats of group I (control) received 1.0 mL of 0.9% NaCl in distilled water (normal saline), group II received 3 mg/kg b. wt. atropine sulphate. Rats of groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark extract and groups VI - VIII received 100, 200 and 400 mg/kg b.wt. of aqueous stem bark extract. Group IX - XI received 100, 200 and 400 mg/kg b. wt. of a mixture of root and stem bark aqueous extract p.o. respectively. After 30 min of the administration, 1 mL of charcoal meal, (10% suspension in 5% gum acacia) was orally administered to rats in each group. The rats were sacrificed by ether (20% v/v) anesthesia and the small intestine was carefully separated from mesentery to avoid being stretched. For each animal, gastrointestinal transit was calculated as percentage distance travelled by charcoal meal to the total length of intestine. The inhibitory effect of the extracts on gastrointestinal transit was calculated relative to respective group.

### **Castor oil-induced enteropooling and electrolyte secretion**

Fifty-five (55) adult rats selected without sex discrimination were fasted for 12 hours and divided into eleven groups of five animals each. Castor oil (1 mL) was administered orally to these rats. One hour later, group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline), group II received standard drug, loperamide (3 mg/kg p.o.). Rats of groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark

extract and groups VI - VIII received 100, 200 and 400 mg/kg b. wt. of aqueous stem bark extract. Group IX - XI received 100, 200 and 400 mg/kg b. wt of a mixture of root and stem bark aqueous extract p.o. respectively. After 2 hours of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to caecum were tied with thread and the intestine removed and weighed. Intestinal fluid was milked into a graduated tube and the volume of the intestinal fluid was taken. The intestine was reweighed and differences between full and empty intestines calculated. The Na<sup>+</sup> and K<sup>+</sup> concentrations in the supernatant, after centrifugation of the intraluminal fluid was measured by flame photometry

### **Statistical analysis**

The computation of the mean and statistical analysis was done using SPSS software version 17.0. Data is expressed as the mean  $\pm$  SD for group of five animals. It was statistically analyzed with one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). For all the tests, results with p values < 0.05 was considered significant.

## **Results**

The percentage yield of the aqueous stem extract was 38.6% while that of the root was 29.2% and the mixture was 35.3%.

Intraperitoneal administration of up to 5000 mg/kg of aqueous extract of the stem, root and mixture of stem and root of *Annona senegalensis* to rats caused no death in the two stages of the test. There were no observable changes in physical behavior of the animals. 100, 200 and 400 mg/kg b. wt. was thus chosen for this study.

Qualitative phytochemical screening shows presence of tannin, flavonoids, steroids, alkaloids, saponin and glycosides in both the root and stem of *A. senegalensis* (Table 1). Reducing sugar was present in the root but absent in the stem. Anthraquinones is absent in both the stem and the root. Quantitative phytochemical analysis shows that glycoside concentration was significantly higher in the stem bark when compared to the ARB and AM.

**Table 1** Quantitative phytochemical screening of the root bark, stem bark and mixture of stem and root bark of *Annona senegalensis* (mg/100mg of extract)

	ARB	ASB	AM
Saponin	0.80 ± 0.02 <sup>a</sup>	0.66 ± 0.34 <sup>a</sup>	0.75 ± 0.03 <sup>a</sup>
Phenol	0.60 ± 0.12 <sup>a</sup>	0.54 ± 0.15 <sup>a</sup>	0.58 ± 0.27 <sup>a</sup>
Flavonoids	0.39 ± 0.22 <sup>a</sup>	0.37 ± 0.33 <sup>a</sup>	0.34 ± 0.02 <sup>a</sup>
Alkaloids	0.62 ± 0.11 <sup>a</sup>	0.83 ± 0.17 <sup>a</sup>	0.71 ± 0.01 <sup>a</sup>
Glycosides	0.03 ± 0.23 <sup>a</sup>	0.11 ± 0.07 <sup>b</sup>	0.06 ± 0.22 <sup>c</sup>
Tannins	0.58 ± 0.22 <sup>a</sup>	0.53 ± 0.19 <sup>a</sup>	0.30 ± 0.13 <sup>a</sup>

Values are mean ± S.D. of six determination. Values with different superscript along the row are significantly different (p < 0.05) from each other

Table 2 presents the zone of inhibition of the aqueous root and stem bark extracts of *A. senegalensis*. The zone of inhibition for all the extracts against all the bacteria was lower than the control. The mixture of stem and root bark extracts (AM) showed significant antimicrobial activity (zone of inhibition > 6mm) against *B. cereus* (10 ± 0.01), *Proteus vulgaris* (9 ± 0.30) and *E. coli* (12 ± 0.32). The root extract (ARB) also showed significant antimicrobial activity (zone of inhibition > 6mm) against *E. coli* (8 ± 0.37). The zone of inhibition for the aqueous stem bark extract (ASB) against all the micro-organisms tested for was ≤ 6 mm.

**Table 2** Antimicrobial activity of aqueous root and stem bark extract of *Annona senegalensis* expressed as zone diameter inhibition (mm)

	<i>Bacillus aureus</i>	<i>Proteus vulgaris</i>	<i>Shigella dysenteriae</i>	<i>E. coli</i>	<i>A. niger</i>	<i>Mucor</i>
ASB	5 ± 0.08	3 ± 0.30	3 ± 0.01	6 ± 0.56	0 ± 0.50	3 ± 0.60
ARB	7 ± 0.65	5 ± 0.10	7 ± 0.10	8 ± 0.37	3 ± 0.45	6 ± 0.47
AM	10 ± 0.01	9 ± 0.30	7 ± 0.10	12 ± 0.32	3 ± 0.12	7 ± 0.90
Control	28 ± 1.0	23 ± 0.45	28 ± 0.01	30 ± 0.34	14 ± 0.35	18 ± 0.23

Values are mean ± SD, n=6, p < 0.05

Table 3 shows the total antioxidant capacity of aqueous root and stem bark extracts of *A. senegalensis*. The aqueous stem bark had the highest total antioxidant capacity. The *in vitro*

antioxidant activity of the root and stem barks of *A. senegalensis* is shown in Table 4. The IC<sub>50</sub> of the aqueous stem bark extract (ASB) was not significantly different ( $p > 0.05$ ) from that of ascorbic acid but ARB and AM was significantly greater ( $p < 0.05$ ). The H<sub>2</sub>O<sub>2</sub> scavenging activity and the ferric reducing assay power of ASB and AM was significantly greater ( $p < 0.05$ ) than ascorbic acid. The ferric thiocyanate scavenging activity and thiobarbituric acid (TBA) activity of all the extracts were significantly greater ( $p < 0.05$ ) than that of ascorbic acids.

**Table 3** Total antioxidant capacity of *Annona senegalensis* root, stem bark and its mixture

	Total antioxidant activity (mg equivalent of ascorbic acid/g of extract )
ASB	564.02 ± 0.18 <sup>c</sup>
ARB	418.17 ± 0.07 <sup>a</sup>
AM	515.58 ± 0.23 <sup>b</sup>

Values are mean ± S.D, n = 6. Values with different superscript down the column are significantly different from the others ( $p < 0.05$ )

**Table 4** *In vitro* antioxidant activity of *Annona senegalensis*

	Ascorbic acid	ARB	ASB	AM
DPPH (IC <sub>50</sub> ) mg/ml	54.10 ± 0.02 <sup>a</sup>	91.80 ± 0.23 <sup>c</sup>	53.45 ± 0.13 <sup>a</sup>	74.30 ± 0.22 <sup>b</sup>
H <sub>2</sub> O <sub>2</sub> Scavenging (% activity)	20.12 ± 0.15 <sup>b</sup>	14.23 ± 0.17 <sup>a</sup>	18.82 ± 0.22 <sup>b</sup>	16.35 ± 0.13 <sup>b</sup>
Ferric thiocyanate (% activity)	63.01 ± 0.45 <sup>a</sup>	86.26 ± 0.32 <sup>b</sup>	85.14 ± 0.21 <sup>b</sup>	85.71 ± 0.11 <sup>b</sup>
Thiobarbituric acid % activity	72.85 ± 0.12 <sup>a</sup>	94.14 ± 0.13 <sup>b</sup>	95.54 ± 0.13 <sup>b</sup>	93.45 ± 0.22 <sup>b</sup>
Ferric reducing activity power (%)	66.29 ± 0.35 <sup>a</sup>	71.10 ± 0.05 <sup>a</sup>	79.13 ± 0.02 <sup>b</sup>	75.92 ± 0.23 <sup>b</sup>

Values are mean ± S.D, (n = 6). Values with different superscript along each row are significantly different from the others ( $p < 0.05$ )

Fig. 1 shows the reduction in number of stool for the first four hours. In the first three hours, there was gradual decline in the number of stool. By the 4<sup>th</sup> hour, there was observable reduction in stool when compared to the control which was still high. Table 5 shows the stool inhibition and gastrointestinal tract transit inhibition by aqueous root and stem bark extracts of *A. senegalensis* in castor oil induced diarrhoeal rats. All of the



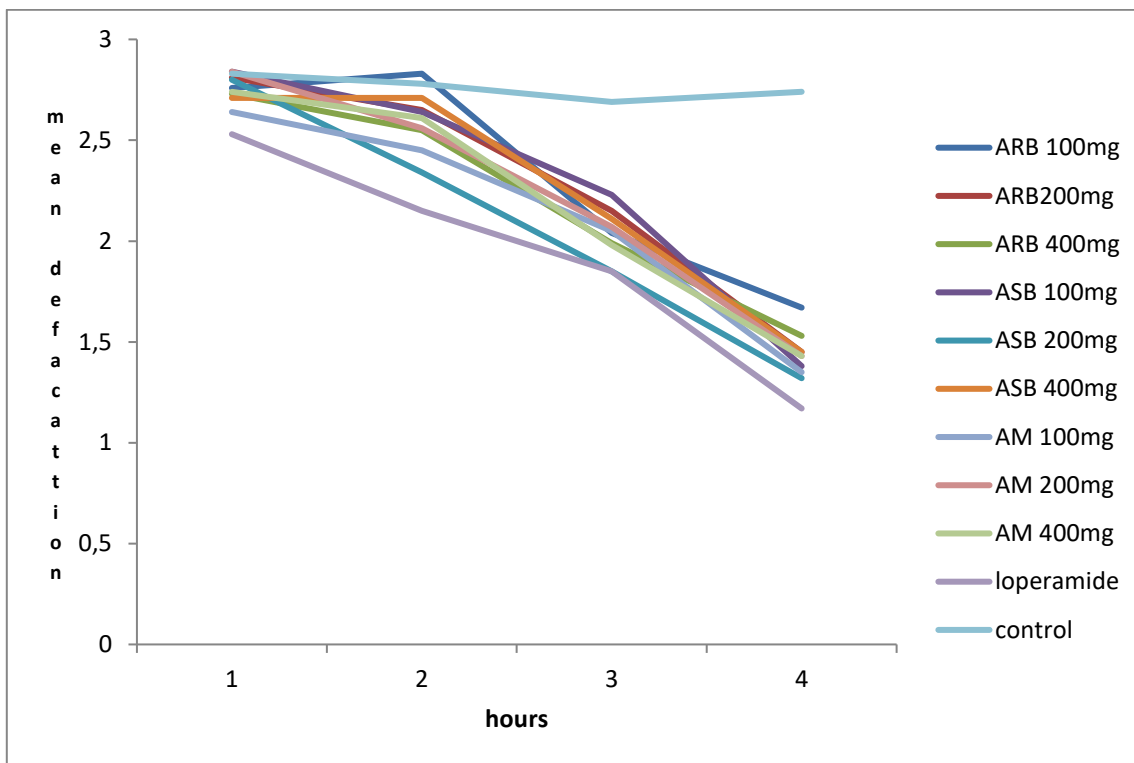
extracts inhibited stool (% ranging from 24.7 – 17) but was significantly lower ( $p < 0.05$ ) than stool inhibition of the standard drug; loperamide.

**Table 5** Stool inhibition and gastrointestinal transit inhibition in castor oil- induced diarrhoeal rats administered aqueous root and stem bark extracts of *Annona senegalensis*

Group	Mean no. defecation	% stool inhibition	%Gastrointestinal transit inhibition
I (control)	11.08 ± 0.20 <sup>g</sup>	-	15.60 ± 0.09 <sup>a</sup>
II (Loperamide)	7.70 ± 0.52 <sup>a</sup>	30	69.90 ± 0.05 <sup>f</sup>
III (100 mg/kg b.wt ARB)	9.30 ± 0.06 <sup>f</sup>	17	49.80 ± 0.10 <sup>d</sup>
IV (200 mg/kg b.wt. ARB)	9.06 ± 0.01 <sup>e</sup>	17.9	25.40 ± 0.10 <sup>b</sup>
V (400 mg/kg b. wt. ARB)	8.81 ± 0.02 <sup>d</sup>	20.29	37.41 ± 0.31 <sup>c</sup>
VI (100 mg/kg b. wt. ASB)	9.09 ± 0.01 <sup>e</sup>	17.7	47.12 ± 0.12 <sup>d</sup>
VII (200 mg/kg b.wt. ASB)	8.31 ± 0.00 <sup>b</sup>	24.7	25.73 ± 0.20 <sup>b</sup>
VIII (400 mg/kg b. wt. ASB)	8.98 ± 0.01 <sup>e</sup>	18.7	38.02 ± 0.21 <sup>c</sup>
IX (100 mg/kg b. wt. AM)	8.65 ± 0.02 <sup>c</sup>	21.7	22.08 ± 0.20 <sup>b</sup>
X (200 mg/kg b.wt AM)	9.09 ± 0.01 <sup>e</sup>	17.6	22.85 ± 0.18 <sup>b</sup>
XI 9400 mg/kg b. wt. AM)	8.71 ± 0.02 <sup>c</sup>	21.9	43.27 ± 0.33 <sup>d</sup>

Values are mean ± S.D of five determinations. Values with different superscript down the column are significantly different from the others ( $P > 0.05$ )

All extracts also reduced the number of defecation when compared to the normal control. Administration of 200 mg/kg b. wt. aqueous stem bark (ASB) was more efficient in reducing the number of stool after loperamide (the standard drug). All of the extracts significantly increased the % gastrointestinal transit inhibition when compared to the negative control but were all significantly lower than the standard drug, loperamide. Amongst the extracts, aqueous root bark extract (ARB) and aqueous stem bark extract (ASB) both at 100 mg/kg b.wt. and 400 mg/kg had the highest % gastrointestinal transit inhibition. There was a significant decrease ( $p < 0.05$ ) in percentage inhibition of gastrointestinal transit of diarrhoeal rats administered higher doses (200 mg/kg and 400 mg/kg b. wt.) of ASB and ARB.



**Fig 1** Hourly stool inhibition in castor oil- induced diarrhoeal rats administered aqueous root and stem bark extracts of *Annona senegalensis*

Table 6 shows the result of enteropooling and electrolyte concentration of the intestinal fluid. All of the extracts significantly decreased ( $p < 0.05$ ) the weight and volume of the intestinal fluid of castor oil- induced diarrhoeal treated rats when compared to the negative control. The weight of the intestinal fluids of rats administered moderate doses (100 and 200 mg/kg b. wt.) of ARB was not significantly different from the standard drug loperamide. The weight of intestinal fluid of rats administered 100 and 200 mg/kg b. wt of ARB significantly decreased ( $p < 0.05$ ) when compared with the negative control and the standard drug, loperamide while 400 mg b. wt. Aqueous mixture (AM) of root and stem bark was not significantly different ( $p > 0.05$ ) from that of the standard drug, loperamide. Aqueous root bark extract (ARB) at 100 mg/ kg b. wt. and 200 and 400 mg/kg b. wt. of ASB significantly decreased the concentration of both  $\text{Na}^+$  and  $\text{K}^+$  when compared to the standard drug.

**Table 6** Effect of administration of aqueous root and stem bark extracts on enteropooling and intestinal fluid electrolyte concentration

Group	Weight of intestinal fluid (mg)	Vol. of intestinal fluid (ml)	K <sup>+</sup> (mol/L)	Na <sup>+</sup> (mol/L)
I (control)	3.27 ± 0.33 <sup>d</sup>	3.00 ± 0.5 <sup>c</sup>	10.36 ± 0.49 <sup>d</sup>	309.00 ± 0.74 <sup>g</sup>
II (Loperamide)	1.63 ± 0.09 <sup>a</sup>	2.03 ± 0.3 <sup>b</sup>	9.37 ± 0.27 <sup>c</sup>	180.23 ± 0.02 <sup>c</sup>
III (100 mg/kg b.wt ARB)	2.28 ± 0.30 <sup>a</sup>	1.43 ± 0.25 <sup>a</sup>	5.46 ± 0.20 <sup>b</sup>	148.68 ± 0.56 <sup>b</sup>
IV (200 mg/kg b.wt. ARB)	2.01 ± 0.50 <sup>a</sup>	1.43 ± 0.22 <sup>a</sup>	10.49 ± 0.19 <sup>d</sup>	288.16 ± 0.23 <sup>b</sup>
V (400 mg/kg b. wt. ARB)	3.94 ± 0.67 <sup>d</sup>	2.63 ± 0.49 <sup>c</sup>	9.26 ± 0.42 <sup>c</sup>	319.70 ± 0.89 <sup>g</sup>
VI (100 mg/kg b. wt. ASB)	2.43 ± 0.43 <sup>b</sup>	2.26 ± 0.15 <sup>b</sup>	11.02 ± 0.19 <sup>e</sup>	303.95 ± 0.08 <sup>f</sup>
VII (200 mg/kg b.wt. ASB)	2.45 ± 0.05 <sup>b</sup>	2.23 ± 0.8 <sup>b</sup>	5.97 ± 0.47 <sup>b</sup>	130.00 ± 0.96 <sup>a</sup>
VIII (400 mg/kg b. wt. ASB)	3.89 ± 0.14 <sup>d</sup>	2.60 ± 0.6 <sup>c</sup>	4.60 ± 0.27 <sup>a</sup>	112.58 ± 0.92 <sup>a</sup>
IX (100 mg/kg b. wt. AM)	3.04 ± 0.29 <sup>c</sup>	1.50 ± 0.25 <sup>a</sup>	10.75 ± 0.60 <sup>d</sup>	274.95 ± 0.02 <sup>e</sup>
X (200 mg/kg b.wt AM)	3.1 ± 0.60 <sup>c</sup>	1.93 ± 0.33 <sup>a</sup>	11.75 ± 0.30 <sup>e</sup>	298.68 ± 0.03 <sup>f</sup>
XI (400 mg/kg b. wt. AM)	2.18 ± 0.27 <sup>a</sup>	2.03 ± 0.30 <sup>b</sup>	10.23 ± 0.32 <sup>d</sup>	265.77 ± 0.16 <sup>d</sup>

Values are mean ± S.D of five determinations. Values with different superscript down the column are significantly different from the others (p < 0.05)

## Discussion

Any substance that is not toxic at 5,000 mg/kg b. wt. is considered safe [45]. The aqueous root and stem bark extracts of *Annona senegalensis* is therefore safe at doses ≤ 5000 mg/kg b.wt.

Antidiarrhoeal activity of medicinal plants is due to the presence of tannins, alkaloids, saponins, sterols, triterpenes and reducing sugar [46]. The presence of these phytochemicals in *A. senegalensis* root and stem barks may be responsible for its antidiarrhoeal activity. Reducing sugar present in ARB but absent in ASB may be responsible for the difference in antidiarrhoeal activity of the two extracts. A zone of inhibition greater than 6 mm indicates antimicrobial activity, thus AM exhibited antimicrobial activity against *E. coli*, *B. cereus* and *Shigella dysenteriae*. The antimicrobial activity exhibited by AM when compared to with the individual plants may be due to synergistic effect. The synergistic effect exhibited by the mixture of root and stem barks (AM) of this plant may be due to formation of complex compounds which are more effective in the inhibition of particular specie of micro-organisms by either inhibiting cell wall synthesis or by causing cell death or lyses

[47]. Also, synergism of different plant parts may lead to slower development of bacterial resistance [12], [48].

The significantly greater total antioxidant capacity, H<sub>2</sub>O<sub>2</sub> scavenging activity and ferric reducing assay power exhibited by ASB indicates its good antioxidant property. Free radicals induce lipid peroxidation which destroys cell membrane and leads to the inability of membranes to maintain ionic gradients which will particularly affect the efflux of sodium and potassium, thus leading to diarrhoea [49]. Plants with antioxidant property will therefore be of advantage in the treatment of diarrhoea, thus, the antioxidant potential of ASB may contribute to the antidiarrhoeal activity of ASB. The good antioxidant property can be attributed to the presence of phenolic compounds in the extract [50]]. Flavonoids and their derivatives are the most active polyphenolic antioxidant [51].

The gradual reduction in the number of stool by the extracts indicates antidiarrhoeal potential of the extracts. The significant reduction in mean number of stool demonstrates the efficacy of these extracts as an effective antidiarrhoeal agent. The significant reduction by aqueous stem bark (ASB) extract of *A. senegalensis* indicates antisecretory activity of the extract. The antisecretory property may be due to its ability to inhibit prostaglandin synthesis which has been reported to inhibit diarrhoea [52]. Flavonoids and glycosides, present in this plant extract, have been reported to inhibit release of autacoids and prostaglandins [53]. Glycoside was significantly low in the root extract when compared to the other extracts. This might be a reason for the decreased antisecretory activity of the root extract.

The inhibitory effect of ARB and ASB extracts on gastrointestinal motility indicates antimotility activity of the extracts which may be due to the presence of antimotility agents such as tannins [54] and flavonoids [55]. Tannic acid form complexes with proteins in the lumen which then lines the intestinal wall, thus reducing peristaltic movement and secretion [54], [56], [57]. It could also bring similar function by reducing intracellular Ca<sup>2+</sup> inward current or by activation of the Ca<sup>2+</sup> pumping system which induces muscle relaxation [58]. Also, castor oil-induced gastro-intestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine and loperamide, known anticholinergic agent [59]. Anticholinergic agents are known to inhibit gastro-intestinal (GI)

hypermotility [60]. Therefore, ARB and ASB may contain anticholinergic compound responsible for their antimotility activity.

Desensitization occurs when there is persistent signal at the receptors [61]. This might be a reason for the decline in the inhibitory effect of the root and stem extract at high dose of 400 mg/kg b.wt. Previous study also reported decrease in inhibitory efficiency of the stem bark on gastrointestinal transit at higher dose ( $\geq 100$  mg/kg) [33].

A decrease in the motility of gastrointestinal tract increases the stay of the substance in the intestine and allows for better reabsorption of water and nutrients [62]. This can be a reason for the antienterpooling activity exhibited by 100 mg/kg b.wt ARB. The significant reduction in intestinal fluid  $\text{Na}^+$  concentration of castor oil- induced diarrhoeal rats administered 200 and 400 mg/kg b. wt. ASB indicates the pro - absorptive property of the extract. This suggests that the plant contains phytochemicals that are able to stimulate  $\text{Na}^+$  absorption by stimulating any of the apical ion transporters. Reabsorption of  $\text{Na}^+$  via any of the channels play an important role in re-absorption of water [63]. This may contribute to the antidiarrhoeal potential of the extract.

### **Conclusion and Recommendation**

Synergistic interaction between aqueous root and stem bark extract of *Annona senegalensis* improved antimicrobial activity against most diarrhoea causing micro-organism studied. The mixture did not show any observable improvement in the *in vivo* diarrhoea models studied. The findings from this study indicates that both the stem and root barks exhibit antidiarrhoeal activity. Both extracts exhibited good antimotility activity. The aqueous stem bark in addition exhibited good antioxidant, antisecretory and proabsorptive properties while the aqueous root bark exhibited good antienterpooling activity. The extracts (ASB and ARB) is more efficient at lower doses (100 – 200 mg/kg b. wt.). Further studies on the isolation of the bioactive compound and its synergistic interactions should be carried out.

### **Abbreviations**

ASB: *Annona senegalensis* stem bark, ARB: *Annona senegalensis* root bark. AM: *Annona senegalensis* mixture of root and stem bark extract. b.wt. body weight, DPPH: 2,2-Diphenyl-1- Picrylhydrazyl,  $\text{IC}_{50}$ : concentration required to attain 50% radical scavenging activity.  $\text{H}_2\text{O}_2$ : Hydrogen peroxide.  $\text{LD}_{50}$ : Lethal dose.

### **Ethics Approval**

Adults albino rats used in this research were handled according to the guidelines for the protection and handling of Laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the ethical committee of the Department of Biochemistry, University of Ilorin, Nigeria.

### **Availability of Data and Material**

All data generated or analysed during this study are included in this published article.

### **Competing interest.**

The authors declare that they have no competing interest.

### **Funding**

No fund was received from any organisation or institution for this study.

### **Authors' contribution**

ARO designed the study; AMU performed the laboratory experiments and drafted part of the manuscripts. SIY participated in the drafting of the manuscripts. All authors read and approved the final manuscripts.

### **Acknowledgement**

The authors wish to thank Mr. Raji for his technical support.

## **References**

1. Baldi, J. and G. Gregorio, Probiotics for treating infectious diseases. *Journal of Pharmacology*. 2009. 4: p 23-30
2. UNICEF Data. Monitoring the situation of children and women. Nov. 2016.
3. World health Organisation (WHO). Diarrhoeal disease. April, 2013.
4. Guerrant, R.L., T. VanGilder and T.S. Steiner. Infectious Diseases Society of America. Practice guidelines for the management of infectious diarrhea. *Clinical Infectious Disease*. 2001. 32 p :331-351.
5. Karam S. and V. Bhavna. An integrated approach towards Diarrhoea. *Research and Reviews: Journal of AYUSH*. 2012. 1(2): p 11-33.
6. Soderholm, J.D and M. H. Perdue. Effect of stress on intestinal mucosal function. In: *Physiology of the gastrointestinal Tract*. Johnson L.R. ed. Academic Press New York 2006. 1: p 763-769.
7. Freeman, H. J. and A.B.R. Thomson, *First principles of gastroenterology. The basis of disease and an approach to management*. 5<sup>th</sup> edition. JANSSEN-ortho. A.B.R. Thomson and E.A. Shaffer Editors. 2004. p 175-256.
8. Hodges, K. and R. Gill. Infectious diarrhoea: Cellular and molecular mechanisms. *Gut Microbes*. 2010. p. 4 -21.
9. Wynn, S. and B. Fougere. *Vertinary herbal medicine*. Mosby Elsevier, St. Louis. 2007.
10. Vitali, F. et al., Inhibition of intestinal motility and secretion by extracts of *Epibobium sp.* in mice. *Journal of Ethnopharmacology*. 2006. 107: p. 342-348.
11. Otimenyin, S., O. M. Uguru, and B. E. Akanbi. Antidiarrhoeal effect of aqueous extracts of *Momordica balsamina* and *Stachytarpheta indica* in Rats. *Journal of Natural Products*. 2006. 1: p. 36-45.
12. Battacharya, A. et al., Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosa diseases. *Physiology Review*. 2014. 94: p. 329-354.
13. Cos, P. et al., Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of concept'. *Journal of Ethnopharmacology*. 2006. 106: p. 290-302.

14. Sarin, R.V and P. A Bafna. Herbal Antidiarrhoeals: A review. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012. 3(2): p. 637-647.
15. Dalziel, J. M. The useful plants in West Tropical Africa. Crown Agents. Longon. 1955. p. 510-519.
16. Ibrahim, M.A. et al., Screening of West African plants for antihelminthic activity. International livestock Centre for Africa (ILCA), Bulletin. Addis Ababa. Ethiopia. 1983. 17: p. 19-22.
17. Nwude, N. and M. A. Ibrahim. Plants used in traditional veterinary Medicine practice in Nigeria. Journal of Pharmacology Therapy. 1980 3: p. 261-273.
18. Abubakar, M.S. et al., The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of northern Nigeria. Journal of Ethnopharmacology. 2007. 111: p. 625-629.
19. Langason, R.B.F., D. N. Akunyili, and P. I. Akubue. A preliminary study of the gastrointestinal effects of some Nigerian medicinal plants. Fitoterapia. 1994. 65: p. 235-240.
20. Muanza, D. N., et al., Antibacterial and antifungal activities of nine medicinal plants from Ziare. International Journal of Pharmacognosy. 1994. 32: p. 337-345.
21. Adzu, B., S. Amos, M. Adamu, and K. Gamaniel. Antinociceptive and anti-inflammatory effects of the methanol extract of *A. senegalensis* root bark. Journal of Natural Remedy. 2003. 3: p. 63-6725.
22. Ajaiyeoba, E. et al., In vivo antimalarial and cytotoxic properties of *A. senegalensis* extract. African Journal of Traditional Complementary and Alternative Medicines. 2006. 3 (1): p. 137-141.
23. Okoli, C.O. et al., Neuropharmacological evaluation of *Annona senegalensis* leaves. African Journal of Biotechnology. 2010. 9(49): p. 8335-8444.
24. Jada, M., W. Usman and Y. Adamu. *In vitro* Antimicrobial Effect of crude Tannins Isolated from the leaf of *Annona senegalensis*. International Journal of Biochemistry Research Review. 2014. 4(6): p. 615-623.
25. Jada, M., W. Usman and A. Olabisi. Crude flavonoids isolated from the stem bark of *Annona senegalensis* have antimicrobial activity. Journal of Advances in Biology and Biotech. 2015. 2(1): p. 24-29.
26. Afolabi, F. and O. Afolabi. Phytochemical Constituents of some medicinal plants in South West, Nigeria. IOSR Journal of Applied Chemistry. 2013. 4(1): p. 76-78
27. You, M., D. et al., Roemerine, an aporphine alkaloid from *Annona senegalensis* that reverses the multidrug- resistance phenotype with cultured cells. Journal of Natural Product. 1995. 58(4): p. 598-604.
28. Ijaiye, I., S. Arzika and M. Abdulkadir. Extraction and phytochemical screening of the root and leave of *Annona senegalensis* (Wild custard apple). Academic Journal. Interdisciplinary. Studies. 2014. 3(7): p. 9-15.
29. Ngamo, T.L. et al., Chronic toxicity of essential oils of 3 local aromatic plants towards *Sitophilus zeamais* Motsch. (Coleoptera Curculinidae). African Journal of Agricultural Research. 2007. 2 (4): p. 164-167.
30. Mpiana, P. Antisickling properties, thermal and phytochemical degradations of anthocyanin extracts from *Annona senegalensis* (Annonaceae). International Journal Biological Chemistry Science. 2012. 6(5): p. 2241-2251.
31. Yisa, J., J. Egila and A. Darlinton. Chemical composition of *Annona senegalensis* from Nupe land, Nigeria. African Journal of Biotechnology. 2010. 2(6): p. 4106-4109
32. Okhale, E.S. et al., *Annona senegalensis* Person (Annonaceae): A review of its ethnomedicinal uses, biological activities and phyto compounds. Journal of Pharmacognosy and Phytochemistry. 2016 5(2): p. 211- 219.
33. Burkill, H. M. The useful plants of West Tropical Africa. Royal Botanical Gardens Kiew. 2000. 5: p. 296 – 297.

34. Suleiman, M.M., T. Dzenda and C.A. Sani. Antidiarrheal activity of the medicinal stem bark extract of *A. Senegalensis*. Journal of Ethnopharmacology. Experiment. 2008. 116 (1): p. 125 – 130.
35. Inui, T. et al., Counter-current chromatography-based analysis of synergy in anti-tuberculosis ethnobotanical. Journal of Chromatography. 2007. 1151: p. 211 - 215.
36. Sofowora, A. Medicinal plants and traditional medicine in Africa. 2<sup>nd</sup> edn. Spectrum Books Limited. Ibadan. Nigeria. 1990.
37. Trease, G. E., and W.C. Evans. A textbook of pharmacognosy, 13<sup>th</sup> edtn. Barlliere Indian Limited. 1989. p. 19 – 21.
38. Harbone, J.B. Phytochemicals Methods. In; A guide to modern techniques of plant analysis (ed J.B. harbone). 1993. p. 182 - 196
39. Lorke, D. A new approach to practical acute toxicity. Archives of Toxicology. 1983. 54: p. 275-287
40. Benzie, I. F. and J. J. Strain. The ferric reducing ability of plasma ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Analytical Biochemistry. 1996. 239: p. 70 – 76.
41. Lingnert, H., K. Vallentin and C.E. Erickson. Measurement of antioxidative effect in model system. Journal of Food Processing Preservation. 1979. 3: p. 87-103.
42. Ruch, R. J., S. J. Cheng and J. E. Waunig. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. Carcinogen. 1989. 10: p. 1003-1008.
43. Kikuzaki, H. and N. Nakatani. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. Journal of Food Science. 1993. 58: p. 1407-1410.
44. Ottolengi, A. Interaction of ascorbic acid and mitochondria lipids. Archives of Biochemistry and Biophysics. 1959. 79: p. 355 - 361.
45. Olila, D., J. Opuda-Asibo and Odyek-Olwa. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. African Health Sciences. 2001 1: p. 66 - 72.
46. Lorke, D. A new approach to practical acute toxicity. Archieves of Toxicology. 1983. 54: p. 275-287
47. Longanga, O.A., A. Verduyze and A. Foriers. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC). Journal of Ethnopharmacology. 2000. 71: p. 411-423.
48. Chanda, S., and K. Rakholiya. Combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technology advances. A. Mendes- Vilas (ed). 2011.
49. Countinho, H.D.M., et al., Herbal therapy associated with antibiotic therapy potentiation of the antibiotic activity against methicillin-resistant *Staphylococcus aureus* by *Turnera ulmifolia* L. BMC Complement. Alternative Medicine. 2009. 13(2): p. 9-15
50. Dudeja, P. K. and K. Ramaswamy. Intestinal Anion absorption. In Physiology of the gastrointestinal Tract. 2006. Vol. 2: p. 1881-1915. Elsevier Inc. DOI: 10.1016/B978-012088394-3/50077-5
51. Rice-Evans. Flavonoids and scavengers of isoflavones; absorption metabolism and bioactivity. Free Radical Biology and Medicine. 2004. 36: p. 827 – 828.
52. Hussein, M.A., et al., Structural features, kinetics and SAR study of radical scavenging and antioxidant activities of phenolic and anilic compounds. Chemistry Central Journal. 2013. 7: p. 53 – 59.
53. Sunil, B., K. Bedi, A. Singla and R.C. Johri. Antidiarrheal activity of piperine in mice. Planta Medica. 2001. 67: P. 284-287.
54. Velga, F., G. Fonte, A. Saya and B. Tita. Inhibition of intestinal motility and secretion by extracts of *Epibobium spp* in mice. Journal of Ethnopharmacology. 2006. 107: p. 342 – 348.



55. Yadav, A.K. and V. Tangpu. Antidiarrheal activity of *Lithocarpus dealbeta* and *Urnealobals* extracts therapeutic implications. *Pharmaceutical Biology*. 2007. 45: p. 223 – 229.
56. Levy, G. Gastrointestinal clearance of drugs with activated charcoal. *New England Journal of Medicine*. 1982. 307: p. 676 – 678.
57. Mukheerjee, P.K., et al., Screening of antidiarrhoeal profile of some plant extracts of a specific region of West Bengal, India. *Journal of Ethnopharmacology*. 1998. 60: p. 85 – 89.
58. Belemtougri, R. G., et al., Effects of two medicinal plants *Psidium guajara* L (Myrtaceae) and *Diospyros mespiliformis* L (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. *Journal of Zhejiang Universal Science. B*. 2006. 7(1): p.56 – 63.
59. Brown, J. H. and P. Taylor. Cholinergic agonist In: Brunton L.L. Lazo. J.S. Parker k.l. (Eds). *The pharmacological basis of Therapeutics*. 11<sup>th</sup> edn. McGraw-Hill. New York. 2006. p.183 - 200.
60. Saralaya, M. G., M. R. Patel and A.N. Patel. Antidiarrhoeal activity of methanolic extract of *Moringa olifera* lam roots in experimental animal models. *International Journal of Pharmacology Research*. 2010. 2(2): p.35-39.
61. Nelson, D.I. and M.M. Cox. *Principles of Biochemistry*. Lehninger. 4<sup>th</sup> edn. W.H. Freeman and Co. New York. 2005. p. 422 - 428.
62. Carlo, D.G., G. Aitore, A.A. Izzo, P. Maibline, N. Mascolo, P. Viola, M.V. Diurno and F. Capasso. Inhibition of intestinal motility and secretion by flavonoids in mice and rats, structure activity relationship. *Journal of Pharmacology*. 1993. 45: p. 1054-1059.
63. Halevey, J., Boldin, M. E., and Boslelt, H. The role of aldosterone in the regulation of sodium and chloride transport in the distal colon of sodium depleted animals. *Gastroenterology*. 1986. 9: 1227 – 1233.