Antidiarrheal Properties of Aqueous Leaf Extract of *Cyathula prostrata* on Castor Oil-Induced Diarrhoea in Wistar Rats

Precious Ojo Uahomo* and Joshua Charles Isirima
Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria

ABSTRACT

Objectives: Diarrheal disease remains a public health problem in Nigeria as a developing country as well as other African countries. In order to alleviate this disease, a wide range of medicinal plants have been explored for which *Cyathula prostrata* is one of them. The leaf of this plant is traditionally used for the treatment of diarrhoea. In addition, this plant is scientifically evaluated to have an antibacterial effect on in vitro study. Phytochemical present in aqueous and ethanolic extract of *Cyathula prostrata* include; flavonoids, saponins, steroids, terpenoids, alkaloids, tannins, and phytosterols. The aim of this study was to investigate the effect of aqueous leaf extract of *C. prostrata* on Castor-oil induced diarrhea in Wistar rats. **Methods:** Twenty healthy Wistar rats were fasted for 6 hours prior to the experiment but allowed free access to water. The experimental rats were completely randomized into five groups of four animals each. Established antidiarrhea models with slight modification were employed. The test groups received various doses (97.65mg/kg, 195.3mg/kg, and 390.6mg/kg) of the extract, whereas positive controls received Loperamide (2.5mg/kg) and negative controls received distilled water (1ml/kg). **Results:** In castor oil induced diarrhoea model, the extract doses, significantly (p<0.05) prolonged onset of diarrhoea, decreased the frequency of defecation, and weight of faeces. **Conclusion:** The results of the present study confirmed antidiarrheal activity of the leaf of *Cyathula prostrata*, thus providing the scientific basis for its traditional use in the treatment of diarrhoea in Nigeria.
Key word: Diarrhoea, *Cyathula prostrata*, Castor Oil, Aqueous Leaf extract, Gastrointestinal Tract, Diarrhoea models

I.   INTRODUCTION

Diarrhea can be defined as an alteration in the normal bowel movement, characterized by a situation in which an adult daily stool exceeds 300g and contains 60 – 95 % water [1]. Diarrhea can cause severe dehydration that can lead to death. In fact, diarrhea remains the second leading cause of mortality among children under five years of age next to respiratory infections and kills more young children than AIDS, malaria, and measles combined [2,3]. This disease predominantly affects developing countries. Of all child deaths from diarrhea, 78% occur in the African and South-East Asian regions. Ethiopia's pneumonia and diarrhea mortality rate are the fifth highest in the world next to India, Nigeria, Pakistan and the Democratic Republic of Congo [4]. Each year, an estimated 2.5 billion cases of diarrhea occur among children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades, more than half of these cases are in Africa and South Asia, where bouts of diarrhea are more likely to result in death or other severe outcomes [5,6].

Some traditional medicinal herbs that have scientifically been evaluated for anti-diarrhea activity include *Khaya senegalensis*, *Xycocarpus muculensis*, *Jatropha curcus* and *Adansonia digitata* [7]. The present plant of study is *Cyathula prostrata* and is locally known as Sowore Pepein Yoruba (Western Nigeria).

Cyathula prostrata (L) Blume (Amaratheceae) is an annual, branched herb/shrub reaching up to 1m with stem trails on the ground and bears leaves which are rhomboid-oblong and adhesive fruits [8]. Traditionally, various preparations of the leaves, stems and roots of this plant are used to treat a range of illnesses including articular rheumatism, cough, skin diseases, scabies, craw-craw, snake bites, bruises, liver problem, dysentery, diarrhea, nausea, cholera vomiting blood, and many others in Nigeria and other African countries [8-10]. Among the Kurichayas tribe of Kannur District, a tea spoon of the dried powdered root is boiled in water and taken thrice daily as cure for fever [11]. When mixed with other plants (*Synedrella nodiflora* and *Aframomum*

melegueta), and clay, it is used to treat heart trouble and bronchial infections while the fruit has been claimed to prevent miscarriages [8]. Scientifically, the methanolic extract of *Cyathula prostrata* has been documented to be relatively non-toxic in albino mice [10]. Also, it was recently documented that the methanolic extract of this plant possesses anti-inflammatory and analgesic properties, justifying its application in the traditional management of ailments associated with pains among others [12]. Despite the arrays of traditional applications to which the leaf, stem and root of *Cyathula prostrata* are subjected to, available literature revealed that there is paucity of information on the scientific elucidation of these plants as remedy for the acclaimed related ailments. Hence, this present investigation seeks to scientifically evaluate the anti-diarrheal activity of the aqueous leaf extract of this plant in diarrhea models using Wistar rats.

II. MATERIALS AND METHODS
Procurement of Experimental Animals
Animals were procured from the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt and were acclimatized for a period of two weeks with their weights checked constantly during this period. All the animals (180±0.02g) were housed in clean plastic cages that were placed in a well-ventilated house (temperature: 22 ± 3^0C; photoperiod: 12 hours; humidity: 45–50%). The animals were fed on rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and clean tap water, except when fasting was required during the experiment. The cages and the animal house were cleaned on daily basis. A total of sixty animals were used in this study. They were divided into three groups of twenty animals per group. Animals in the first group were used to investigate the effect of aqueous extract of *Cyathula prostrata* on Castor oil-induced diarrhoea, animals in the second group were used to investigate the effect of aqueous extract of *Cyathula prostrata* on Castor oil-induced enteropooling, while the animals in the third group were used to investigate the effect of aqueous extract of *Cyathula prostrata* on gastrointestinal motility.

Plant collection
Fresh apical shoot of *Cyathula prostrata* were collected from Omuihechi Community in Aluu in Ikwerre Local Government Area of Rivers State, Nigeria. The plant was identified and authenticated by Dr. Ekeke in the Department of Plant Science and Biotechnology, University of Port Harcourt, Herbarium and voucher number UPH/V/1308 was assigned.

Extraction of Plant
Plant tissue homogenization method as described by Pandey and Tripathi [13] was used in the extraction of the fresh plant part in distilled water. Wet, fresh plant parts were grinded in a blender to fine particles and a measured volume of distilled water was added into it and was shaken vigorously for 5-10 minutes after which the extract was filtered. The filtrate was then centrifuged for clarification of the extract [14]. This was re-dissolved in the solvent to determine the concentration during administration to the animals.

Acute toxicity study
The acute toxicity study was carried out according to the OECD-425 guidelines [15]. Wistar rats were selected by random sampling. The animals for the study were kept fasting overnight and were provided with water only. 2000mg/kg body weight of the extract was administered orally by gastric intubations to a single rat and it was observed for 7 days. Mortality was observed in the animal therefore the dose administered was assigned toxic dose. The dose of the extract administered was stepped down by a factor of 3.2 (625mg/kg body weight). Mortality was still observed in the animal and the dose of the extract administered was further stepped down by a factor of 3.2 (195.3mg/kg body weight). No mortality was observed at this dose. Therefore, the same dose was repeated in three other animals to confirm the safe dose of the extract. This dose was used as the effective dose, while half of this dose was used as the low dose and double the dose was used as the high dose.

Drugs and Chemicals
Loperamide hydrochloride was a product of Laborate Pharmaceuticals (India) and Richy Gold International Limited (Nigeria), respectively. Castor oil was a product of Bills Sons and Company Limited (Druggist), Southport, England.

Experimental Design
Castor Oil-Induced Diarrhoea in Rats
Twenty healthy Wistar rats were fasted for 6 hours prior to the experiment but allowed free access to water. The experimental rats were completely randomized into five groups of four animals each. The procedure described by Bajad *et*

al. [16] was adopted with slight modification. Animals in group A which received 1.0ml of distilled water served as negative control, while those in groups B (positive control), C, D, and E (test groups) received 1.0ml each corresponding to 2.5mg/kg body weight of loperamide (a reference drug), 97.65mg/kg, 195.3mg/kg and 390.6mg/kg of the extract, respectively. Thirty minutes after administration, all the animals were orally administered 1ml of castor oil and thereafter placed in cages lined with a pre-weighed transparent paper. During the 6-hour observation period, 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 pre-weighed transparent paper and fresh weight of the stool. The dry weight of the faeces was obtained by drying the fresh faeces in the laboratory oven (Uniscope Laboratory oven, SM9053, Surgifriend Medicals, England) at 100ºC until constant weight was obtained. The difference in the fresh weight of the faeces and dry weight of the faeces gives the water content of the faeces. At the end of the 6-hour exposure period, the animals were sacrificed under the effect of diethyl ether anaesthesia and the small intestine of the animals were dissected and removed. Thereafter, the contents of the small intestines were squeezed out

Castor Oil-Induced Entero-pooling

The procedure described by Chitme *et al.* [17] was adopted for the castor oil-induced entero-pooling study. The animals were fasted without food for 6 hours prior to the experiment but were allowed free access to water. Four animals were randomly selected for each group and were placed in their respective cages. Animals in the negative control group (group A) received 1.0ml of distilled water, while those in the positive control group (group B) received 1.0ml of loperamide (2.5mg/kg body weight). Rats in groups C, D, and E (test groups) were orally administered 97.65mg/kg, 195.3mg/kg and 390.6mg/kg of the aqueous extract, respectively. Immediately afterwards, 1.0ml of castor oil was administered orally to each of the rats in all the groups. After 30 minutes, each rat was sacrificed according to the method described by Akanji and Yakubu [18] and the ends of the pylorus and caecum of the small intestine were tied. The small intestine was dissected and its intestinal content squeezed into a measuring cylinder. The volumes and the masses of the

intestinal contents were noted and used to compute the percentage of inhibition of intestinal content.

Gastrointestinal Motility

The method described by Teke *et al.* [19] was adopted for the evaluation of the effect of the extract on gastrointestinal transit in the rats. Twenty, healthy Wistar rats were fasted for 6 hours prior to the experiment but were allowed free access to water. The experimental rats were completely randomized into five groups of four animals each. The negative control group (group A) received 1.0ml of distilled water while the positive control group (group A) received 1.0ml of loperamide (0.6mg/ml) intramuscularly. Animals in groups C, D, and E (test groups) were orally administered 97.65mg/kg, 195.3mg/kg and 390.6mg/kg of the extract, respectively. Charcoal meal (10% charcoal suspension in 5% agarose agar, prepared by weighing 10g of charcoal powder and 5g of agarose agar into 100ml distilled water and mixed thoroughly) was administered orally, 30 minutes after the administration of loperamide and the extract. The animals were then sacrificed after 45 minutes of charcoal administration, using the diethyl ether as anesthesia as described by Akanji and Yakubu [18]. The small intestine was removed very carefully and the length of the intestine as well as distance travelled by the charcoal meal through the intestine was measured. The percentage of gastrointestinal motility was computed as the ratio of distance moved by the charcoal meal to the length of the small intestine.

Methods of Data Analysis

The experimental results were analyzed using the Statistical Package for the Social Sciences (SPSS), version 16.0 software. Results were expressed as a mean ± standard error of the mean (SEM), and statistical analyses were carried out by employing one-way analysis of variance (ANOVA), followed by Tukey's post Hoc test to compare results of controls and the groups. In all cases, statistical significance was set at $p < 0.05$.

Ethical Approval

Ethical approval was obtained from the University of Port Harcourt Ethical Committee for the purpose of use, control of experimental animals and all ethical standards were strictly adhered to throughout the duration of this research.

III. RESULTS

Acute oral toxicity test

Oral administration aqueous leaves extract of *Cyathula prostrata* produced overt toxic signs and death during the observation period of 7 days after a single administration of 2000mg/kg. This could be due to the lack of access to food and water to the experimental rat during this period of toxicity evaluation. Hence the toxic dose (LD_{50}) of aqueous extract of *Cyathula prostrata* was pegged at 2000mg/kg in Wistar rats not allowed access to water and food.

Effect of aqueous extract of *Cyathula prostrata* on Castor oil- induced diarrhoea

Castor oil administration to Wistar rats induced diarrhoea within 63.25±0.48 minutes in the control group. This condition was remarkably delayed by loperamide with 121.75±0.53 minutes (52%), the aqueous extract of *Cyathula prostrata* with the maximum effect observed at 390.6mg/kg with a maximum 140.00±0.82 minutes (86%) as shown in Table 1. Oral pretreatment of Wistar rats with different doses of aqueous extract of *Cyathula prostrata* showed a significant ($p<0.05$) delay on the onset of diarrhoea, with the higher dose of the extract exhibiting a better effect. In addition, the extract significantly reduced the frequency of defecation and the number of wet stools when compared with control ($p<0.05$). The percentage of total faecal output, output of water content and the average weight of wet faeces were significantly reduced by different doses of the extract, in which the higher dose of the extract (390.6mg/kg) produced a better effect compared to any of the groups as depicted in Table 1. The high dose of aqueous extract of *Cyathula prostrata* significantly ($p<0.05$) reduced the onset of diarrhea, number of defecations, number of wet stools, fresh weight of stool and water content of stool when compared with positive control (2.5mg/kg loperamide).

Table 1: Effects of *Cyathula prostrata* extract on Castor oil-induced diarrhoea in Wistar rats

Group	MOT (min)	MND	MNWS	MNDS	MFWS (g)	MWCS (g)
Control	63.25±0.48	6.00±0.41	2.25±0.25	1.75±0.48	4.75±0.25	2.20±0.04
Loperamide	121.75±0.53	2.50±0.29	1.25±0.24	1.25±0.25	0.82±0.03	0.47±0.03*
Low Dose	82.00±0.71*	3.25±0.25*	1.75±0.23*	1.50±0.29*	0.95±0.03*	0.52±0.03*
Medium Dose	100.75±0.48*	2.75±0.25*	1.00±0.01*	1.75±0.25*	0.82±0.03*	0.42±0.03*
High Dose	140.00±0.82*#	2.25±0.25*#	0.75±0.29*#	1.50±0.29*	0.72±0.03*#	0.35±0.03*#

* = significant at $p<0.05$ when compared with negative control (1ml distilled water) and # = significant at $p<0.05$ when compared with positive control (2.5mg/kg loperamide)

MOT = Mean Onset Time; **MND** = Mean Number of Defecations; **MNWS** = Mean Number of Wet Stool; **MNDS** =Mean Number of Dry Stool; **MFWS** = Mean Fresh Weight of Stool; **MWCS** = Mean Water Content of Stool; **Low Dose =** 97.65mg/kg; **Medium Dose =** 195.3mg/kg; **High Dose** = 390.6mg/kg

Effect of aqueous extract of *Cyathula prostrata* on Castor oil-induced Enteropooling

The weight and volume of intestinal contents (MWIC and MVIC) of the negative control (1ml distilled water) were 2.88±0.14 and 2.53±0.05 respectively. All doses of the plant extract were able to significantly inhibited castor oil–induced gastrointestinal fluid accumulation. Accordingly, the volume of intestinal contents for extract-treated groups at doses of 97.65, 195.3, and 390.6mg/kg were 2.18±0.05 ($p<0.05$), 1.73±0.06 ($p<0.05$), and 1.30±0.04 ($p<0.05$) respectively. In addition, the plant extract significantly decreased the weight of intestinal contents at 97.65mg/kg (2.50±0.11, $p<0.05$), 195.3mg/kg (2.10±0.10, $p<0.05$), and 390.6mg/kg (1.23±0.05, $p<0.05$) as compared with the negative control group as shown in Table 2.The highest effect on both weight and volume of intestinal content (MWIC and MVIC) was achieved at a dose of 390.6mg/kg of aqueous extract of *Cyathula prostrata* and it was significant at $p<0.05$ when compared with the negative control (1ml distilled water) and positive control (2.5mg/kg loperamide) as shown in Table 2.

Table 2: Effects of *Cyathula prostrata* extract on castor oil-induced Entero-pooling in Wistar rats

Group	MWIC	MVIC
Control	2.88±0.14	2.53±0.05
Loperamide	1.63±0.09*	1.60±0.07*
Low Dose	2.50±0.11*	2.18±0.05*
Medium Dose	2.10±0.10*	1.73±0.06*
High Dose	1.23±0.05*#	1.30±0.04*#

* = significant at $p<0.05$ when compared with negative control (1ml distilled water) and # = significant at $p<0.05$ when compared with positive control (2.5mg/kg loperamide)

MWIC = Mean Weight of Intestinal Content; **MVIC** = Mean Volume of Intestinal Content
Low Dose = 97.65mg/kg; **Medium Dose =** 195.3mg/kg; **High Dose** = 390.6mg/kg

Effect of aqueous extract of *Cyathula prostrata* on Gastrointestinal Motility/Gastrointestinal Transit

The aqueous extract of *Cyathula prostrata* reduced normal gastro-intestinal motility and castor oil induced motility significantly ($p<0.05$) at all doses when compared with control as shown in Tables 3 and 4 respectively and the high dose of 390.6mg/kg had the highest effect on normal gastrointestinal movement (60.52%) when compared with the positive control (2.5mg/kg loperamide). A dose dependent increase in the mean onset time (MOT) and percentage inhibition in terms of control of onset of diarrheal stool was observed in the aqueous extract of *Cyathula prostrata* from 29.64±0.48, 58.10±0.08 and 121.34±0.71 at doses of 97.65mg/kg, 195.3mg/kg and 390.6mg/kg respectively as shown in Table 5.

In the negative control group, the distance traveled by the charcoal meal was 77.25±0.63 and its peristaltic index (PI) was 70.23±1.13. The plant extract was able to significantly reduce the distance traveled by the charcoal meal at doses of 97.65mg/kg, 195.3mg/kg and 390.6mg/kg ($p<0.05$). A dose-dependent reduction in the distance traveled by the charcoal meal in Wistar rats was observed as shown in Table 4. The standard drug, loperamide, showed a significant reduction ($p<0.05$) in the distance traveled by the charcoal meal and resulted in a percentage of inhibition (85%) as compared with the negative control group as shown in Table 11.

Table 3: Effects of *Cyathula prostrata* extract on charcoal meal distance travelled in Wistar rats

Parameter/Dose (ml)	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	**97.65**	**195.3**	**390.6**
Distance travelled by Charcoal meal (cm)	77	35	44	35	29
Distance travelled by Charcoal meal (cm)	79	36	46	34	31
Distance travelled by Charcoal meal (cm)	77	36	44	33	29
Distance travelled by Charcoal meal (cm)	76	35	43	37	33

Table 4: Effects of *Cyathula prostrata* extract on Castor oil-induced transit time in Wistar rats

Group	MDTCM
Control	77.25±0.63
Loperamide	35.50±0.29
Low Dose	44.25±0.63*
Medium Dose	34.75±0.85*
High Dose	30.50±0.96*#

* = significant at $p<0.05$ when compared with negative control (1ml distilled water) and # = significant at $p<0.05$ when compared with positive control (2.5mg/kg loperamide)

MDTCM = Mean Distance Travelled by Charcoal Meal
Low Dose = 97.65mg/kg; **Medium Dose =** 195.3mg/kg; **High Dose** = 390.6mg/kg

Table 5: Effects of *Cyathula prostrata* extract on the onset of diarrhoea in Castor Oil-induced diarrhoea in Wistar rats

Parameter	Distilled Water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MOT	63.25±0.48	121.75±0.53	82.00±0.71	100.75±0.52	140.00±0.82
D_{freq}		92.49±0.09	29.64±0.48	58.10±0.08	121.34±0.71*

* = significant at p<0.05 when compared with positive control (loperamide)

$$D_{freq} = \frac{MODTG - MODNG}{MODNG} x100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{ Equation 1 [20]}$$

Where;
MOT = Mean Onset Time, **D_{freq}**= Percentage inhibition in terms of control in onset of diarrheal stool, **MODTG** = Mean Onset of Diarrhea in Treated Group and **MODNG** = Mean Onset of Diarrhea in Negative Control

Table 6: Effects of *Cyathula prostrata* extract on mean number of defecations in castor oil-induced diarrhoea in Wistar rats

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MND	6.00±0.41	2.50±0.29	3.25±0.25	2.75±0.25	2.25±0.25
PID		58.33±0.41	45.83±0.64	54.17±0.64	62.5±0.64*

* = significant at p<0.05 when compared with positive control (loperamide)

$$PID = \frac{MNDCC - MNDCD/E}{MNDCC} x 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{ Equation 2 [21]}$$

Where;
MND = Mean Number of Defecations, **PID** = Percentage Inhibition of Defecation, **MNDCC** = Mean Number of Defecations Caused by Castor oil, **MNDCD/E**= Mean Number of Defecations Caused by Drug/Extract

Table 7: Effects of *Cyathula prostrata* extract on total number of wet faeces in Castor oil-induced diarrhoea in Wistar rats

Parameter /Dose (ml)	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MNWS	2.25±0.25	1.25±0.24	1.75±0.23	1.00±0.01	0.75±0.29
P_{freq}		44.44±0.04	22.22±0.08	55.56±0.96*	66.67±0.14*

* = significant at p<0.05 when compared with positive control (loperamide)

$$P_{freq} = \frac{MNWSC - MNWST}{MNWSC} x 100 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{ Equation 3}$$

Where;

MNWS = Mean Number of Wet Stool, P_{freq} = Percentage Inhibition in terms of the purging frequency (number of wet stools), **MNWSC** = Mean Number of Wet Stool in Control group, and **MNWST** =Mean Number of Wet Stool in Treated group

Table 8: Effects of *Cyathula prostrata* extract on number of dry faeces, fresh weight of faeces and water content of faeces in castor oil-induced diarrhoea in Wistar rats

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MNDF	1.75±0.48	1.25±0.25	1.50±0.29	1.75±0.25	1.50±0.29
MFWF	4.75±0.25	0.82±0.03	0.95±0.03	0.82±0.03	0.72±0.03
MWCF	2.20±0.04	0.47±0.03	0.52±0.03	0.42±0.03	0.35±0.03

Where;
MNDF = Mean Number of Dry Faeces, **MFWF** = Mean Fresh Weight of Faeces and **MWCF** = Mean Water Content of Faeces

Table 9: Effects of *Cyathula prostrata* extract on weight of intestinal content in castor oil-induced diarrhoea in Wistar rats

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MWIC	2.89±0.14	1.63±0.09	2.50±0.11	2.10±0.11	1.23±0.05
PIIC		43.60±0.36	13.49±0.21	27.34±0.29	57.44±0.64*

* = significant at p<0.05 when compared with positive control (loperamide)

$$PIIC = \left(\frac{MWICC - MWICT}{MWICC}\right) x\ 100 \ \text{...}\ \textbf{Equation 4 [22]}$$

Where;
MWIC = Mean Weight of the Intestinal Content, **PIIC** = Percentage Inhibition of Intestinal Content, **MWICC** = Mean Weight of the Intestinal Content of the Control group and **MWICT** = Mean Weight of the Intestinal Content of the Test group

Table 10: Effects of *Cyathula prostrata* extract on Volume of intestinal content in castor oil-induced diarrhoea in Wistar rats

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MVIC	2.53±0.05	1.60±0.07	2.18±0.05	1.73±0.06	1.30±0.04
PIIFV		36.76±0.29	13.83±0.29	31.62±0.17	48.62±0.17*

* = significant at p<0.05 when compared with positive control (loperamide)

$$PIIFV = \left(\frac{MVICC - MVICT}{MVICC}\right) x\ 100 \text{...}\ \textbf{Equation 5 [23]}$$

Where;
MVIC = Mean Volume of the Intestinal Content, **PIIFV** = Percentage Inhibition of Intestinal Fluid Volume,**MVICC** = Mean Volume of the Intestinal Content of the Control group, and **MVICT** = Mean Volume of the Intestinal Content of the Test group

Table 11: Effects of *Cyathula prostrata* extract on charcoal meal distance travelled in Wistar rats.

Parameter	Distilled water (ml)	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	1	2.5	97.65	195.3	390.6
MDTCM (cm)	77.25±0.63	35.50±0.29	44.25±0.63	34.75±0.85	30.50 ±0.96
PI	70.23±1.13	32.27±0.52	40.23±1.00	31.59±1.52	27.73 ±1.71 *
G_{meq}	-	54.05±0.54	42.72±0.12	55.02±0.26	60.52 ±0.34 *

* = significant at p<0.05 when compared with negative control (Distilled water) and positive control (loperamide)

Mean Length of Small Intestine (MLSI) = 110±0.56cm

$$PI = \frac{MDTCM}{MLSI} \; x \; 100 \; \text{………… Equation 6 [24]}$$

Where;
PI = Peristaltic Index, **MDTCM** = Mean Distance Travelled by Charcoal Meal and **MLSI** = Mean Length of the Small Intestine

$$PIT = \frac{MDTCM}{MLSI} \; x \; 100 \; \text{……….. Equation 7 [21]}$$

Where;
PIT = Percentage Intestinal Transit, **MDTCM** = Mean Distance Travelled by Charcoal Meal, and **MLSI** = Mean Length of Small Intestine

$$PI \, (G_{meg}) = \frac{PIC-PIT}{PIC} \; x \; 100 \; \text{… Equation 8 [25]}$$

Where;
G_{meq} = Percentage Inhibition, **PIC** = Peristaltic Index of Control and **PIT** = peristaltic Index of Test group
Or,

$$G_{meq} = \frac{PIC-PIT}{MPICG} \; x \; 100 \; \text{……… Equation 9}$$

Where;
G_{meq} = Percentage Inhibition in terms of control in the Gut travelled distance, **MPICG** = Mean Peristaltic Index of Control Group and **MPITG** = Mean peristaltic Index of treated group.

In vivo anti-diarrheal index (ADI_{in vivo})
The result from in vivo ADI revealed that there was a dose-dependent increment in the anti-diarrheal index (ADI) value of aqueous extract of *Cyathula prostrata*. The in vivo ADI at 97.65mg/kg, 195.3mg/kg and 390.6mg/kg dose were 30.41±0.166, 56.21±0.271 and 79.82±0.323 respectively while the in vivo ADI for the standard drug, loperamide was 60.56±0.125. However, the highest anti-diarrheal index was observed at the maximum dose, 390.6mg/kg, of the extract as shown in Table 12. The high dose of the extract was more significant (p<0.05) when compared with the standard drug (loperamide),

Table 12: The in vivo Anti-Diarrheal Index (ADI _{in vivo})

Parameter	Loperamide (mg/kg body weight)	*Cyathula prostrata* (mg/kg)		
	2.5	97.65	195.3	390.6
D_{freq}	92.49±0.09	29.64±0.48	58.10±0.08	121.34±0.71*
G_{meq}	54.05±0.54	42.72±0.12	55.02±0.26*	60.52±0.34*
P_{freq}	44.44±0.04	22.22±0.08	55.56±0.96*	66.67±0.14*
ADI _{in vivo}	60.56±0.125	30.41±0.166	56.21±0.271	79.82±0.323*

* = significant at p<0.05 when compared with positive control (loperamide)

$$ADI_{invivo} = \sqrt[3]{D_{freq} x G_{meq} x P_{feq}} \; \text{……. Equation 10 [26,27]}$$

Where;
D_{freq}= Percentage inhibition in terms of control in onset of diarrheal stool, **G_{meq}** = Percentage

Inhibition, P_{freq} = Percentage Inhibition in terms of the purging frequency (number of wet stools)

ADI$_{in vivo}$ for Loperamide (2.5mg/kg)

$ADI_{invivo} = \sqrt[3]{(92.49x54.05x44.44)}$

$ADI_{invivo} = \sqrt[3]{222,159.32}$

$ADI_{invivo} = 60.56$

$ADI_{invivo} = \sqrt[3]{(0.09x0.54x0.04)}$

$ADI_{invivo} = \sqrt[3]{0.001944}$

$ADI_{invivo} = 0.125$

ADI$_{in vivo}$ for Low Dose (97.65mg/kg)

$ADI_{invivo} = \sqrt[3]{(29.64x42.72x22.22)}$

$ADI_{invivo} = \sqrt[3]{28,135.43}$

$ADI_{invivo} = 30.41$

$ADI_{invivo} = \sqrt[3]{(0.48x0.12x0.08)}$

$ADI_{invivo} = \sqrt[3]{0.00461}$

$ADI_{invivo} = 0.166$

ADI$_{in vivo}$ for medium Dose (195.3mg/kg)

$ADI_{invivo} = \sqrt[3]{(58.10x55.02x55.56)}$

$ADI_{invivo} = \sqrt[3]{177,606.54}$

$ADI_{invivo} = 56.21$

$ADI_{invivo} = \sqrt[3]{(0.08x0.26x0.96)}$

$ADI_{invivo} = \sqrt[3]{0.01997}$

$ADI_{invivo} = 0.271$

ADI$_{in vivo}$ for High Dose (390.6mg/kg)

$ADI_{invivo} = \sqrt[3]{(121.34x60.52x66.67)}$

$ADI_{invivo} = \sqrt[3]{489,590.93}$

$ADI_{invivo} = 79.82$

$ADI_{invivo} = \sqrt[3]{(0.71x0.34x0.14)}$

$ADI_{invivo} = \sqrt[3]{0.033796}$

$ADI_{invivo} = 0.323$

IV. DISCUSSION

The present study aimed at evaluating the antidiarrheal activity of the aqueous extract of C. prostrate by using different experimental models of diarrhoea in Wistar rats. In all models, diarrhoea was induced by administering castor oil to each Wistar rat. Castor oil produces diarrhea due to its active metabolite, ricinoleic acid which is liberated by the action of lipases in the upper part of the small intestine [28]. It mediates its action by binding to EP3 prostanoid receptors on smooth muscle cells [29], and facilitates the accumulation of fluid in the intestine by inhibiting absorption and enhancing secretion of fluid and electrolytes [30]. Furthermore, this metabolite also alters the motility of GI smooth muscles [31]. In the castor oil induced diarrhoea model; the extract produced a significant effect on all parameters measured: onset of diarrhoea, the number of wet and total stools and weight of wet stools. There are several mechanisms proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na+K+ATPase activity, consequently reducing normal fluid absorption, activation of adenylatecyclase or mucosal cyclic adenosine monophosphate (cAMP)-mediated active secretion, and stimulation of prostaglandin formation and platelet activating factor (PAF); prolong onset of diarrhea, reduction of gastrointestinal motility and inhibition of synthesis of prostaglandins observed in this study to support traditional uses of *Cyathula prostrata* [32,33]. *Cyathula prostrata* as a plant has proved to be of importance in inflammation, hypertension, in microbial and bacterial infections. It has also proved to be excellent in wound healing due to the antioxidant nature of its phyto-constituents [32].

In the Castor oil-induced diarrhoea model, the extract produced a significant effect on all parameters measured: onset of diarrhoea, the number of stool, number of wet stool, and weight of wet stools. Study by Gideon *et al*. [32] and Ojekale *et al*. [33] suggest that the anti-bacterial, analgesic and anti-inflammatory activity demonstrated by *C. prostrata* was due to the

inhibition of prostaglandin biosynthesis. Thus, the antidiarrheal action exerted by aqueous extract of *C. prostrata* may also be associated with the inhibition of prostaglandin formation. This suggestion is validated by the facts that castor oil-induced diarrhoea is related to stimulation of prostaglandins biosynthesis [34,35]. The phytochemical analysis of the extract of *C. prostrata* by Gideon *et al*. [32], Ojekale *et al*. [33] and Uahomo *et al*. [36] revealed the presence of different bioactive agents. Among the secondary metabolite identified, flavonoids, terperniods and phytosterols are known to modify the production of cyclooxygenase 1 and 2 (COX-1, COX-2) and lipooxygenase (LOX) thereby inhibiting prostaglandin production [37,38]. Tannins present in the extract precipitate proteins in the intestinal mucosa by forming the protein tannates, which make the intestinal mucosa more resistance to chemical alteration and hence reduce the peristaltic movements and intestinal secretion [39]. Therefore, the anti-diarrheal activity of *C. prostrata* aqueous extract observed in this study may be attributed to the presence of flavonoids, alkaloids, tannins and phytosterols in the aqueous extract.

Mostly, antidiarrheal agents act by decreasing secretion and/or reducing the propulsive movement of GI smooth muscles. So, to further get information about the mechanism for the antidiarrheal activity, the aqueous extract was evaluated by using enteropooling and motility tests. In the castor oil-induced enteropooling assay, the extract significantly reduced the intraluminal fluid accumulation when compared to the negative control and standard drug (loperamide). This result is in line with the report of studies done on other plants present with similar phytochemical constituents such as *Dodonaea viscosa* [40], *Lantana camara* [41,42], aqueous extract of *Phoenix dactylifera* [43] and *Amaranthus spinosus* [44].

The maximal effect of the extract was similar to Loperamide, which is one of the most widely used drugs against diarrhoea disorder; as shown in present study Loperamide effectively antagonized diarrhoea induced by castor oil [45]. The active metabolite of castor oil, ricinoleic acid, induces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins. The prostaglandins released thus stimulate secretion by preventing their absorption of sodium chloride and water [46]. Thus, it is possible that the extract significantly inhibits gastrointestinal hyper-secretion and enteropooling by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid. The anti-enteropooling activity of the extract could also probably be related to the existence of phytochemical constituent including flavonoids, steroids and tannins. Flavonoids and steroids inhibit the release of prostaglandins; thereby inhibiting secretion induced by castor oil and facilitates absorption of electrolytes [37,38]. Tannins decrease fluid secretion by inhibiting cystic fibrosis transmembrane conductance regulator (CFTR) and calcium-activated chloride channels (CaCC), by generating a protein-precipitating reaction to the GI mucosa [39,47].

In the gastrointestinal transit test, the extract was able to inhibit intestinal motility; a rising tendency of the inhibitory effect on the gastrointestinal motility was observed when the dose was increased. During the experiment, the charcoal meal method was selected to follow the displacement of the gastrointestinal content because the reduction of gastrointestinal motility is one mechanism by which many antidiarrheal agents can act [48,49]. The extract significantly reduced intestinal transit as observed by the decrease in GI motility of the charcoal meal. This finding suggests that the extracts act on all parts of the intestine. A decrease in the motility of gut muscles increases the stay of substances in the intestine. This allows a greater time for absorption [50]. Thus, the reduction in the intestinal propulsive movement in the charcoal meal model may be due to the anti-motility property of the extract. This assumption correlates with the findings of Tadesse *et al*. [41] on the anti-motility effect of the leaves of L. camara and, its constituents and solvent fractions of the leaves of this plant [42,51]. Drugs that inhibit intestinal transit in pathophysiological states are effective in relieving diarrhoea [52].

From the analysis of the result of findings, the extract significantly inhibited GI transit in the pathophysiological state as compared with the control. However, the extract was more effective in the normal intestinal transit than castor oil induced intestinal transit. This finding may be due to the constipating activity of the extract at different elected doses. Secondary metabolites such as flavonoids [53] and tannins [39] are reported to possess antidiarrheal activity due to their ability to inhibit intestinal motility. Hence, the significant anti-motility effect of the extract may be related to the synergistic inhibitory effect of flavonoids and tannins on castor oil-induced gastrointestinal motility.

Like the castor oil-induced and enteropooling diarrheal model, maximum effect was observed with the highest dose of the extract rather than the standard drug in charcoal meal test. This might be due to different secondary metabolites in the extract that may prolong the time for absorption of water and electrolytes through hampering the peristaltic movement of the intestine. Clinically, diarrhoea may result from disturbed bowel function, in which case, there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes and a rapid bowel transit [54]. In vivo, ADI is a measure of the combined effects of the different components of diarrhoea, including purging frequency, the onset of diarrheal stools and frequency of intestinal movement [26]. Besides, higher ADI value is a measure of the effectiveness of an extract in curing diarrhoea. The ADI value increased with dose, suggesting the dose dependency of this parameter. The highest selected dose of the extract, with the highest ADI value, is endowed with the best antidiarrheal activity when compared with other selected doses as indicated in the analysis of results of findings. This is supported by the findings of Tadesse *et al.* [41].

V. CONCLUSIONS

The results of this study revealed that the aqueous extract of *C. prostrata* is endowed with significant antidiarrheal activity. It inhibited the frequency of defecation and reduced greatly the wetness of faecal excretion. Moreover, it also produces an inhibitory effect on castor oil-induced intestinal secretion and gastrointestinal propulsion. These antidiarrheal activities of the extract may be attributed to the presence of phytochemicals including tannins, alkaloids, terpenoids, saponins, phytosterols and flavonoids that act individually or collectively. These findings provide a scientific support for a traditional use of the fresh leaves of *C. prostrata* as remedy for diarrhoea. Further studies to evaluate the anti-diarrhea activity of the aqueous leaves extract of *C. prostrata* in other models to corroborate the findings of this study are encouraged. The isolation of the active compound, its evaluation in experimental model and study of mechanisms of action will eventually yield and develop new drugs.

Conflict of Interest

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES
[1]. Guerrant RL, Van Gilder T, Steiner TS, Theilman MN, Slutsker L. Practice Guidelines for the management of infectious diarrhea. Journal of Infectious Diseases, 2001; 32: 331-351.
[2]. Liu L, Hope LJ, Simon C, Jamie P, Susana S, Joy EL, Igor R, Harry C, Richard C, Mengying L, Colin M, Robert EB, Child Health Epidemiology Reference Group of WHO and UNICEF. 2012. Child health epidemiology reference group of WHO and UNICEF. Global, regional and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet, 379:2151–61. https://doi.org/10.1016/S0140-6736(12)60560-1
[3]. WHO, 2013. Diarrheal disease; Cited 6th October, 2021, Available at: http://www.who.int/mediacentre/factsheets/fs330/en/, Accessed 5th June, 2021
[4]. International Vaccine Access Center IVAC], 2014. Pneumonia and Diarrhea Progress Report. Available at: http://www.jhsph.edu/research/centers-and-institutes/ivac/resources/IVAC-2014Pneumonia-Diarrhea-ProgressReport.pdf, Accessed 6th June, 2021
[5]. Ehiri J. 2009. Maternal and Child Health; New York, Springer; 2nd Edition, Page: 58
[6]. Raji MIO, Ibrahim YKE. 2011, Prevalence of waterborne infections in Northwest Nigeria: A retrospective study. Journal of Public Health Epidemiolog, 3(9):382-385.
[7]. Afroz S, Alamgir M, Khan MT, Jabbar S, Nahar N and Choudhuri MS. 2006. Antidiarrhoeal activity of the ethanol extract

of Paederia foetida Linn. (Rubiaceae). Journal of Ethnopharmacology, 105(1-2): 125–130. https://doi.org/10.1016/j.jep.2005.10.0044

[8]. Burkhill HM. 1985. The useful Plants of West Tropical Africa. Volume 1, Fam. A-D, Royal Botanical Gardens, Kew, Kew Publishing, Page: 324

[9]. Odugbemi T, Odunayo A. 2006. Nature of medicinal plants. In outline and Pictures of Medicinal Plants from Nigeria. Tolu Odugbemi (Editor). University of Lagos Press, Lagos, Nigeria, 73-161.

[10]. Kannappan P, Sundaram KS. 2009. Toxicity assessment of the medicinal plant *Cyathula prostrata*. Journal of Applied Bioscience, 13:681-687. Available via http://www.m.elewa.org/JABS/2009/13/1.pdf

[11]. Rajith NP, & Ramachandran VS. 2010. Ethnomedicines of Kurichyas, Kannur district, Western Ghats, Kerala. Indian Journal of Natural Products and Resources, 1, 249-253.

[12]. Ibrahim B, Sowemimo A, van Rooyen A, & Van de Venter M. 2012. Antiinflammatory, analgesic and antioxidant activities of *Cyathula prostrata* (Linn.) Blume (Amaranthaceae). Journal of Ethnopharmacology, 141(1), 282–289. https://doi.org/10.1016/j.jep.2012.02.032

[13]. Pandey AK, & Tripathi S. 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2, 115-119.

[14]. Das K, Tiwari RKS and Shrivastava DK. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 4(2):104-111. https://doi.org/10.5897/JMPR09.030

[15]. OECD (Organization for Economic Cooperation and Development). 1996. OECD Guidelines for the testing of chemicals/section 4: Health Effects Test No.425; Acute Oral Toxicity-Acute Toxic class method, OECD, Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2001)4&doclanguage=en, Accessed 4th June, 2021

[16]. Bajad S, Bedi KL, Singla AK and Johri RK. 2001. Antidiarrhoeal activity of piperine in mice. Planta Medica, 67(3): pp. 284–287. https://doi.org/10.1055/s-2001-11999

[17]. Chitme HR, Chandra R and Kaushik S. 2004. Studies on antidiarrheal activity of Calotropis gigantea R. Br. in experimental animals. Journal of Pharmacy & Pharmaceutical Sciences, 7(1): pp. 70–75.

[18]. Akanji MA and Yakubu MT. 2000. α-tocopherol protects against metabisulphite-induced tissue damage in rats," Nigerian Journal of Biochemistry & Molecular Biology, 15(2), 179–183.

[19]. Teke GN, Kuiate JR, Ngouateu OB and Gatsing D. 2007. Antidiarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. Journal of Ethnopharmacology, 112(2): pp. 278–283

[20]. Schuster BG. 2001. A new integrated program for natural product development and the value of an ethnomedical approach. Journal of Alternative and Complementary Medicine, 7(1):61-72.

[21]. Kola-Mustapha AT, Ghazali YO, Ayotunde HT, Atunwa SA and Usman SO. 2019. Evaluation of the antidiarrheal activity of the leaf extract of Parquetinanigrescens and formulation into oral suspensions: Journal of Experimental Pharmacology, 11; 65-72

[22]. Giday, M., Asfaw, Z., & Woldu, Z. Ethnomedicinal study of plants used by Sheko ethnic group of Ethiopia. Journal of ethnopharmacology, 2010, 132(1), 75–85. https://doi.org/10.1016/j.jep.2010.07.046

[23]. Ekor M. 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacology, 4:177.

[24]. Agegnehu MD, Zeleke LB, Goshu YA, Ortibo YL, Mehretie AY. 2019. Diarrhea prevention practice and associated factors among caregivers of under-five children in Enemay district, Northwest Ethiopia. Journal of Environmental Public Health; 5490716.

[25]. Bern C, Martines J, De Zoysa I, Glass RI. 1992. The magnitude of the global problem of diarrhoeal disease: A ten-year update. Bulletin of World Health Organization, 70:705-714.

[26]. Than A, Kulkarni HJ, Hmone W, Tha SJ. 1989. Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. International Journal of Crude Drug Research, 27:195–200

[27]. Hussain Z, Amresh G, Singh S, Rao CV. 2009. Antidiarrheal and antiulcer activity of Amaranthus spinosus in experimental animals. Pharmaceutical Biology; 47(10): 932-939

[28]. Kulkami SR, Pandit AB. 2005. Enzymatic hydrolysis of castor oil: an approach for rate enhancement and enzyme economy. Indian Journal of Biotechnology, 4:241–5

[29]. Tunaru S, Althoff TF, Nüsing RM, Diener M, Offermanns S. 2012. Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. PNAS; 109: 9179–84.

[30]. Racusen LC, Binder HJ. 1979. Ricinoleic acid stimulation of active anionsecretion in colonic mucosa of the rat. Journal of Clinical Investigations, 63:743–9.

[31]. Mathias JR, Martin JL, Burns TW, Carlson GM, & Shields RP. 1978. Ricinoleic acid effect on the electrical activity of the small intestine in rabbits. The Journal of clinical investigation, 61(3), 640–644. https://doi.org/10.1172/JCI108975

[32]. Gideon IO, Williams OT, Perpetua UN, Bright EI. 2012. Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* (L) Blume against some human pathogens. Journal of Intercultural Ethnopharmacology, 1(1):35-43

[33]. Ojekale AB, Oladipupo AL and Malik OL. 2016. *Cyathula prostrata*: A Potential Herbal Hope for Hypertensives, an Animal Model Study and Its Secondary Metabolites Assessment via GC-MS. European Journal of Medicinal Plants, 14(2): 1-10

[34]. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. 1976. Enteropooling assay: a test fordiarrhea produced by prostaglandins. Prostaglandins, 11:809–14.

[35]. Taufiq-Ur-Rahman M, Shilpi JA, Ahmed M, Hossain CF. 2005. Preliminary pharmacological studies on Piperchaba stem bark. Journal of Ethnopharmacology, 99(2):203–9.

[36]. Uahomo PO, Isirima JC and Akoko S. 2022. Evaluation of Phytochemicals and Bioactive Properties in Leaf and Root Parts of *Cyathula prostrata* (Pasture Weed) – A Qualitative and Quantitative Analysis. Asian Plant Research Journal, 9(3), 8-16.

[37]. Awad AB, Toczek J, & Fink CS. 2004. Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. Prostaglandins, leukotrienes, and essential fatty acids, 70(6), 511–520. https://doi.org/10.1016/j.plefa.2003.11.005

[38]. Hämäläinen M, Nieminen R, Asmawi MZ, Vuorela P, Vapaatalo H, & Moilanen E. 2011. Effects of flavonoids on prostaglandin E2 production and on COX-2 and mPGES-1 expressions in activated macrophages. Planta medica, 77(13), 1504–1511. https://doi.org/10.1055/s-0030-1270762

[39]. Ashok PK, Upadhyay K. 2012. Tannins are astringent. Journal of Pharmacognosy and Phytochemistry, 1(3):45.

[40]. Jemal A. 2019. Evaluation of In Vivo Antidiarrheal Activities of Hydroalcoholic Leaf Extract of Dodonaea viscosa L. (Sapindaceae) in Swiss Albino Mice. Journal of Evidence-Based Integrative Medicine, 24:1-10

[41]. Tadesse E, Ephrem E, Teshome N and Getnet M. 2017. Evaluation of the anti-diarrheal activity of the aqueous stem extract of Lantana camara Linn (Verbenaceae) in mice. BMC Complementary and Alternative Medicine, 17:190

[42]. Mengistu G, Engidawork E, Nedi T. 2015. Evaluation of the antidiarrhoeal activity of 80% methanol extract and solvent fractions of the leaves of Lantana camara linn (Verbenaceae) in mice. Ethiopia Pharmaceutical Journal, 31:107–21.

[43]. Agbon AN, Kwaneshie HO, Hamman WO. 2013. Antidiarrheal activity of aqueous fruit extract of Phoenix dactylifera (Date Palm) in Wistar rats. British Journal of Pharmacology and Toxicology, 4(3):121–7.

[44]. Zeashan H, Amresh G, Satyawan S and Chandana VR. 2009. Antidiarrheal and antiulcer activity of Amaranthus spinosus in experimental animals. Pharmaceutical Biology, 47(10), 932-939

[45]. Begum VH, Dhanalakshmi M, Muthukumaran P. 2013. In vivo evaluation of antidiarrhoeal activity of the leaves of Azima tetracantha Linn. International Journal of Nutrition and Metabolism, 5(8):140–4.

[46]. Pierce NF, Carpenter CC, Elliot HL, Greenough WB. 1971. Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology, 60(1):22–32.

[47]. Wongsamitkul N, Sirianant L, Muanprasat C, Chatsudthipong V. 2010. A plant derived hydrolysable tannin inhibits CFTR chloride channel: A potential treatment of diarrhea. Pharmaceutical Research, 27(3):490–7.

[48]. Qnais EY, Abdulla FA, Abu Ghalyun YY. 2005. Antidiarrheal effects of Juniperus phoenicia L. leaves extract in rats. Pakistan Journal Biological Science, 8(6):867–71.

[49]. Ezekwesili JO, Nkemdilim UU, Okeke CU 2010. Mechanism of antidiarrhoeal effect of ethanolic extract of Psidium guajava leaves. Biokemistri, 22(2):85–90.

[50]. Islam MM, Rashna SP, Kazi SE, Jesmin C, Fahima A, Nahida P, Sharmin A. 2013. Antidiarrheal activities of Dillenia indica bark extract. International Journal of Pharmaceutical Sciences and Research, 4(2):682–8.

[51]. Sagar L, Sehgal R, Ojha S. 2005. Evaluation of antimotility effect of Lantana camara L. var. acuelata constituents on neostigmine induced gastrointestinal transit in mice. BMC Complementary and Alternative Medicine, 5:18

[52]. Silva PCB, Neto JC, da Silva ADS, Silva KM, Silva TMS, Agra MF, *et al*. 2012. Antidiarrheal activity of Solanuma sterophorum in mice. Brazilian Journal of Pharmacognosy, 22(1):131–6.

[53]. Di Carlo G, Autore G, Izzo AA, Maiolino P, Mascolo N, Viola P. 1993. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: Structure activity relationships. Journal of Pharmacy and Pharmacology, 45(12):1054–9.

[54]. John AO, Emmanuel OA, Witness DH. 2008. Antidiarrhoeal activity of Psidium guajava Linn. (Myrtaceae) leaf aqueous extract in rodents. Journal of Smooth Muscle Research, 44(6):195–207.