Trop J Phytochem Pharm Sci, May 2025; 4(5): 198 - 203 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

In vivo Evaluation of the Anti-Diarrhoeal Therapeutic Effects of *Costus afer* Methanol Extract

Ugochi O Njoku[1]., Martin O Ogugofor[2]., John O Ogbodo[3*] and Chizaramekpere G Ogbodo[4]

[1]*Department of Biochemistry, Faculty of Biological Science, University of Nigeria*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Coal City University, Nigeria*
[3]*Department of Science Laboratory Technology, Faculty of Physical Science, University of Nigeria*
[4]*Department of Clinical Pharmacy, Faculty of Pharmaceutical Science, University of Nigeria.*

ABSTRACT

Herbal medicines are traditionally used for prophylaxis and therapeutic purposes. This research work explored the anti-diarrhoeal properties of methanol extract of *Costus afer* stem (MECAS) on diarrhea induced by castor oil in male albino rats. The experimental animals were randomly separated into six groups, each consisting of six (6) albino rats. Group 1 served as the standard control; group 2 was administered normal saline while group 3 was given loperamide (2 mg/kg); groups 4, 5, and 6 were treated with varying doses of the MECA (200, 400, and 600 mg/kg b.w). The effects of the MECAS on standard defecation showed that the groups treated with 200 mg/kg b.w MECAS and loperamide reduced defecation compared to those that received 400 and 600 mg/kg b.w MECAS with 2.33 ± 1.53 and 3.67 ±1.36 fecal droppings respectively. The result indicated a significant ($p<0.05$) dose-dependent increase in the percentage inhibition of propulsion (35.16%) and enteropooling inhibition in the group administered 200 mg/kg b.w MECAS compared to the groups that received 400 and 600 mg/kg b.w MECAS respectively. This research demonstrated that the *Costus afer* exhibits anti-diarrhoeal effects at lower doses for the treatment of diarrhea patients and scouring animals.

Keywords: *Costus afer*, Diarrhoea, Castor oil, Gastrointestinal motility, Enteropooling.

Received 27 March 2025
Revised 19 May 2025
Accepted 31 May 2025
Published online 01 June 2025

Introduction

Medicinal plants can be defined as plants that contain phytochemical properties or compounds that can be used for therapeutic and prophylaxis purposes. From ancient times till date, many indigenous plants have always been used locally as a significant alternative source of affordable medicine, food, and spices. They are regarded as potent therapeutic agents due to their pharmacological activities[2]. These pharmacological activities are evidence of therapeutic efficacy and the plant physiochemical properties such as vitamins and minerals, which contribute to their wide range of biological effects resulting in their protective or disease-preventive properties derived from plant parts[3]. Plants carry out the following mechanisms: antioxidant actions, hormonal regulations, stimulation of enzymes, interference with DNA replications, anti-microbial effects, and physical actions as a result of their photochemical properties[4]. However, many of these claims of plants that are traditionally used for herbal medicines are being investigated for their therapeutic efficacy.

*Corresponding author. Email: john.ogbodo@unn.edu.ng

Tel: +2348037331402

Citation: Njoku UO, Ogugofor MO, Ogbodo JO, Ogbodo CG.In Vivo Evaluation of the Anti-Diarrhoeal Therapeutic Effects of *Costus afer* Methanol Extract. Trop J Phytochem Pharm Sci. 2025; 4 (5): 198 – 203 http://www.doi.org/10.26538/tjpps/v4i5.1

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Diarrhea is seen scientifically as an alteration in the normal bowel movement; it is characterized by increased frequency of bowel movement and sound and with a sign and symptoms of wet stool and abdominal pain. It is caused by increased secretion of fluid into the intestine, reduced absorption of fluid from the intestine, and rapid passage of stool (wet) through the intestine[5]. Clinically, it is used to describe increased stool liquidity, which is usually associated with increased stool weight and frequency[6]. Medically, diarrhea can be classified as chronic or acute. Currently, rotavirus is the primary source of diarrhea infection, especially in infants. The high child mortality rate associated with diarrhea cases is combated through the applications of Oral Rehydration Therapy (ORT), even though it does not decline the volume or duration of diarrhea[7]. Antibiotics and gut motility-suppressing agents serve as an alternative therapeutic approach, wherein leading to reverse dehydration, hastening patient recovery from the illness, and reducing individual periods of infection[8]. A large number of people in the remote parts of Africa prefer to handle diarrhea cases with medicinal plants in spite of the availability of an extensive diarrhea management approach (using synthetic drug/orthodox) medicine due to the high-cost effect of drugs[9].

Costus afer (family Costaceae), being one of the medicinal plants, thrives well in humid lowland and upland tropical areas and is locally known as bush cane[9]. The plant parts, like flowers, fruits, leaves, and root or root bark, are from *Costus. After* plants have been used orally as a medicine for treating diarrhea and diabetes[10], this plant also contains anti-inflammatory properties such as anti-lithic, anti-ulcerogenic, hypoglycemic, hypolipidemic, and antioxidant actions[10]. This study aimed to evaluate the effect of *Costus after* stem methanol extract on castor oil-induced diarrhea for the amelioration of diarrhea patients.

Figure 1: *Costus afer* stem in its natural habitat

Materials and Methods

Collection of Plant Material
Fresh *Costus afer* were obtained from Ozom mgbagbu Owa, Enugu State of Nigeria (https://www.mindat.org/feature-2330182.html) on November 20th, 2021, and authenticated by the Bioresources Conservation and Development Center, Enugu State, Nigeria authority.

Analytical Instrument
The analytical instruments used for this study are from the Department of Biochemistry University of Nigeria Lab and include Measuring Bottles, weighing balance, Rotary Evaporator (Reflux condenser), Test tubes, cylindrical beaker, Funnel, Cotton wool, Hand gloves, Rat cages, Syringes, a Spoon, Beakers, stop clock, Measuring cylinder, Whatman filter paper No. 1, Ruler, Aluminum foil, Rat oral feeder, and a Dissecting set.

Chemicals/Reagents
The chemicals used were of pharmacological and analytical grade, including methanol (Sigma Chemical Company Ltd, St. Louis, U.S.A.). Castor oil, Loperamide (Standard drug), Costus *afer* extract, Distilled water, and Gentian violet

Qualitative and Quantitative Phytochemical Analysis of Costus afer Stem
This was carried out as described by [12-14].

MECAS Preparation
The fresh stems of *Costus afer* were washed with distilled water, chopped into smaller pieces, air dried in the laboratory for three weeks, and then pulverized to powder. The pulverized sample (1084 g) was macerated with 3.8 liters of methanol for 48 hrs under constant stirring. The mixture was filtered using a filter cloth. The resulting filtrate was further subjected to further filtration using Whatman filter paper No.1 to get a pure filtrate. The filtrate was concentrated using a rotary evaporator to get a semi-solid extract.

Experimental Animals
A total number of Thirty-six (36) adult male Wister albino rats with average body weights of 135-140g were used. The rats were acquired from the University of Nigeria, Nsukka, Department of Zoology and Environmental Biology's animal house. They were treated in accordance with conventional animal protocol, exposed to a 12-hour light-dark cycle, and acclimated for 14 days in a cage with adequate ventilation. The animals were given unlimited access to clean water and a regular rat diet (Pecco Foods, Enugu, Nigeria). The Ethics and Biosafety Committee of the Faculty of Biological Science, University of Nigeria Nsukka, granted ethical approval (REF: UNN/FBS/EC/1072) on November 16th, 2021.

Experimental design
The experimental animals were randomly separated into six groups, each consisting of six (6) albino rats. Group 1 served as the standard control; group 2 was administered normal saline while group 3 was given loperamide (2 mg/kg); groups 4, 5, and 6 were treated with varying doses of the MECA (200, 400, and 600 mg/kg b.w).

Determination of Test Parameters
Acute Toxicity Test
The acute toxicity of MECAS was investigated using the approach described by Lorke[11]. In this investigation, eighteen albino mice were used. There were two stages to the test. In the first phase, nine mice were randomly categorized into three groups and given 10, 100, and 1000 mg/kg body weight (through a cannula) of MECAS. A new set of nine experimental mice was randomly divided into three groups of three mice each and administered 1600, 2900, and 5000 mg/kg body weight of MECAS, respectively, based on the results of the phase one investigation. During 24 hours of constant monitoring, the mice were monitored for indicators of toxicity and death.

Normal Defecation Test
This was studied using a modified method of Izzo et al.[15]. In a random way, the experimental rats that received oral administration of MECAS 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively, were fasted for 24 hours although with free access to water. The group used for control received the vehicle (gum acacia dissolved in normal saline (5 ml/kg) or loperamide (2 mg/kg). The experimental rats were kept in separate animal cages, and the feces collected from the white sheath of paper were inserted under the animal cages. The number of feces produced by each animal was counted and recorded every four hours.

Castor Oil-Induced Diarrhoea Test
This was evaluated using the method of Awouters et al.[16] and Ezike et al.[17]. Experimental rats to receive oral administration of MECAS (200 mg/kg), (400 mg/kg) and (600 mg/kg), respectively, were randomly distributed in separate cages in groups (n = 6) and fasted for 24 hours, however with no restriction to water. The control groups were given the vehicle (gum acacia dissolved in (5 mL/kg) normal saline) and (2 mg/kg) loperamide. Each of the rats was given 2 ml castor oil (p.o) after one hour of their treatments and then placed in a separate animal metal cage for easy collection of the fecal matter from the white spray underneath the cages.
The Percentage Inhibition and Severity were calculated via Equation 1

$$\text{Percentage inhibition} = \frac{(T0-T1)}{T0} \times 100$$

T_0 = number of wet stools in the control group
T_1 = number of wet stools in the test group

$$\text{Severity} = \frac{T1}{T0} \times 100$$

Gastrointestinal Enteropooling Test
This was done using the method of Akah,[17]. A period of one hour was given before the oral administration of castor oil; those in group one was given normal saline (NaCl 0.9%) 5mg/kg orally, serving as the control. Group 2 animals were given loperamide, the standard drug (2 mg/kg body weight). The other three experimental animal groups were given the MECA at doses of 200, 400, and 600 mg/kg body weight. The experimental rats were sacrificed after two hours, and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were deposited into a graduated tube, and their volume was measured.

Gastrointestinal Motility Test
This was determined using the charcoal meal test method of Ezike et al.[18], modified by Tiwari et al.[19]. The effect of the extract and fractions on intestinal propulsion was determined using the charcoal meal test method Ezike et al.[18], with slight modifications Tiwari et al.[19]. Swiss albino mice fasted for 24 h; with no restriction to water was orally given

MECA of 200 mg/kg, 400 mg/kg, and 600 mg/kg respectively. The control groups were given the vehicle (acacia gum dissolved in normal saline) and Loperamide (5 mL/kg). Thirty minutes later, 5% activated charcoal suspended in 10% aqueous solution of acacia gum (charcoal meal) was given to each of the animals orally. After the charcoal meal, the animals were left for another 30 minutes, then sacrificed in a chloroform chamber, and the intestine was carefully isolated. The distance traveled by the charcoal meal from the stomach into the duodenum (pylorus) was measured.

The Percentage of Transit Inhibition was estimated using Equation 2

Percentage inhibition $= \frac{(T0-T1)}{T0} \times 100$

T_0 = total length of intestine

T_1 = charcoal distance of test group

Statistics Analysis

IBM Statistical Product and Service Solution (IBM-SPSS) version 21 (Chicago, IL) was used to analyze the study's data. Duncan's homogenous subset, post hoc multiple comparisons, and one-way analysis of variance (ANOVA) were used to determine whether the means differed significantly. The means ± standard deviation of repeated measurements were used to express the results. Significant mean values were those with $p < 0.05$.

Results and Discussion

The demand for effective and safe anti-diarrhoeal agents has made anti-diarrhea research an important area of active research. The solvent extraction technique used during extraction gave a percentage yield (11.49 %), indicating the presence of bioactive compounds[20] in *Costus af*ter the stem. Methanol is a polar solvent used to extract various polar compounds [21]. The methanol extracts of *the Costus afer* (MECAS) stem were well accepted by the animals when it was administered orally. There was no sign of acute toxicity change such as tremors, change in feeding behavior, restlessness, or death during the period of observation. Acute toxicity test of extract (MECAS) in mice established a high lethal dose (LD_{50}) value up to 5000 mg/kg body weight (table 1), suggesting that the extract may be regarded as safe and practically non-toxic with a remote risk of acute intoxication for human and livestock consumption[22]. The phytochemical analyses of the extract (MECAS) (table 2) revealed the presence of the following secondary metabolites: flavonoid (7.93 ± 1.22 mg/g), terpenoids (6.68±1.08), alkaloids (6.34±0.52), cardiac glycosides (6.23±0.47), saponins (3.82 ± 0.74), and tannins (1.39 ± 0.40).

Flavonoids, which are present in the highest amounts, have been shown to have free radical scavenging ability and antioxidant properties, which might be linked to the anti-diarrhea and wound healing properties [23]. It inhibits spasmogen-induced contractions of smooth muscles, intestinal secretion, small intestinal transit, prostaglandins, and autacoid release, resulting in a reduction of motility and secretion induced by castor oil[17, 24-26]. Also, alkaloids present in the extract (6.34±0.52) could bring relief as a result of analgesic and anti-spasmodic effect[s13] and prevent the release of autacoids and prostaglandins, thereby inhibiting secretion induced by castor oil. In addition, flavonoids and saponins have been reported to yield profound stabilizing effects on lysosomal membranes, while saponins and tannins bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules [27]. Previous studies showed that tannins stimulate the normalization of the deranged water transport across the mucosal cells and thus reduce intestinal transit[29]. In addition, saponins and flavonoids have been reported to exert profound stabilizing effects on lysosomal membranes, while tannins and saponins bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules[27]. Sometimes, phytoconstituents like tannins present in extracts may denature proteins, resulting in a reduction in intestinal secretion[28]. Tannins act locally on the gut wall, thereby inhibiting intestinal motility and thus possess anti-secretory effects [28, 30]. Previous studies showed that tannins stimulate the normalization of the deranged water transport across the mucosal cells and thus reduce intestinal transit[29].

The results from this experiment showed that the phytochemicals present in the methanol extract of the *Costus afer* (MECA) stem may have resulted in antidiarrhoeal activities of the extract[8]. These biologically active compounds present in this plant extract could serve as potential sources of drugs. In this study, diarrhea was induced by castor oil. It has been scientifically proven that castor oil can be used to induce diarrhea because it contains one of the most active metabolites such as ricinoleic acid, that has hypersecretory activity, which stimulates peristaltic activity in the small intestine, resulting in changes in the permeabilities of electrolyte in the intestinal mucosa [31,32]. Its action stimulates the release of endogenous prostaglandin E and F, leading to stomach cramps and diarrhea as a result of its effect or impact on the smooth muscle and secretion [33]. The stimulation of prostaglandin formation and activation of adenylate cyclase or mucosal cAMP-mediated active secretion are among the proposed mechanisms that explain the action of castor oil in inducing diarrhea[34].

Table 1: Acute toxicity (LD_{50}) of the methanol extract of *Costus afer* stem on mice

Group	Phase I dosage (mg/kg b.w)	Phase I report	Phase II dosage (mg/kg b.w)	Phase II report
Group 1	10	0/3	1600	0/3
Group 2	100	0/3	2900	0/3
Group 3	1000	0/3	5000	0/3

n= 3

Table 2: Quantitative phytochemical analyses of *Costus safer* stem methanol extract

Phytochemical constituents (mg/g)	Qualitative	Quantitative (Mean± SD)
Flavonoids	+	7.93±1.22
Terpenoids	+	6.68±1.08
Alkaloids	+	6.3 ±0.52
Cardiac glycosides	+	6.23±0.47
Saponins	+	3.82±0.74
Tannins	+	1.39±0.40

Key: ++ moderate, + relatively low

All the rats in the untreated group produced copious diarrhea (0 % protection). Pretreatment with 200, 400, and 600 mg/kg showed protection in a decrease of 50, 25, and 25 %, respectively, while the standard drug (loperamide) exhibited 100 % inhibition of defecation, as shown in Table 3. Pretreatment with *Costus after* stem extracts elicited a significant ($p<0.05$) and dose-dependent decrease in the total number of stool and wet stools as well as a significant ($p < 0.05$) increase in the number of dry stools in rats pretreated with the extract when compared to the untreated group. A similar effect was observed in the loperamide-pretreated group, as represented in Table 3.

In this study, loperamide was adopted as the standard drug, which is currently one of the most efficacious and widely applied anti-diarrhoeal drugs[35]. It regulates the gastrointestinal tract, slows down intestinal transit, colon flow rate and any effect on colonic motility. The therapeutic action of loperamide is believed to be associated with its antimotility and antisecretory properties[34].

Table 3: Protective Impact of Methanol Extract of *Costus afer* (MECAS) Stem in Castor Oil-Induced Diarrhea in Albino Rats

Group	Total No. of stool	No. of wet stool	Severity (%)	Inhibition (%)
Control	5.75±1.26 [ab]	0.00 [a]	0	**100**
Untreated	10.25±1.71[d]	7.25±1.26[d]	10	0.00
Loperamide (2 mg/kg)	5.50±1.29 [a]	0.00 [a]	0	100.00
MECA (200 mg/kg)	6.25±0.96 [ab]	2.0 ± 0.82 [b]	27.59	72.41
MECA (400 mg/kg)	8.50±0.58 [cd]	2.90 ± 0.50[c]	40.00	60.00
MECA (600 mg/kg)	7.50±1.29 [bc]	3.0 ± 0.82 [c]	41.38	58.62

n= 6. Values are presented as SD ± Mean. Values in the same column with different letters, such as a-d, are considered significantly different at $p < 0.05$.

Table 4: Intestinal Content Accumulation (Enteropooling) Test

Group	Weight of intestinal content (g)	Volume of intestinal content (g)	Differences (g)
Normal	0.39 ± 0.02 [a]	0.90± 0.26[ab]	-0.51
Untreated	1.29 ± 0.28 [c]	1.05 ± 0.53 [b]	0.24
Loperamide (2 mg/kg)	0.37 ± 0.06 [a]	0.53 ± 0.10 [a]	-0.16
MECA (200 mg/kg)	0.84 ± 0.05 [b]	0.95± 0.30[ab]	-0.11
MECA (400 mg/kg)	1.20 ± 0.19 [c]	1.00 ± 0.16 [b]	0.2
MECA (600 mg/kg)	1.22 ± 0.12 [c]	1.05 ± o.19 [b]	0.17

n= 6. Values are presented as SD ± Mean. Values in the same column with different letters, such as a-d, are considered significantly different at $p < 0.05$.

Inhibition of the frequency of defecation, reduction in faecal output and wetness of faeces were used as indices of anti-diarrhoeal action [36,34].
The study results showed that rats treated with the extract (200 mg/kg) b.w and loperamide (2 mg/kg) showed no defecation and had the ability to protect the animal against castor oil-induced diarrhea. The reduction in normal defecation is likely to be a result of the interference of loperamide with gastrointestinal peristalsis and motility, as proposed by Akah[17]; there was a significant ($p < 0.05$) reduction in the incidence and severity of diarrhoeal stool excreted from the experimental animals (adult male Wister rats). The increase in a mean number of fecal matter in the rats administered with only castor oil could be a result of the release of ricinoleic acid from the enzymatic breakdown of castor oil in the gut [29]. Also, the total amount of watery feces excreted by animals administered 200, 400, and 600 mg/kg of the *Costus after* stem methanol extract was significantly ($p <0.05$) lower compared with rats administered with castor oil. It could be seen that the extract was able to reduce the frequency of diarrhea stool. However, the lowest dose of the extract (200 mg/kg) conferred greater protection against castor oil-induced diarrhea than 400 and 600 mg/kg b.w of extract, as seen in (Table 3). The reason could suggest that 200 mg/kg b.w is the optimum effective dose or that the extract possessed a biphasic activity. Alternatively, the reason for this could also be that the intestine may poorly absorb the extract at high doses, and their stimulatory properties may cause cramping and diarrhea, as seen in well-known natural isoquinoline alkaloids called berberine extracted from various plants used in traditional Chinese medicine[37,38].
Administration of *Costus afer* stem methanol extract demonstrated a significant ($P < 0.05$) dose-dependent reduction in the intestinal content weight and volume of rats in the groups treated with 200 mg/kg of the extract and loperamide (2 mg/kg) and non-significant ($p > 0.05$) decrease in the groups treated with 400 and 600 mg/kg respectively when compared to the untreated animals as shown in Table 7. The percentage (%) inhibition of the volume and weight of intestinal content of rats treated with loperamide (2 mg/kg), 200, 400, and 600 mg/kg b.w decreased, respectively. The untreated group has 0 % inhibition (Table 4).
In the intestinal content accumulation test (enteropooling) as shown in table 4), the extract (200 mg/kg) significantly ($p <0.05$) reduced both the volume and weight of intestinal content. In contrast, the reduction in that of 400 and 600 mg/kg b.w was non-significant ($p > 0.05$) compared to the untreated (Table 2). The effect of decreasing volume, as seen in groups 3, 4, 5, and 6 relatives to the weight of the intestinal content is a direct consequence of reduced water and electrolyte secretion into the small intestine, which suggests that the extract may enhance electrolyte absorption from the intestinal lumen and is consistent with inhibition of hyper-secretion.
There was a significant ($p > 0.05$) decrease in the distance traveled by the charcoal meal in groups 4 (MECA (200mg/kg), 5 (MECA (400mg/kg), and 6 (MECA (600mg/kg) in table 5 relative to the untreated group and a significant ($p < 0.05$) increase in ascending order when compared with the group administered with the standard drugs (Loperamide(2mg/kg). Also, the result revealed a significant ($p > 0.05$) decrease in descending order relative to the untreated group. However, this result is inversely proportional to transit inhibition in that there was a significant ($p<0.05$) increase relative to the untreated group.
Nonetheless, since electrolyte absorption determines the efficiency of nutrient absorption in a living organism, the enhanced electrolyte absorption by the extract may have improved the absorption of other intestinal contents. Additionally, solute absorption in any region of the intestine is a function of the rate of water uptake in that region.
These findings logically imply that the extract improves the absorption of water, solutes, and electrolytes from the intestinal lumen/luminous flow, hence inhibiting enteropooling and gastrointestinal hyper-secretion. Because prostaglandins are agents linked to intestinal fluid accumulation, it may also indicate that the extract was able to block prostaglandin biosynthesis at low doses 39, 40.

Table 5: Impact of Methanol Extract of *Costus afer* (MECAS) Stem on Gastrointestinal Motility

Groups	The total length of the intestine (cm)	Distance traveled by charcoal meal	Propulsion (%)	Inhibition of Transit
Normal	75.38 ± 6.70[b]	32.83 ±3.04 [a]	43.55	56.45
Untreated	60.95±7.78[a]	36.28± 2.59[a]	59.52	40.48
Loperamide (2 mg/kg)	78.78 ± 5.10 [b]	21.84 ± 1.63 [a]	27.72	72.28
MECA (200 mg/kg)	62.58 ± 8.28 [a]	24.15 ± 0.13 [a]	38.59	61.41
MECA (400 mg/kg)	73.38 ± 10.00 [a]	29.13 ± 3.75 [a]	39.69	60.30
MECA (600 mg/kg)	61.93 ± 3.71 [a]	25.00 ± 2.51 [a]	40.37	59.63

n= 6. Values are presented as SD ± Mean. Values in the same column with different letters, such as a-d, are considered significantly different at $p < 0.05$.

Conclusion

Costus afer extract is safe even at high oral acute dose levels and can inhibit castor oil-induced diarrhea and enteropooling in rats, although it is more effective at a low dose of 200 mg/kg as a result of the abundance of flavonoids, tannins, alkaloids, cardiac glycosides and terpenoids found in the extract. Therefore, it can be used for medicinal purposes for the treatment of diarrhea but in lower doses as it was seen to possess a biphasic effect on the intestine.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

References

1. Egba IS, Ogbodo JO, Ogbodo PO, Obike CA. Toxicological evaluation of two named herbal remedies sold across Orumba South Local Government Area of Anambra State, South-Eastern Nigeria. Asian J Biochem 2018;1(1):1–6.
2. Njoku UO, Nwodo OFC, Ogugofor MO. Cardioprotective potential of methanol extract of *Costus afer* leaf on carbon tetrachloride-induced cardiotoxicity in albino rats and Health Care. Asian J Pharm Res 2017;9(2):51-58.
3. Okwu DE. Phytochemicals, vitamins and mineral content of two Nigeria medicinal plants. Int J Mol Adv Sci 2005;1(4):375 – 381.
4. Schroeter H, Boyd C, Spencer JP, Williams RJ, Cadenas E, Rice-Evans C. "Signaling in Nuerodegradation: Influences of Flavonoids and Nitric Oxide." Neurobiol. Aging. 2002;23: 861-880.
5. Guerrant RL, Van Gilder T, Steiner TS, Theilman MN, Slutsker L, Tauxe RV. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32(3):331-351.
6. Suleiman MM, Dzenda T, Sani CA. Antidiarrhoeal activity of the methanol stem-bark extract of *Annona senegalensis* (Annonaceae). J Ethnopharmacol 2008;116(1):125-130.
7. Subbotina MD, Timchenko VN, Vorobyov MM, Konunova YS, Aleksandrovih YS, Shushunov S. Effect of oral administration of tormentil root extract (*Potentilla tormentilla*) on rotavirus diarrhea in children: a randomized, double blind, controlled trial. Pediatr Infect Dis J 2003;22(8):706-711.
8. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhea: Modes of action and effects on intestinal function. Phyther Resour 2006;20(9):717-724.
9. Singh A, Saharan VA, Ram V, Ram V, Bhandari A. Evaluation of anti-diarrhoeal activity of *Elytraria acaulis* extracts on magnesium sulphate- and castor oil-induced diarrhoea in wistar rats. Malay J Pharm Sci 2013;11(2):31–39.
10. Omokhua GE. Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. Afr Resour Rev 2011;5:282-287.
11. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-287.
12. Harborne JB. "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis,." *2nd Edition, Chapmann and Hall Publishers, London 1998;*236-250.
13. Trease GE, Evans WC. "Involvement of Nitric Oxide from Nerves in Diarrhea Induced by Castor Oil in Rats." Japanese J Pharmacol. 2002;82: 168-170.
14. Soni A, Sosa S. "Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts." J Pharmacognos Phytochem. 2013;2(4): 22-29.
15. Izzo AA, Nicoletti M, Giannattasio B, Capasso F. Antidiarrheal Activity of *Terminalia sericea-burch.* ex DC Extracts. Nat Drugs Dig Tract, Capasso, F Mascolo, N (Eds), EMSI, Rome 1992;225-256.
16. Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA. Delay of castor oil diarrhea in rats: A new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharmacogn Pharmacol 1978;30:41–45.
16. Ezike AC, Akah PA, Okoli CO, Ufere IK, Ezeudu E, Okoye CF, Ashara C, Igbokwe IN. Studies on Gastrointestinal Effects of *Desmodium velutinum*: A Traditional Remedy for Diarrhea. Am J Pharmacol Toxicol. 2014;9(2):114-124.
17. Akah P. Purgative potentials of *Euphorbia heterocycle*. Fitoterapia 19;60:45–8.
18. Ezike AC, Akah PA, Okoli CO, Ezeuchenne NA, Ezeugwu S. *Carica papaya* (paw-paw) unripe fruit may be beneficial in ulcer. J Med Foods. 2009;12:1268-1273.
19. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharm. 2011;1(1):1-9.
20. Begona G. Density dynamic viscosity and derived properties of binary mixtures of methanol or ethanol with water, ethyl acetate and methyl acetate at T= (293.15, 298.15 and 303.15) K. J Chem Thermodyn. 2007;39(12):1578-1588.
21. Tijani AY, Uguru MO, Salawu OA. Anti-pyretic, anti-inflammatory and anti-diarrhoeal Properties of *Faidherbia albida* in Rats. Afr J Biotechnol. 2008;7(6):696-700.
22. Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M, Venkataraman S. Evaluation of antidiarrhoeal activities of *Magnifera indica*. J Ethnopharmacol. 2003;(84):11-15.
24. Veiga VF, Zunino L, Calixto JB, Patitucci ML, Pinto AC. "Phytochemical and Antioedematogenic Studies of Commercial Copaiba Oils Available in Brazil,." Phytoth Res. 2001;15: 476–480.

25. Akuodor GC, Muazzam IM, Usman-Idris UA, Megwas JL, Akpan KC, Chilaka N, Okoroafor DO, Osunkwo UA. "Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of *Bombax buonopozense* in Rats." Ibnosina J Med Biomed Sci. 2011;3(1): 15–20.
26. Abel NA, Helen OK, Wilson OH. "Antidiarrheal Activity of Aqueous Fruit Extract of *Phoenix dactylifera* in Winstar Rats (DATE PALM)." British J Pharma Toxi 2013;4(3): 121–27.
27. Oyedapo OO, Akinpelu BA, Orefuwa SA. Anti-inflammatory effect of *Threobroma cacao*, root extract. J Med Plants 2004;5:161-166.
28. Tripathi KD. Non-steroidal anti-inflammatory drugs and antipyretic analgesics. Essentials Med Pharmacol 2003; 4edn Jaypee Brother New Delhi.
29. Ramesh C, Kumar DBK, Einstein JW, Saleem BS, Kumar VY. Antidiarrhoeal activity of leaf extracts of *Mimosa pudica*. J Pharm Biomed Sci 2010;2(7):1-4.
30. Kumar S, Dewan S, Sangraula H, Kumar VL. "Antidiarrhoeal Activity of the Latex of *Calotropis procera*." J Ethnopharm 2001;76: 115-118.
31. Hardman JG, Limbird LE. "Drugs Affecting Gastrointestinal Function" *In: Goodman, L.S. and A. Gilman (Eds.),* Pharma Basis Therap. *10th Edition, Macgraw Hill, New York* , 2001;1023-1024.
32. Bakare RI, Magbagbeola OA, Akinwande AI, Okunowo OW, Green M. . "Antidiarrhoeal Activity of Aqueous Leaf Extract of *Momordica charantia* in Rats." J Pharmacogn Phyto. 2011;3(1): 1–7.
33. Saito T, Mizutani F, Iwanaga Y, Morikawa K, Kato H. Laxative and anti-diarrheal activity of polycarbophil in mice and rats. Japanese J Pharmacol 200289:133-141.
34. Agbon AN, Kwaneshie HO, Oliver W. Antidiarrheal Activity of Aqueous Fruit Extract of *Phoenix dactylifera* (DATE PALM) in Wistar Rats. Br J Pharmacol Toxicol. 2013;4(3):121–7.
35. Balogun SO, Tanayen JK, Ajayi AM, Ibrahim A, Ezeonwumelu JOC, Oyewale AA, Oloro OJ, Goji ADT, Kiplagat DM, Adzu B. Preliminary evaluation of anti-diarrheal, ulcerprotective and acute toxicity of aqueous ethanolic stem bark extract of *Ficus trichopoda* in experimental rodents. Asian J Med Sci. 2011;3(1):37-42.
36.Ammon PJ, Thomas PS (1974) "Effects of Oleic and Recinoleic Acids Net Jejunal Water and Electrolyte Movement." J Clin Invest. 1974;53: 374–79.
37.Khin-Maung U, Nyunt-Nyunt W, Tin U. "Clinical Trial of Biberine in Acute Watery Diarrhea." British Med J (Clin Res Edn) 1985;291(6509): 1601-1605.
38. Fan D, Wu X, Dong W, Sun W, Li J, Tang X. "Enhancement by Sodium Caprate and Sodium Deoxycholate of Gastrointestinal Absorption of Biberine Chloride in Rats." Drug Dev Ind Pharm 2013;39(9): 1447-1456.
39. Shah S. "Evaluation of Diarrhea: The Challenge Continues, Part 1." Indian J Med Sci 2004;58: 75-78.
40. Rao NV, Prakash KC, Shanta KS. Pharmacological investigation of *Cardiospermum haliccabum* (Linn) in different animal models of diarrhea. Indian J. Pharm. 2006; 38:346-349.