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RESEARCH PAPER

ANTIDIARRHOEAL PROPERTY OF THE METHANOL EXTRACT OF *ANACARDIUM OCCIDENTALE* LINN STEM BARK IN LABORATORY RODENTS

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ABSTRACT

Diarrhoea is a disease characterised by gut secretory and motility dysfunction. *Anacardium occidentale* stem bark decoction is used locally in management of diarrhea. This study investigated the antidairrhoeal property of the methanol extract of *Anacardium occidentale* stem bark in laboratory rodents. The antidiarrhoeal property of the stem bark methanol extract of *Anacardium occidentale* was investigated using castor oil induced diarrhoeal model in mice and Charcoal meal transit in Albino rats. There were no incidences of mortality recorded up to the dose of 5000 mg/kg extract used in the acute toxicity test. The Gas chromatography mass spectrophotometry revealed oleic-acid (45.51 %) as the predominant component. The Phytochemistry showed the presence of alkaloids, saponins, steroids, flavonoids and tannins.In the castor oil model, all the test doses of the extract significantly delayed the onset of diarrheoal and decreased the total number loose faeaces. Significant reduction in the whole number and weight of feaces were observed only with 400 and 800mg/kg doses. All the three doses of the extract delayed the gastrointestinal transit of charcoal meal.The present findings demonstrated the antidiarheoal property of the stem bark extract of *Anacardium occidentale* being able to delay the onset of diarrhea and decrease gastrointestinal transit.

Keywords: *Anacardium occidentale*, Diarrheoal , motility, castor oil, charcoal meal

INTRODUCTION

Diarrhoea is a health problem of large concern especially in developing countries contributing increased child death in Africa. Each year, there are approximately 4 billon cases of diarrhoea worldwide leading to 4 million deaths especially among children in this age group (Azubuike and Nkagineme, 2007). The most accessible modern method of managing diarrhoea is targeted at preventing dehydration and fluid loss through oral rehydration with salts and zinc tablets. In the situation of severe fluid loss additional pharmacological agents such as antispasmodic or antimotility agents are used to prevent further fluid loss.Anti-motility drugs are frequently limited by side effects such as colonic dilatation accompanied by perforation and increased carriage of gutenteropathogens (Henry *et al*, 2001).

Traditionally, diarrhoea is managed with the use of herbs. The use of herbs formulation in the diarrhoea management is a regular activity in most part of African nations. These nations still rely on herbal concoction from plants for their health management despite the brillant progress and discovery in medical sciences (Agunu *et al*., 2005)*Anacardium occidentale* is one of the most common herbs use in managing diarrhoea in local community of Nigeria (Etuk, *et al*,

2009) and Africa. *Ancardium Occidentale* (A.O) is a member of the Anacadiacae popularly known as cashew. Scientific reports on the extract has demonstrated its protection against inflammation (Olajide *et al*, 2004), bacterial (Akinpelu, 2001) and diarrheal (Omoboyowa *et al*, 2013). There is an overwhelming evidence of the use of its various parts (leaves, stem, bark, kernel, flowers, fruits and roots) in disease management such as colic and diarrhoea (Leslie, 2005). The bark decoction is particularly used locally in management of severe diarrhoea (Ayepola and Ishola, 2009).

The work was therefore undertaken to evaluate the antidiarhoeal activity of the stem bark of *Anacardium occidentale* in laboratory rodents.

MATERIALS AND METHODS

Collection and extraction of stem bark of *Anacardium occidentale*: The stem bark of *Anacardium occidental*e was collected at Abeokuta, Ogun State. The plant was authenticated by a staff of the Herbarium, Mr Esimelekuai D.P.O., University of Ibadan, Ibadan, Nigeria and deposited with herbarium no.: UIH-22599. The stem bark was dried under shade and pulverized. The obtained pulverised sample (500 g) was packed into a soxhlex extractor and extracted with methanol. Methanol was later removed from the resultant mixture with the aid of a rotatory evaporator under reduced pressure of 52^{o}C. The solid sample of the extract obtained was kept in the refrigerator. The crude methanol extract was designated as AoME.

Acute toxicity test: This test was carried on methanol extract of *Anacardium occidentale* (AoME) following Lorke's method (Lorke, 1983). A total number of eighteen mice were used for this study. They were distributed into six groups of three (3) mice each. Groups 1, 2 and 3 animals received oral treatment of the extract (200,400 and 800mg/kg po respectively). Thereafter, the animals were transferred into a transparent chamber where toxicity manifestations and death record were observed for a period of 12hours. When there was no observed death of these animals, further specific doses of AoME (1600, 2900 and 5000mg/kg po) were given orally to animals in group 4, 5 and 6 respectively to establish the actual LD50 value.

Phytochemistry screening: The protocols outlined by Trease and Evans (1989) were used for the screening of the AoME. Saponins, tannins, flavonoids sterol, and alkaloids were assessed.

Gas Chromatograpy Mass-Spectrometry analysis (GC-MS): The GC-MS analysis on the AoME was carried out using Gas Chromatograph equipped and coupled to a mass spectrometer in National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State.

Castor oil induced diarrhea: The Castor oil induced diarrhoea study on AoME was done using the procedure explained by Musa *et al* (2015).On the commencement of this work, twenty five (25) healthy adult Swiss Mice used for were deprived assess to food for a period 6 hours but allowed to drink water.
They were later grouped and treated as follows:
Group 1 received distleed water (0.1 mL/10 g p.o)
Group 2 received loperamide (2.5 mg/kg p.o)
Group 3,4 and 5 received AoME (200,400 and 800 mg/kg p. o respectively)

Thirty (30) minutes after drug and extract treatment, all animal were given 0.2 mL of castor oil orally and thereafter placed in cages line with pre-weighted transparent paper and served as the first(1^{st}) weight of the paper (g). During the 4-hour observation period the time of onset of diarrhoea, Whole number of faeces and the number of loose stool were recorded. After the experiment, the transparent paper containing the faeces was weighed again and served as the second (2^{nd}) weight of the paper (g). The transparent paper was later dried in an ovum for forty (45) minutes and weighted the third time. The Whole weight of stool was computed as follows:
Whole weight of stool(g) = 2^{nd} weight of paper(g)-1^{st} weight of paper(g)

Charcoal Meal transit study: The procedure explained by Abdullahi *et al* (2001) was employed to study the effect of methanol extracts of *Anacardium occidentale* on charcoal meal transit. On the day of the work, thirty (30) Albino wistar rats were not allowed to eat for 18 h before the commencement of the experiment but were not denied drinking water. They were later distributed and treated as shown below:

Group 1 received normosaline (1 mL/10 g p.o) and served as control
Group 2 received Carbachol (1 mg/kg p.o)
Group 3 received Atropine (1 mg/kg p.o)
Group 4,5 and 6 received AoME (200,400 and 800 mg/kg p.o)

Thirty minutes after these administrations, 0.5 mL of charcoal meal was given to the all the animals and were later kept in their respective cages. After forty five (45) minutes of the administration of charcoal meal, the animals from the various groups were sacrificed using slight decapitation and the small intestine from the level of the pylorus to caecum was carefully removed in each animal. The length of the small intestine, and the distance travelled by charcoal meal were measured.

For each animal, the percentage of the distance travelled by the charcoal meal against length of the small intestine is regarded as the gastrointestinal transit.

Ethical Considerations: Ethical Statement All the experimental animals were treated following the Ethical Principles and Guidelines in accordance with established protocols under the guidelines of the Principle of Laboratory Animal Care (National Institute of Health publication No. 85-23) (1996).

Statistical Analysis: Data were expressed as mean ± Standard Error of Mean (SEM). Comparisons between groups were made using the one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test, 95% confidence level, and at $p < 0.05$ was considered statistically significant.

RESULT

Acute toxicity study in mice: The toxicity test of the extract (AoME) on mice is represented on Table 1. There were no incidences of mortality recorded with all doses of the extract used. The LD_{50} is greater than 5g/kg body weight orally of the extract.

Phytochemical composition of methanol extract of the stem bark of *Anacardium occidentale*: The qualitative phytochemical compositions revealed the presence of high amount of steroids, flavonoids and phenols in the methanolic extract of the stem bark of *Anacardium occidentale* (AoME). The level of alkaloids, saponins and tannins in AoME were as shown in Table 2.

Gas chromatography mass spectrophotometry composition of AoME: GCMS analysis of the AoME revealed the presence of 15 components (Table 3). However, the predominant components are the oleic-acid (45.51 %) and hexadecanoic acid (20.57 %).

Effect of methanol extract of the stem bark of *Anacardium occidentale* on castor oil induced diarrhoea
***Number of loose faeces*:** The extract (AoME) in all the doses administered significantly reduced the number of loose feaces after a four hour observation period when compared to the values obtained in the control animals ($P<0.05$) (figure 1) The reduction in loose faecal matter is dose dependent.

Whole number of faeces: The extract (AoME) in all the doses administered reduced the total number of faeces after a four hour observation period when compared to the values obtained in the control animals (figure 2), however, this reduction was significant only in animals that received 400 and 800 mg/kg of AoME ($P<0.05$). This reduction was not different from than that of loperamide (an antidiarrhoeal drug).

Total weight of faeces: As shown in figure 3, the extract (AoME) in all the doses administered decreased the total weight of feaces after a four hour observation period when compared to the values obtained in the control animals. However, this reduction was significant only in animals that received 400 and 800 mg/kg of AoME ($P<0.05$). The reduction in animals that received high dose of the extract was more pronounced than that obtained by loperamide (an antidiarrheal drug).

Onset of diarrhoea: As observed in figure 4, the extract (AoME) in all the doses administered significantly delayed the onset of diarrhea observed over a four hour period when compared to the values obtained in the control animals. ($P<0.05$) The reduction in animals that received 400 and 800 mg/kg of the AoME was more pronounced than that obtained by loperamide (an antidiarrheal drug).

Effect of the methanol stem bark of *Anacardium occidentale* on intestinal transit (%) in rats

As shown in the figure 5, the extract (AoME) at doses of 200, 400 and 800 mg/kg produced a significant decreased in the intestinal transit of the intestine when compared to the control animals which received 1 mg/100 g normalsaline ($P<0.05$). The reduction produced by the extract was not higher than that of atropine (an antagonist of cholinergic receptors). Carbachol at 1 mg/kg significantly increased charcoal meal transit compared with normal saline (1 mg/100 g p.o) ($p<0.05$)

Table 1: Preliminary qualitative phytochemical analysis of AoME

Phases	Doses (mg/kg p.o)	Number of death
Phase I	10.0	0 (3)
	100.0	0 (3)
	1000.0	0 (3)
Phase II	1600.0	0 (3)
	2900.0	0 (3)
	5000.0	0 (3)

Table 2: Preliminary qualitative phytochemical analysis of AoME

Phytos	AoME	Phytos
Alkaloids	++	Alkaloids
Saponins	+	Saponins
Steroids	++++	Steroids
Flavonoids	+++	Flavonoids
Phenols	++++	Phenols
Tanins	++	Tanins

+ = Low; ++ = Moderate; +++ = High; ++++ = Extremely High

Table 3: GC-MSC analysis of AoME

COMPONENTS	RT	%
Glyceritol	4.28	1.14
Myristic acid	14.21	0.63
Pentadecanoic acid 14 methyl ester	16.83	1.34
Tetradecanoic acd	17.44	1.59
Stearic acid	20.99	16.14
9,12 octadecadien-1-ol	19.86	1.45
11-Octadecanoic cid ME	19.95	2.65
Octadecanoic acid ME	20.31	0.53
Oleic acid	20.72	45.51*
Hexadecanoic acid	17.87	20.57
1,E-11,Z-13-0ctadecatriene	21.73	1.33
Octadecanoic acid,2-hydroxyl-1,3-propanediyl ester	22.42	1.17
2-methyl-Z,Z-3,13-octadecadienol	24.24	1.58
Octadecanoic acid, 2-hydroxyl 1,3-propanediyl ester	24.46	1.18
1,2-Epoxycycyclooct-3-ene,5,5-dimethyl-8-methylene	26.38	3.18

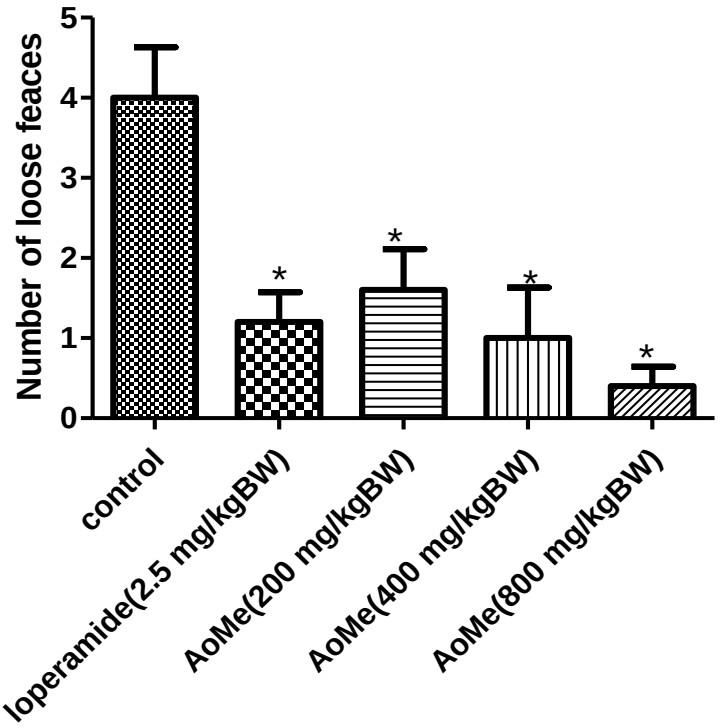

Figure 1: Effect of AoME on Number of loose faeces

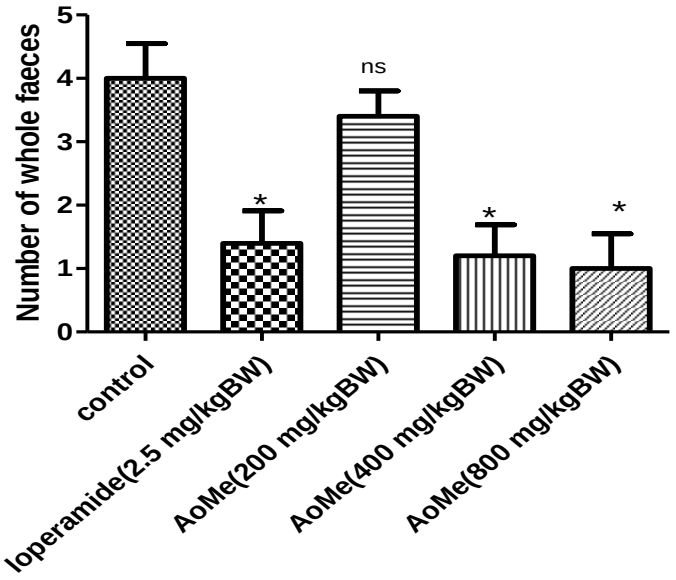

Figure 2: Effect of AoME on Number of whole feaces

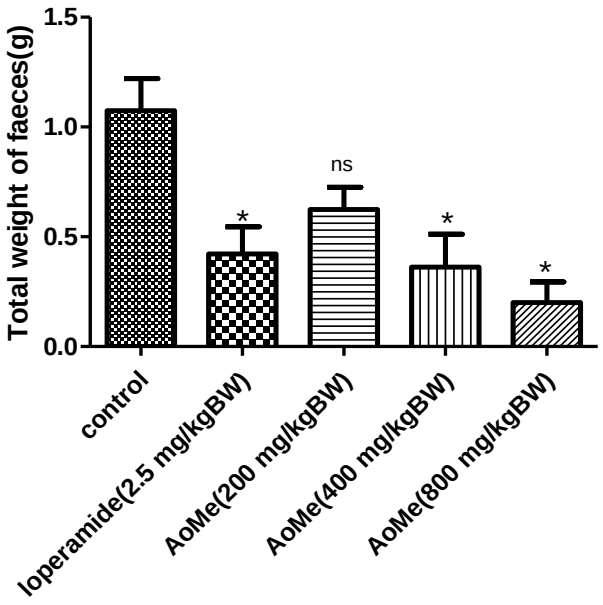

Figure 3: Effect of AoME on total weight of feaces

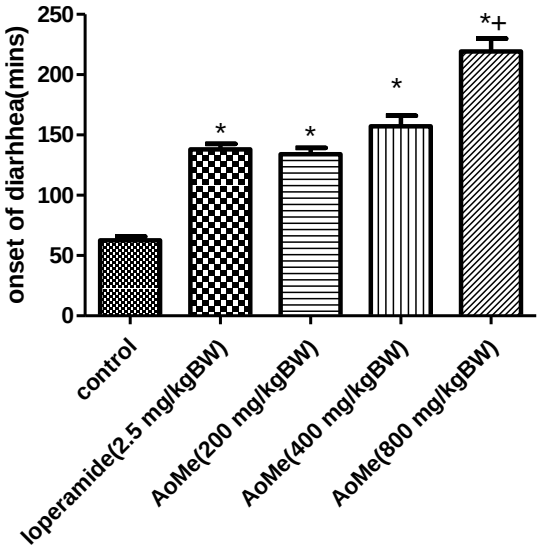

Figure 4: Effect of AoME onset of diarrhoea

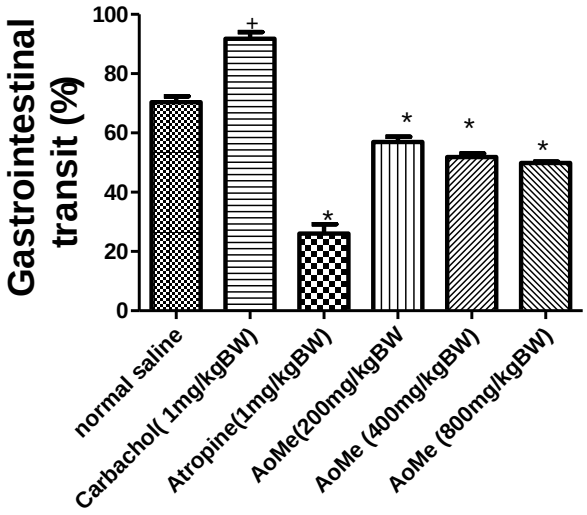

Fig 5: Effect of AoME on Intestinal transit of Charcoal meal

DISCUSSION

The toxicity test revealed no incidences of mortality up to 5000mg/kg dose. According to Lorke (1983), the extract is suggested to be safe showing no mortality at that dose. The observed level of safety is also agreement with its use in herbal medicine.

The Phytochemical evaluation showed the presence of high amount of flavonoids, steroids and phenols in AoME while akaloids, saponins and tannins were moderately present. Flavonoids and alkaloids are compounds that exhibit

remarkable physiological and pharmacological activities that affect the motility of the gastrointestinal tract (Sammy and Gopalakrishakone, 2008). Flavonoids have being demonstrated to relax gut smooth muscle in a manner that depends on concentration (Marcelly, *et al* 2012). Alkaloids have also been shown to have antidiarrhoeal effect through decreasing intestinal transit of substance (Cowan, 1999). Tannin salt has been known to interfere with secretory process of the gut by making its mucosa more resistant (Tripathi, 1994). It is expected that the presence of these phytos might impact antidiarheal property on the plant.

On the other hand, the GC-MS analysis revealed oleic-acid as the predominant components. Oleic acid has been demonstrated in humans (Henry *et al*, 2001) to slow down gastrointestinal transit in patient with chronic diarrhoea by activating nutrient-triggered inhibitory feedback mechanisms in the ileum through the activation of neural pathways such as those involving endogenous opoids (Lin *et al*, 1996; Zhao, 2000). The predominant of oleic acid composition in the extract may justify the local usefulness of the extract in management of severe diarrhoea.

Evaluation of the effect of methanol stem bark extract of *Anacardium occidentale* on diarrhoea induced by castor oil in mice showed that it prolonged the onset of diarrhoea, Whole number of stool, number of loose stool and fresh weight of stool when compared with the untreated animals. This extract effect is similar to that obtained for loperamide. Loperamide acts on opiod receptors to slow down GI motility and increase the intestinal fluid reabsorption (Holzer, 2009).These findings are indication of antidiarrhoeal action and could justify the basis for its folkloric usage. The observed result is consistent with the work of Aruojo (2015) where the gum exudates of the stem bark was reported to possess significant antidiarrhoea property.

Castor oil induces diarrhoea in experimental animals and humans through its active metabolite, ricinoleic acid. Ricinoleic acid causes diarrhoea through a series of events which include decreasing gastrointestinal transit and inhibiting the activity of Na^{+}-K^{+} ATPase (Musa 2015). The latter event leads to accumulation of fluid in the intestine while the decrease in gastrointestinal transit will prevent re-absorption of fluid in the intestine which eventually leads to its loss in the feaces. The antidarrhoeal effect of AoME may be via any of these mechanisms.

Transit time of charcoal in acacia meal provides an assessment of gastrointestinal motilityin rats. (Peddireddy, 2010)All the doses of AoME significantly decreased the gastrointestinal transit when compared to the control group. This observed ability of the extract to reduce gastrointestinal transit could explain the delay in onset of diarrhoeal and decrease in the frequency of stool and total number of faeces by the extract in this study. Decreasing the movement of the intestine will increased fluid reabsorption thereby minimizing the loss of water in the faeces resulting in the decreased in number of loose faeces. This may suggest that the extract antidiarrhoeal effect may be considered to be more of gastrointestinal motility mechanism rather than secretory.

In conclusion, the present findings demonstrated the antidiarheoal property of the stem bark extract of *Anacardium occidentale* being able to delay the onset of diarrhea and decrease gastrointestinal transit.

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REFERENCES

Abdullahi, A.L., Agho, M.O., Amos, S., Gamaniel, K.S. and Watanabe, C. (2001). Antidiarrhoeal activity of the aqueous extract of *terminalia avicennoides roots. Phytother Res*; 15:431-434.

Agunu, A., Yusuf, S. Andrew, G.O., Zezi, A.U. and Abdulrahman, E.M.(2005). Evaluation of five medical plants used in diarrhoea treatment in Nigeria. *Journal of Ethnopharmacology*; 100: 27-30.

Akinpelu, D.A. (2001). Antimicrobial activity of *anacardiumoccidentale* bark. *Fitoterapia*; 72 : 286-287.

Araújo Thiago, Douglas, S.C., Nayara, A. S., Luan, K.M., Simone de Araújo, Ana Patrícia Oliveira, Francisca Beatriz M., Sousa, Durcilene A. Silva, André L.R. Barbosa, José Roberto S.A. Leite and Jand Venes R. Medeiros

(2015) Antidiarrheal activity of cashew GUM, a complex heteropolysaccharide extracted from exudate of *Anacardium occidentale* L. in rodents. *Journal of Ethnopharmacology*; 174:299-307.

Ayepola, O.O. and Ishola, R.O. (2009). Evaluation of antimicrobial activity of *anacardium occidentale* (linn.). *Adv.In Med. Dent. Sci*; 3(1): 1-3.

Azubuike, J. C. and Nkagineme, K.E.(2007). Pediatrics and child health in a tropical region. African Educational Publishers Ltd., Owerri. p. 4.

Cowan, M.M.(1999)/ Plant products as antimicrobial agents. *Clinical Microbiology Review*; 12(4): 564-582.

Etuk, E.U., Ugwah, M.O., Ajagbonna, O.P. and Onyeyili, P.A.(2009). Ethnobotanical survey preliminary evaluation of medicinal plants with antidiarrhoea properties in Sokoto State, Nigeria . Journal of Medicinal Plants Research; 3(10), pp. 763

Henry, P., Ronnie Fass and Amy E. Foxx-Orenstein (2001). Treatment of Patients with Diabetic Gastroparesis. *Gastroenterol Hepatol (NY)*. 6(6 Suppl 9): 1–16.

Holzer, P. (2004). Opioids and opioid receptors in the enteric nervous system: from a problem in opioid analgesia to a possible new prokinetic therapy in humans. *Neurosci Lett.*; 361(1-3):192-195.

Leslie Taylor. (2005). The healing power of rainforest herbs. RainTree Nutrition, Inc. Carson City, NV89701

Lin, H.C., Zhao, X.T. and Wang, L.J. (1996). Intestinal transit of fat in proximal gut depends on accelerating effect of CCK and slowing effect of opioid pathway. *Dig Dis Sci* 41:1884.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archive of Toxicology*; 54: 275-287.

Musa, T. Yakubu, Quadri O. Nurudeen, Saoban, S. Salimon, Monsurat O. Yakubu, Rukayat O. Jimoh, Mikhail O. Nafiu, Musbau A. Akanji, Adenike T. Oladiji, and Felicia Williams (2015) Antidiarrhoeal Activity of *Musa paradisiacal* Sap in Wistar Rats. *Alternat Med*; 683726.

Olajide, O.A., Aderogba, M.A., Aduragbenro, D.A. and Makinde, J.M. (2004). Effects of anacardium occidentale stem bark extract on in vivo inflammatory models. *Journal of Ethnopharmacology*; 95(2-3): 139-142.

Omoboyowa, D.A., Nwodo, F.C. and Joshua, P.E. (2013). Antidiahoeal activity of chloroform ethanol extracts of Cashew (*Anacardium occidentale*) Kernels. *J.Nat. Pro*; 6:109-117.

Peddireddy, M.K.R. (2010). In vivo Methods for Evaluation of Drugs for the Treatment of Gastrointestinal Motility Disorders. *Indian Journal of Pharmaceutical Education*; 44(1):42-48 ·

Sammy, R.P. and Gopalakrishnakone, P. (2008). Review: Therapeutic potential of plants as anti-microbials for drug discovery. *Journal of Evidence-Based Complementary and Alternative medicine*; 7(3): 283-294 doi:10.1093/ecam/nen036

Trease, G.E. and Evans, W.C. (1998). A textbook of Pharmacognosy, 13th edn.Bailliere-tindall ltd., London;. p. 19-21:139–142

Tripathi, K.D (1994). Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers (P), New Delhi;p.775

Zhao, X.T., Wang, L.J. and Lin, H.C. (2000). Slowing of intestinal transit by fat depends on naloxone-blockable efferent, opioid pathway. *Am J Physiol Gastrointest Liver Physiol*; 278: G866–G870.

AUTHOR CONTRIBUTIONS

Dr. F.S. Oluwole supervised this research work from designed to execution.Dr. Blessing Omolaso designed and coordinated all laboratory experiments analyzed and interpreted results. Dr. Ajayi Abayomi provided support in the conduction of the experiment and data analysis. All authors read and approved the manuscript.

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