JOURNAL OF VETERINARY AND APPLIED SCIENCES
2023 VOL. 13(2): 273 – 284
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Ex vivo evaluation of the antispasmodic effects of fractions derived from *Anacardium occidentale* leaf extract

Daniel C. Ifenkwe*, Kelechi G. Madubuike, Samuel O. Onoja, Emmanuel Ewa, Jessica I. Ukwueze, Macnissi U. Chima, Oluchi N. Nwankudu, Stella C. Akomas

Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

==========

Abstract

Diarrhoeal diseases and disorders affect both humans and animals, and there is a global search, particularly among herbs, for bioactive anti-diarrhoeal agents. This study evaluated the antispasmodic activity of fractions derived from partitioning of *Anacardium occidentale* leaf extract, on isolated rabbit smooth muscle contractility. Leaves of *A. occidentale* were collected, dried, pulverized, extracted by cold maceration using methanol, and the resulting crude extract was partitioned following the modified solvent-solvent protocol to yield three fractions: methanol, chloroform and petroleum ether fractions. Rabbit jejunum of about 2 – 3 cm long was dissected out, placed on a petri dish containing freshly prepared and oxygenated Tyrode solution at room temperature. The tissue was mounted and allowed to record its basal rhythmic contraction for two minutes before the effect of the fractions were evaluated at different concentrations. The effects of standard autonomic modification drugs, acetylcholine, histamine, and the muscarinic inhibitor atropine sulphate at different concentrations were tested in the presence/absence of varying concentrations of the test fractions. Results showed that the fractions derived from *A. occidentale* leaf extract provoked significant ($p < 0.05$) concentration dependent relaxing effect on contracting smooth muscles isolated from rabbit jejunum. The fractions partially inhibited acetylcholine-induced smooth muscle contractions, whereas, the histamine receptors were almost completely blocked, indicating more anti-histaminic, than anti-cholinergic effects. The chloroform fraction possessed active principle(s) with overt antispasmodic activities than other fractions. It was concluded that the fractions derived from extracts of *A. occidentale* leaves exhibited good anti-diarrhoeal potential, principally via the histaminic pathway, lending credence to the folkloric claim that *A. occidentale* leaves can be used to manage diarrhoea.

Keywords: *Anacardium occidentale* leaves; Crude extract fractions; Antispasmodic activity; *Ex vivo* evaluation; Rabbit jejenum..

***Correspondence:** Daniel C. Ifenkwe; E-mail: ifenkwe.daniel@mouau.edu.ng; Phone: +2347030863788

Article History: Initial submission received: July 24, 2023; Final revised form received: November 03, 2023; Accepted for publication: November 14, 2023; Published: November 21, 2023.

Introduction

Diarrhoea affects both humans and animals, especially the young, and has remained a global concern that requires urgent attention. It is the second most common cause of death among children below the age of five, after pneumonia (Johansson *et al.,* 2009). The World Health Organization (WHO) classified diarrhoeal diseases as among the world's top rated communicable diseases with very high mortality rates, especially among children below the age of five and infants (WHO, 2014). In addition to the high mortality associated with diarrhoea in neonates, the condition has a lot of economic implications on food animals and livestock production, as it results in wasting among animals, and thus ultimately to decreased animal protein production and very serious losses to livestock farmers.

Diarrhoea manifests as increased passage of loose or watery stools daily or abnormally for an individual or animal. It is a major symptom of gastro-intestinal disorders, characterized by increased gastro-intestinal motility and secretions (Ezeja *et al.,* 2012). Diarrhoea can be caused by bacterial toxins, viral or parasitic organisms, as well as non-infectious agents. Although, diarrhea, like vomiting, is normally a protective mechanism, which helps remove irritants or toxic substances from the gastrointestinal tract, it often results in altered body physiology with attendant loss of electrolytes, vital minerals and salts, acute dehydration and sometimes death.

Principally, diarrhoea occurs for the reason that the balance between fluid secretion into and fluid absorption from the intestinal lumen is altered resulting in a net increase in fluidity of the faeces. For instance, some diseases that decrease the absorption of fluid or intestinal contents (such as is seen in transmissible gastroenteritis in swine or parvovirus enteritis of dogs) result in a net increase in fluid in the gastric lumen (Gricilda and Molly, 2001). Diarrhoea occurs in three forms: secretory form (caused mostly by bacterial enterotoxins), exudative form (as a result of increased permeability of the intestinal mucosa either due to inflammation or infections) or osmotic form (commonly associated with mal-digestion or mal-absorption of food). Typically, most diarrhoea syndromes are a combination of these forms. Therefore, a good anti-diarrhoeal agent should be able to principally decrease intestinal secretions, cause dysmotility or reverse the underlying problem that produced the observed changes in secretions or motility (Tadesse *et al.,* 2014).

Antispasmodics are a group of medications used to reduce excessive gastrointestinal smooth muscle contractility and spasm (Wald, 2020). Antispasmodic agents are classified based on their action into anti-cholinergics and direct smooth muscle relaxers (Song, 2022).

Herbal plants have become one of the basis of modern medicine (Syned *et al.,* 2011; Umer *et al.,* 2013). It was estimated that in Africa alone, that with the use of herbal medications, cases of diarrhoea among children below the age of five has decreased from 177.0 per 1000 live birth to 81.3 per 1000 live birth (WHO and UNICEF, 2017), which is an indication that alternative herbal remedy is gaining more focus especially in Africa. *Anacardium occidentale* plant is one of these herbal remedies scientifically reported to control diarrhoea of both infectious and non-infectious origins (Akinpelu, 2001; Odugbenmi and Akinsulire, 2006; Mustapha and Hafsat, 2007; Ezeigbo *et al.,* 2011). This present study evaluated the *ex vivo* antispasmodic activity of fractions derived by partitioning the crude extract of *A. occidentale* leaves.

Materials and Methods

Description of Study Area: This study was carried out in the Animal Research Laboratory of the Department of Veterinary Physiology and Pharmacology, College of Veterinary

Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU).

Plant Material (Collection, Extraction and Partitioning): Fresh leaves of *Anacardium occidentale* earlier pre-screened for efficacy in experimentally-induced diarrhoea (Ezeigbo *et al.,* 2011), were collected within the premises of Veterinary College, Michael Okpara University of Agriculture, Umudike (MOUAU). They were identified and authenticated by a taxonomist from the Department of Botany, College of Natural Sciences, MOUAU. Voucher specimens were deposited at the herbarium of the College of Veterinary Medicine (CVM), MOUAU, and voucher number assigned was MOUAU/CVM/VPP/2017/015.

The leaves collected were separated from debris, washed in running tap water, and subsequently air-dried, before being pulverized into fine powder using a stainless steel laboratory blender (Ansah *et al.,* 2011). The pulverized dried leaves were extracted by cold maceration (1: 5 weight per volume in methanol) for 72 hours and with intermittent vigorous agitation every two hours. The crude extract obtained was subjected to qualitative phytochemical analysis following standard methods and procedures, as previously described Trease and Evans (1989), Sofowora (1996, Harborne (1998) and Brusotti *et al*. (2014), and further partitioned following the solvent-solvent protocol described by Kupchan and Tsou (1973) as modified by Houghton and Raman (1998) in a 9:1 concentration of extracting solvent (methanol). Thereafter, the resultant solution was then partitioned successively in equal volumes of immiscible organic solvents of increasing polarity, first petroleum ether (Pet Ether) which was well shaken, then chloroform ($CHCl_3$). Before adding the next solvent, the fraction formed by the former was decanted. Thereafter the next solvent was added, shaken well and allowed to settle. At the end, all the three fractions obtained (methanol, chloroform and petroleum ether fractions) were evaporated to dryness using a rotary evaporator, and transferred into pre-weighed, labelled clean beakers. The percentage yields of the different fractions were calculated, using the standard formula.

Experimental Animals: Three matured rabbit bucks weighing between 1.80 kg and 1.82 kg were used for the study, one buck for each partitioned extract (fraction) of *A. occidentale*.

***Ex vivo* Evaluation of the Effects of Derived Partitions on Isolated Rabbit Jejunum:** Evaluation of the activities of the fractions on the isolated rabbit jejunum were done following the methods and procedures described in earlier reports by Amos *et al* (1998). A matured rabbit buck, fasted overnight (18 hours) was properly restrained and exsanguinated. The fur around the rabbit abdomen was carefully shaved and a mid-line incision through the skin and abdominal muscle along the *linea alba* made using a pair of coarse scissors. The jejenum segment of the rabbit small intestine of about 2 – 3 cm long was dissected out and placed on a Petri dish containing freshly prepared and oxygenated Tyrode solution at room temperature. Using a pair of fine curved-forceps, a length of thread was passed through both ends of the dissected guts and was mounted on to an aerated (95% O_2 / 5% CO_2) 30 ml capacity organ bath connected to a pressure transducer (HL Scientific Industries, Amabala Cantt, India) maintained at 37 ± 1°C. The intestinal content was removed by three times intermittent flushing with Tyrode solution over two minutes. Mounted intestinal tissues recorded basal rhythmic contraction for about two minutes before the effect of the fractions were evaluated (at different concentrations).

Thereafter, a 60 minutes equilibration period was allowed during which, the physiological solutions were changed at 15 minutes intervals. At the end of the equilibration period, the effects of standard autonomic modification drugs, acetylcholine (0.33 mg/ml – 0.99 mg/ml), histamine (0.33 mg/ml – 0.99

mg/ml) and the muscarinic inhibitor atropine sulphate (0.33 mg/ml – 0.66 mg/ml) were investigated in the presence/absence of varying concentrations of the fractions (0.4 and 0.8 mg/ml). The contact time for each assay was one minute; thereafter three brisk washes and a 15 minutes rest period was observed before the next addition. All assays were repeated in triplicates to ensure consistency of responses.

Ethical Considerations: All procedures on the rabbits were carried out in strict compliance to the institutional ethical instructions for the use of animals for research, as well as with adequate consultations to the Experimental Ethic Committee (EEC) guidelines to laboratory animal care and use (Louhimies, 2012). Ethical Approval Number: MOUAU/CVM/REC/202224.

Statistical Analysis: Data obtained were analysed using GraphPad Prism statistical package, and expressed in terms of means ± standard errors as well as percentage fold-changes, where appropriate. Dose-dependent effect on anti-diarrhoea index *in vivo* (ADI), peristaltic index (PI), etc., were subjected to one way analysis of variance (ANOVA) coupled with appropriate post-hoc statistics, while multiple comparisons (comparing the effect of the fraction-treated groups with the control-untreated group, and between the three fractions) was conducted using multiple linear regression analysis (MLRA), while statistical confidence was set at 95 % ($p < 0.05$).

Results

Phytochemical Analysis: The phytochemical screening of *A. occidentale* leaf crude extract and its derived fractions revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinone, steroids, phenolics, resins, terpenoids, and cardiac glycosides, with more of these phytochemicals present in methanol and chloroform partitions (Table 1).

Yield of the Partitioning: The partitioning of the crude extract yielded 8 g (40 %), 7 g (35 %) and 4 g (20 %) of methanol, chloroform and petroleum ether fractions, respectively.

Table 1. Results of the qualitative phytochemical screening of *A. occidentale* leaf extract and its derived fractions.

Metabolites	Crude extract	Methanol fraction	Petroleum ether fraction	Chloroform fraction
Alkaloids	+	+	+	+
Glycosides	+	+	+	-
Flavonoids	+	+	+	+
Tannins	+	+	-	+
Saponins	+	+	+	+
Anthraquinone	+	-	-	+
Steroids	+	+	+	+
Phenolic acid	+	-	-	+
Resins	+	-	+	-
Terpenoids	+	+	-	+
Cardiac Glycosides	+	+	-	-

Key: + means Present, - means Absent.

Effect of Different Fractions of the *A. occidentale* Extract on the Isolated Rabbit Jejunum: The results of the effects of the fractions of the *A. occidentale* extract on the isolated rabbit jejunum are presented in Table 2. The percentage relaxing activity of each partition increased as their concentrations increased (Table 2). The highest percentage relaxing activity of the chloroform fraction (88.81%) and methanol fraction (83.65%), and petroleum ether fractions (81.41%) occurred at 0.8 mg/ml concentration (Table 2). A comparison of the three fractions showed that the relaxant effect occured in the decreasing order: Chloroform fraction > Methanol fraction > Petroleum ether fraction (Table 2).

Table 2. Effects of *A. occidentale* extract fractions on the isolated rabbit jejunum.

Fractions and their test concentrations (mg/ml).	Basal Rhythmic Contraction (mm)	Response to isolated rabbit jejunum (mm)	Percentage relaxing activity (%)
Chloroform fraction			
0.2 mg/ml	9.00 ± 0.40	1.97 ± 0.02	77.91 ± 1.07
0.4 mg/ml	9.00 ± 0.40	1. 62 ± 0.17	81.94 ± 1.82
0.8 mg/ml	8.75 ± 0.25	0.97 ± 0.08	88.81 ± 1.04
Methanol fraction			
0.2 mg/ml	7.50 ± 0.28	2.08 ± 0.07	72.27 ± 3.68
0.4 mg/ml	5.75 ± 0.25	1.33 ± 0.19	77.08 ± 2.91
0.8 mg/ml	7.25 ± 0.25	1.13 ± 0.14	83.65 ± 1.94
Petroleum ether fraction			
0.2 mg/ml	5.25 ± 0.25	1.58 ± 0.21	69.75 ± 4.66
0.4 mg/ml	4.75 ± 0.25	1.13 ± 0.14	76.50 ± 2.36
0.8 mg/ml	5.25 ± 0.25	0.95 ± 0.15	81.41 ± 3.51

Effect of Acetylcholine on the Isolated Rabbit Jejenum: Upon administration of acetylcholine (standard muscarinic), after the smooth muscle was allowed to record its basal rhythmic contraction for two minutes, a forceful contraction of the rabbit Jejunum was elicited which increased in both amplitude and duration in a concentration dependent manner (Table 3). Acetylcholine induced its maximum response (83.61% contractile effect) on the isolated rabbit jejunum at the highest concentration (11.50 mm; 0.99 mg/ml) and this response was significantly ($p < 0.05$) higher when compared to the response at the concentrations of 0.33 mg/ml and 0.66 mg/ml, which elicited maximum responses of 9.25 mm and 9.75 mm with percentage activity of 64.51, and 71.66, respectively (Table 3).

Effect of Atropine sulphate on the Isolated Rabbit Jejunum: Atropine (a standard anti-muscarinic) at 0.33 and 0.66 mg/ml inhibited the normal smooth muscle contraction by 65.90% (from 9.25 mm to 3.12 mm) and

75.69% (from 8.25 mm to 2.00 mm), respectively (Table 4). This tissue response to atropine sulphate occurred in a concentration dependent manner (Table 4).

Competitive Inhibition on Contractile Effect of Acetylcholine by the Different Fractions: The relaxant effect of the different fractions on acetylcholine-induced contraction of the rabbit jejunum is presented in Table 5. The different fractions, even at the highest concentration did not significantly ($p > 0.05$) inhibit the contractile effect of acetylcholine on the isolated smooth muscle of the rabbit jejunum, as the various percentage inhibiting effect of methanol, chloroform and petroleum ether fractions were < 50% (14.80, 31.81 and 15.90 % respectively), when compared with 93.45% response (competitive inhibitory effect) of the anti-muscarinic agent, atropine sulphate (Table 5). However, chloroform partition performed relatively better with an inhibitory effect of 31.81% at its highest concentration, than other partitions.

Effect of Histamine on the Isolated Rabbit Jejunum: The contractile effect of histamine on the rabbit jejunum occurred in a concentration dependent manner: the highest activity (87.26 %) was recorded at 0.99 mg/ml, while 0.33 mg/ml did not elicit up to 50 % contractile activity on the isolated jejenum (Table 6).

Competitive inhibition on contractile effect of histamine by the different fractions: The effect of the different fractions on the contraction induced by histamine on rabbit jejunum is presented in Table 7. The methanol, chloroform and petroleum ether fractions significantly ($p < 0.05$) inhibited the contractile effect of histamine on the isolated rabbit jejunum with blocking effects of 88.88, 91.78 and 87.92% respectively, (concentration dependent) (Table 7). The chloroform partition at the 0.8 mg/ml concentration almost completely abolished (91.78%) the response effect of histamine, exhibiting a better antispasmodic effect than other partitions (Table 7).

Table 3. Effect of acetylcholine on the isolated rabbit jejunum

Dose of Acetylcholine (mg/ml)	Basal rhythmic contraction (mm)	Response to isolated rabbit Jejunum (mm)	Percentage contracting activity (%)
0.33 mg/ml	3.25 ± 0.25	9.25 ± 0.47	64.50 ± 3.47
0.66 mg/ml	2.75 ± 0.25	9.75 ± 0.25*	71.70 ± 2.89*
0.99 mg/ml	1.87 ± 0.12	11.50 ± 0.29**	83.60 ± 1.34**
R^2		**0.737**	**0.713**

$*p < 0.05$; $**p < 0.01$; $***\ p < 0.001$; comparing the response of the varied concentrations of acetylcholine.

Table 4. Effect of atropine sulphate on the isolated rabbit jejenum.

Dose of Atropine sulphate (mg/ml)	Basal Rhythmic Contraction (mm)	Response to isolated rabbit jejunum (mm)	Percentage activity (%)
0.33 mg/ml	9.25 ± 0.47	3.12 ± 0.12	65.90 ± 2.38
0.66 mg/ml	8.25 ± 0.25	2.00 ± 0.00	75.69 ± 0.69

Table 5. Competitive inhibition of contractile effect of Acetylcholine (ACh) by different concentrations of the derived fractions of *Anarcadium occidentale* extract.

Treatments	Mean Inhibition ± S.E. (mm)	Percentage inhibition (%)
Acetylcholine (ACh)	11.00 ± 0.40	-
ACh + Methanol fraction		
0.66 ACh + 0.4 MF	10.00 ± 0.40	9.09
0.66 ACh + 0.8 MF	9.37 ± 0.23	14.80
ACh + Chloroform fraction		
0.66 ACh + 0.4 CF	9.25 ± 0.25	15.90
0.66 ACh + 0.8 CF	7.50 ± 0.28	31.81
ACh + Petroleum ether fraction		
0.66 ACh + 0.4 PEF	9.75 ± 0.12	11.36
0.66 ACh + 0.8 PEF	9.25 ± 0.25	15.90
ACh + Atropine sulphate		
0.66 ACh + 0.4 AS	0.72 ± 0.07	93.45
0.66 ACh + 0.8 AS	1.07 ± 0.14	90.27

ACh – Acetylcholine; MF – Methanol fraction; CF – Chloroform fraction; PEF – Petroleum ether fraction; AS – Atropine sulphate.

Table 6. Effect of histamine on tension generated by the rabbit jejenum.

Dose of Histamine (mg/ml)	Basal rhythmic contraction (mm)	Response of isolated rabbit jejunum (mm)	Percentage activity (%)
0.33 mg/ml	2.17 ± 0.11	4.22 ± 0.19	48.53 ± 1.24
0.66 mg/ml	0.77 ± 0.08	2.07 ± 0.07	63.23 ± 3.23**
0.99 mg/ml	0.35 ± 0.06	2.60 ± 0.19	87.26 ± 2.87***
R^2			0.926

$*\ p$ <0.05; $**\ p$ < 0.01 $***p$ < 0.001; comparing different concentrations of histamine.

Table 7. Comparative inhibitory effects of different concentrations of *Anarcadium occidentale* derived fractions on histamine-induced contraction of the rabbit jejunum (blocking effects).

Treatments	Mean inhibition ± S.E	Inhibitory effect (%)
Histamine	2.07 ± 0.07	-
Histamine + Methanol fraction		
0.66 H + 0.4 MF	0.30 ± 0.06	85.55
0.66 H + 0.8 MF	0.23 ± 0.13	88.88
Histamine + Chloroform fraction		
0.66 H + 0.4 CF	0.21 ± 0.11	89.85
0.66 H + 0.8 CF	0.17 ± 0.04	91.78
Histamine + Petroleum ether fraction		
0.66 H + 0.4 PEF	0.25 ± 0.12	87.92
0.66 H + 0.8 PEF	0.40 ± 0.04	80.67

H – Histamine; MF – Methanol fraction; CF – Chloroform fraction; PEF – Petroleum ether fraction.

Discussion and Conclusion

Out of the three solvents used for partitioning, methanol and chloroform gave higher fraction yield than petroleum ether; this suggests that the extractive index of *A. occidentale* leaf increases with polarity of the solvent.

The phytochemical analysis of the *A. occidentale* leaf crude extract revealed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, anthraquinone, steroids, phenolics, resins, terpenoids, and cardiac glycosides. This finding is in agreement with the reports of Bicalho *et al.* (2001) and Onoja *et al* (2019), and most of these secondary metabolites such as tannins, alkaloids, saponins, flavonoids, steroids, phenolics and reducing sugars, have been shown to possess anti-diarrhoeal property (Longanga-Otshudi, 1999; Umer *et al.,* 2013).

The different partitions/fractions of *A. occidentale* elicited concentration dependent relaxing effect on the isolated rabbit jejunum. The highest antispasmodic effect of the chloroform (88.81%), methanol (83.65%), and petroleum ether fractions (81.41%) occurred at 0.8 mg/ml. Though the three fractions induced a relaxing effect on the isolated rabbit jejunum, above 50%, even at the lowest concentration, the response (relaxant) was highest with the chloroform partition. It is thought that this is due to the fact that the chloroform fraction contains greater amount of basic phytochemicals with anti-diarrhoeal activity such as tannins and flavonoids, in addition to the presence of anthraquinone and phenols, which were absent in other partitions. This could be, among other scientific interpretations, the reason why the chloroform fraction performed more satisfactorily as an antispasmodic agent than other fractions. Earlier reports by Korn-Steiner *et al* (2006) and Brijesh *et al* (2009) showed that plant extracts with high amount of anthraquinone and phenols were potential anti-diarrhoeal agent.

Acetylcholine is a neurotransmitter commonly associated with parasympathetic effects. It binds to muscarinic receptors on smooth

muscles causing the receptor-operated channel to open, thus allowing sodium influx, which causes depolarization of the cell membrane. This depolarization opens voltage-dependent calcium channels, and calcium ions enter the cell to induce the release of calcium from sarcoplasmic reticulum. The cytosolic calcium then binds to calmodulin to elicit a contraction (Akuodor *et al.,* 2011). Similarly, histamine binds to H_1 receptor on gastrointestinal smooth muscle to initiate the same sequence of events (Khan *et al.,* 2013). An elevation of intracellular Ca^{2+} level by influx from extracellular compartment or release from intracellular store also results in contraction (Shamkuwar and Shahi, 2012). Antispasmodic agents are known to reduce excessive gastrointestinal smooth muscle contractility and spasm, causes smooth muscle relaxation, and reduce the activation of parasympathetic nervous system due to their anticholinergic action (Rittler, 2020). Most muscarinic receptor antagonists are synthetic chemicals, however, the two most commonly used anti-cholinergics are scopolamine and atropine, and are extracted naturally from *Atropa belladonma* plant (Hadley and Gaarder, 2005). Antispasmodic agents are classified based on their action into anti-cholinergics and direct smooth muscle relaxants (Song, 2022).

Acetylcholine induced its maximum contractile effect on the isolated rabbit jejunum at the highest concentration (0.99 mg/ml, 83.61%), whereas, atropine sulphate completely blocked the acetylcholine-induced contraction as a known anti-muscarinic agent (Shamkuwar and Shali, 2012). The partial blocking of acetylcholine-induced contraction of the rabbit jejunum by the different fractions suggest partial interactions with muscarinic receptors antagonists by preventing the binding of calcium to calmodulin (different pathway), rather than preventing the release of calcium which induces contraction of the smooth muscle resulting in diarrhoea (Hodges and Gill, 2010).

Similarly, histamine elicited its maximum (87.26%) contractile effect on the rabbit jejunum in a concentration dependent manner, and the inhibition of histamine-induced contraction of the rabbit jejunum by the different fractions of *A. occidentale* suggests their interaction with histaminic receptors (anti-histaminic pathway), and indicates that the different fractions could have acted more as smooth muscle relaxant antispasmodic agent by preventing the calcium influx through the voltage operated channels, inhibiting the calcium induced-calcium release mechanism, preventing the release of calcium from the sarcoplasmic reticulum, closing of sodium and calcium ion channels, activation of second messengers like cAMP, or prevention of binding of calcium to calmodulin (Hajhashemi *et al* 2000; Field, 2003; Sahoo *et al*. 2014).

Conclusion: The different fractions of *Anacardium occidentale* extract used in the study exhibited varying degrees of inhibitory (antispasmodic) activities on smooth muscle contraction in a concentration dependent manner. The partial inhibition of acetylcholine (compared with atropine sulphate) and the complete inhibition of histamine-induced contraction of the rabbit jejunum by the different fractions suggested that the fractions interacted more with histaminic receptors (histaminic pathway) than muscarinic receptors (muscarinic pathway) antagonists, and that they acted more as a smooth muscle relaxant antispasmodic agent. The antispasmodic effect recorded in the present study occurred in the order: chloroform fraction > methanol fraction > petroleum ether fraction.

Conflict of Interest:

The authors declare no conflict of interest.

References

Akinpelu DA (2001). Antimicrobial activity of *Anacardium occidentalis* bark. *Filoterapia*, 72(3): 286 – 287.

Akuodor GC, Idris-Usman M, Ugwu TC, Akpan JL, Irogbeyi LA and Iwuanyanwu TC (2011). Ethanolic leaf extract of *Verbena hastata* produces antidiarrhoeal and gastrointestinal motility slowing effects in albino rats. *Journal of Medicinal Plants Research*, 4: 1624 – 1627.

Amos S, Okwuasaba FK, Gamaniel K, Akah P and Wambebe C (1998). Inhibitory effects of the aqueous extract of *Pavetta crassipes* leaves on gastrointestinal and uterine smooth muscle preparations isolated from rabbits, guinea pigs and rats. *Journal of Ethnopharmacology,* 61: 209 – 213.

Ansah MO, Asare DK, Amoatey HM, Gyamfi ET and Bentil NO (2011). Mineral composition and assessment of human ingestion risk of twelve accessions of *Moringa oleifera Lam*. *Journal of Ecobiotechnology*, 3: 29 – 33.

Bicalho B, Dave R and Chanda S (2001). Volatile compounds of cashew apple (*Anacardium occidentale* L.). Z. Naturforsch. *Research Journal of Medicinal Plant*, 56(1–2): 35 – 39.

Brijesh S, Daswani P, Tetali P, Antia N and Birdi T (2009). Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: validating its traditional usage. *BMC Complementary and Alternative Medicine*, 9(47): 50 – 62.

Brusotti G, Cesari I, Dentamaro A, Caccialanza G and Massolini G (2014). Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethno-pharmacological approach. *Journal of Pharmaceutical and Biomedical Analysis*, 87: 218 – 228.

Ezeigbo II, Ezeja MI, Madubuike KG and Ifenkwe DC (2011). Evaluation of *Anacardium occidentale*, methanol leaf extract in experimental diarrhoea of mice. *Nigerian Veterinary Journal* 33: 624 – 629.

Ezeigbo II, Ezeja MI, Madubuike KG, Ifenkwe DC, Ukweni IA, Udeh NE and Akomas SC (2012). Antidiarrhoeal activity of leaf methanolic extract of *Rauwolfia serpentine. Asian Pacific Journal of Tropical Biomedicine,* 6: 115 – 117.

Ezeja IM, Ezeigbo II, Madubuike KG, Udeh NE, Ukweni IA, Akomas SC and Ifenkwe DC (2012). Anti-diarrhoeal activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea. *Asian Pacific Journal of Tropical Medicine*, 5(2): 147 – 150.

Field M (2003). Intestinal ion transport and the pathophysiology of diarrhoea. *Clinical Investigation,* 111: 931 – 943.

Gricilda SF and Molly T (2001). Study of anti-diarrhoeal activity. *Journal of Ethnopharmacology,* 62(2): 95 – 99.

Gurgel LA, Santos FA, Martins DTO, Mattos PO and Rao VSN (2001). Studies on the antidiarrhoeal effect of dragon's blood from *Croton urucurana*. *Physiotherapy Research*, 15: 319 – 322.

Hadley SK and Gaarder SM (2005). Treatment of irritable bowel syndrome. *American Family Physician*, 72(12): 2501 – 2506.

Hajhashemi V, Sadraei H, Ghannadi AR and Mohseni M (2000). Antispasmodic and anti- diarrhoeal effect of *Satureja hortensis* L. essential oil. *Journal of Ethnopharmacology*, 71(1-2): 187 – 192.

Harborne AJ (1998). Phytochemical methods a guide to modern techniques of plant analysis. *S*pringer Science and Business Media; pp. 15 – 75.

Hodges K and Gill R (2010). Infectious diarrhoea: Cellular and molecular mechanisms. *Gut Microbes,* 1(1): 4 – 21.

Houghton PJ and Raman A. (1998). Laboratory handbook for the fractionation of natural extracts. Springer Science and Business Media; pp. 22 – 23.

Johansson EW, Wardlaw T, Binkin N, Brocklehurst C, Dooley T, Salama P and Young M (2009). Diarrhoea: Why children are still dying and what can be done. New York, USA and Geneva, Switzerland: The United Nations Children's Fund (UNICEF)/World Health Organization (WHO), pp. 16 – 17.

Khan IN, Jahan S, Bhuiya MAM, Mazumder K and Saha BK (2013). Anti-diarrhoeal potential of ethanol and water extracts of *Euphorbia hirta* whole plant on experimental animals: A Comparative Study Scholars. *Journal of Applied Medical Sciences,* 21: 199 – 204.

Korn-Steiner M, Wager KH and Elmadifa I (2006). Tocopherols and total phenolics in ten different nut types. *Food Chemistry*, 98: 381 – 387.

Kupchan SM and Tsou G (1973). Tumor inhibitors. A new antileukemia simaroubolide from *Brucea antidysenterica*. *Journal of Organic Chemistry,* 38:178 – 179.

Longanga-Otshudi A, Foriers A, Vercruysse A, Van-Zeebroeck A and Lauwers S (1999). *In vitro* antimicrobial activity of six medicinal plants traditional used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo (DRC). *Phytomedicine*, 7: 162 – 172.

Louhimies S (2002). Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. *Alternatives to Laboratory Animals (ATLA),* 30: 217 – 219.

Mustapha Y and Hafsat S (2007). Antibacteria activities of *Anacardium occidentalis* isolates*. Institute for Laboratory Animal Research Journal,* 1: 40 – 43.

Odugbemi T and Akinsulire O (2006). Medicinal plants by Species names and uses: In Odugbemi T. Outlines and pictures of medicinal plants from Nigeria University of Lagos press, Lagos (1st Edition); pp. 51 – 65.

Onoja SO, Ifenkwe DC, Daniel-Igwe G, Ezeh GC, Ezeja MI and Anaga AO (2019). Gastroprotective effects of polyphenol rich extract of *Anacardium occidentale* L. leaf. *Nigerian Veterinary Journal*, 40(2): 110 – 117.

Ritter J (2020). Rang and Dale's Pharmacology. In: Flower RJ, Henderson G, Loke YK, MacEwan DJ, and Rang HP. Edinburgh (9th Edition); pp. 377.

Sahoo HB, Sahoo SK, Sarangi SP, Sagar R and Kori ML (2014). Anti-diarrhoeal investigation from aqueous extract of *Cuminum cyminum* Linn. seed in albino rats. *Pharmacogenesis Resources*, 6: 204 – 209.

Shamkuwar PB and Shahi SR (2012). Study of antidiarrhoeal activity of piperine. *Der Pharmacia Lettre*, 4: 217 – 221.

Sofowora, A. (1993). Medicinal Plants and Tradition Medicine in Africa. Spectrum Books Ltd Ibadan, Nigeria; pp. 25 – 30.

Song KH (2022). Antispasmodics. *The Korean Journal of Helicobacter and Upper Gastrointestinal Research,* 22: 102 – 107.

Tadesse WTH, Abebe EG, Abyot EM and Abraham F (2014). Experimental assessment of antidiarrhoeal and antisecretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. *BMC Complementary and Alternative Medicine*, 14 (1): 460.

Trease GE and Evans WC (1989). Trease and Evans' Pharmacognosy. Bailliere Tindall; pp. 45 – 50.

Umer S, Tekewe A and Kebede N (2013). Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complementary and Alternative Medicine*, 13: 21 – 25.

Wald A (2020). Treatment of irritable bowel syndrome in adults. In Grover S. (Ed.), UpToDate. Retrieved July 11, 2021, from https://www.uptodate.com/contents/treatment-of-irritable-bowel-syndrome-in-adults

WHO and UNICEF (2017). WHO/UNICEF, UN DESA/population Division, level and Trends in Child Mortality; Joint statement: clinical management of acute diarrhea, pp. 23 – 35.

WHO (World Health Organization) (2014). Diarrhoeal disease: Fact Sheet No. 330. World Health Organization, Geneva.