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Research Paper

Anti- diarrhoeal activity of chloroform-ethanol extracts of Cashew (*Anacardium occidentale*) kernel

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ABSTRACT

The anti-diarrhoeal activity of ethanol-chloroform extracts of *Anacardium occidentale* kernel at the dose of 21mg/kg and 84mg/kg body weight were studied using rat models of diarrhoea, enteropooling and gastro-intestinal motility induced by castor oil. Acute toxicity and lethality (LD_{50}) and phytochemical constituents of the extracts were also evaluated. The results showed that the extracts significantly ($P<0.05$) reduced the watery texture and number of fecal droppings over 5 hours compared with the untreated group. It also significantly ($P<0.05$) reduced the volume and weight of intestinal content compared to the control animals. On gastro-intestinal motility, the extracts significantly ($P<0.05$) reduced the small intestinal transit of charcoal meal in rats induced with castor oil. The results of the qualitative phytochemical analysis showed that the ethanol-chloroform extract (ethanol, chloroform and middle layers) tested positively to flavonoids, alkaloids saponin, reducing sugars, glycosides and steroids while, chloroform layer and middle layer tested positive to fat and oil. Acute toxicity and lethality studies on ethanol-chloroform extracts revealed an oral LD_{50} equal or more than 5000mg/kg body weight in mice. These results showed that kernels of *A. occidentale* possess anti-diarrhoeal properties through inhibition of hyper-secretion, enteropooling and gastro-intestinal motility which can substantiate its use in the treatment of diarrhoea in traditional medicine.

Keywords: Diarrhoea; Gastro-intestinal; Enteropooling; Phytochemical; LD_{50}

INTRODUCTION

Plants and their derivatives play key role in world health and have long been known to possess biological activity. 30% of all modern drugs are derived from plants (Riaz, et al., 2010). According to the WHO, about 80% of the world's population relies essentially on plants for primary health care (Mckay and Blumberg, 2007). Diarrhoea is the condition of having three or more loose or liquid bowel movements per day, it results from an imbalance between the absorptive and secretary mechanisms in the intestinal tract accompanied by hyper-motility, resulting in excess loss of fluid in the feces (Currie, et al., 1992). It is a common cause of death in developing countries and the second most common cause of infant deaths (16%) after pneumonia (17%) worldwide. In 2009, diarrhea was estimated to have caused 1.1 million deaths in

people aged 5 and over and 1.5 million deaths in children under the age of 5 (WHO, 1990). Oral rehydration salts and zinc tablets are the treatment of choice and have been estimated to have saved 50 million children in the past 25 years. The loss of fluids through diarrhea can cause dehydration and electrolyte imbalances (Wilson, 2005). The incident of diarrhoeal diseases still remains high despite the intervention of government agencies and international organization to halt the trend (Magaji, et al., 2010). The use of herbal drugs in the treatment of diarrhoea is common in many African countries (Agunu, et al., 2005). Despite immense technology and advances in medicine, many people in developing countries still rely on traditional healing practices and medicinal plants for their health care need (Ojewole, 2004). WHO encourage studies into traditional medicinal prevention of diarrhoeal diseases (Atta and Mouneir, 2004). Therefore, there is urgent need for intensification of research into claim of the use of medicinal plants in the management of diarrhoeal diseases (Mohammed, et al., 2009). Brazil, India and Mozambique are the leading cashew nuts producers in the world (Pimentel, et al., 2009). The kernels of cashew (*A. occidentale)* plant have been reported by Orwa, et al. (2009) to be used for curing diarrhoea in some parts of the world. This research attempts at a possible investigation on the anti-diarrhoeal properties of ethanol and chloroform extracts of *Anacardium occidentale* kernel in experimentally induced acute diarrhoeal rats.

MATERIALS AND METHODS

All reagents used were of analytical grade. Drug (Lomotil) used were supplied by Emzor Pharm Ind. Ltd., Nigeria

Plant material: Cashew (*Anacardium occidentale*) (Family-*Anacardiaceae*) nuts were collected in Obolloafor, Enugu State, Nigeria, in March-2011. They were authenticated by Mr. A. Ozioko of the Bioresource Development and Conservation Programme (BDCP) Research Centre in Nsukka.

Extraction Procedure: The cashew kernels were isolated using a simple cutter knife. This was used to slit each nut open and a pointed knife employed to remove the kernel immediately from the shell to minimize contamination with the cashew nut shell liquid (CNSL). The kernel was then subjected to roasting at 80°C for one hour to remove the testa as described by Onilude, et al. (2010). The roasted kernel was ground into coarse form.

The pulverized kernel (1071g) was macerated in 3L mixture of chloroform and ethanol (2:1) for 48 hours. The macerate was passed through Whatman No 4 filter paper. The filtrate was shaken with 20% of distilled water to obtain three (3) layers.

The upper layer (ethanol layer) was drawn out; the middle layer was also separated from the lower layer (chloroform layer). The three layers were concentrated with a rotatory evaporator and dried in a boiling water bath. The weight of the sample was taken after drying. The extract yields were 0.53%, 1.48% and 0.74% for upper, middle and lower layers respectively.

Experimental Animal: Adult albino rats (200-250g) and mice (20-30g) of either sex obtained from the Laboratory animal facility of the Faculty of Biological Sciences, University of Nigeria, Nsukka were used. The animals were housed within the facility and maintained on standard rodent pellets and water *ad libitum.* On transfer to the work area, the animals were allowed 14 days for acclimatization.

All animal experiments were in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23 revised 1985).

Phytochemical Test: Basic qualitative phytochemical screening of the ethanol, chloroform and middle layers of the water-treated extract of the kernel sample was

carried out by testing for the presence or absence of the following plant constituents: flavonoids, tannins, saponins, glycosides, fat and oil, sterol, alkaloids, reducing sugar and carbohydrate. The phytochemical analysis of the sample was carried out using procedures outlined by Harborne (1989) and Trease and Evans (1989).

***Acute toxicity and lethality (LD_{50}) test*:** The acute toxicity and lethality of chloroform-ethanol extract of the cashew kernel was determined using the modified method of Lorke (1983). The test was divided into two stages. In stage one, twenty-seven (27) randomly selected adult mice were divided into nine groups, three per group (n=3) and received 10, 100 and 1000mg/kg body weights of the ethanol, middle layer and chloroform extracts respectively and the signs of toxicity and number of death for a period of 24-hours were recorded. After 24 - hour observation, the doses for the second phase were determined based on the outcome of the results of the first phase. Since there was zero death, a fresh batch of animals were used following the same procedure in phase I but with higher dose ranges of 1900, 2600 and 5000mg/kg body weights of the extract. The animals were also observed for 24-hours for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose (Lorke, 1983).

Anti-diarrhea studies: *Castor oil-induced diarrhoea test:* The effect of chloroform-ethanol extract on diarrhoea was evaluated in rats using the castor oil-induced diarrhoea method (Awouters, et al., 1978), as modified by Nwodo and Alumanah (1991). Adult rats selected without sex discrimination were divided into eight groups (n = 4). Group I received 2ml/kg of Tween 20 (3% v/v) and served as control. Animals in group II received lomotil (2.5mg/kg) and served as the standard while groups III, IV, V, VI, VII and VIII received 21 and 84mg/kg of chloroform-ethanol extract respectively. The extracts were suspended in Tween 20 (3% v/v). One hour after intraperitoneal (i.p) administration of treatments, the animals received castor oil (1ml) orally and were individually placed in a cage, the bottom of which was covered with a white sheet of paper for observation of the number and consistency of fecal droppings. The number of wet droppings was counted every 30 min for 4 hours and the white paper changed after each evaluation. The means of the stools passed by the treated groups were compared with that of the control. The mean number of diarrheic feces pooled by the control group was considered as 100%. The level of inhibition (%) of wetness of feces caused by chloroform-ethanol extracts were calculated relative to the control using the relation:

$$\text{Inhibition of defecation (\%)} = \frac{\text{Mean No of Faeces of Control} - \text{Mean No of Treated Group}}{\text{Mean No of Faeces of Control}}$$

Castor oil-induced enteropooling test: The effect of chloroform-ethanol extracts on pooling of enteric contents was assessed using the castor oil-induced enteropooling method (Robert, et al., 1976). Briefly, adult rats selected without sex discrimination were fasted for 18 h and divided into eight groups (n = 4). Castor oil (1ml) was orally administered to these animals. One hour later, animals in group I received 2 ml/kg of Tween 20 (3% v/v) which served as control. Group II received lomotil (2.5mg/kg) and served as the standard while groups III, IV, V, VI, VII, VIII received chloroform-ethanol extracts (21 and 84mg/kg body weight respectively) suspended in Tween 20 (3%,v/v). Two hours after i.p administration of treatments, animals were sacrificed by overdose of ether anaesthesia and small intestine removed after tying both ends with ligature and weighed. Intestinal contents were collected by milking into a graduated tube and their volumes were measured. The intestine was re-weighed and the

difference between full and empty intestines calculated. The level of reduction in the volume and weight of intestinal content was calculated relative to the control. The mean weight and volume of intestinal content of control rats were considered as 100%.

Gastro-intestinal motility test: The effect of chloroform-ethanol extracts on gastrointestinal motility was assessed using the castor oil-induced intestinal motility in rat model (Mascolo, et al., 1994). Briefly, adult rats of either sex were fasted for 18 h and divided into eight groups (n = 4). Castor oil (1 ml) was administered orally to the animals. One hour later, animals in group I received 2 ml/kg Tween 20 (3%,v/v) and served as control, group II received lomotil (2.5 mg/kg) and served as the standard while groups III, IV, V, VI, VII and VIII received chloroform-ethanol extracts (21 and 84mg/kg respectively). One hour after i.p administration of treatments, animals received 0.2ml of charcoal meal (0.5ml of 10% charcoal suspended in 10% gum acacia) orally. One hour later, the animals were sacrificed by overdose of ether anesthesia and the small intestine was carefully separated from the mesenterum avoiding being stretched. The length of intestine from pyloric sphincter to the ileo-cecal junction (caecum) and the distance traveled by the charcoal meal were measured. For each animal, gastrointestinal transit was calculated as the percentage distance traveled by the charcoal meal relative to the total length of the intestine. The inhibitory effect of chloroform-ethanol extracts on gastrointestinal transit was calculated relative to the control.

Statistical Analysis: The data obtained was analyzed using One Way Analysis of Variance. The data was further subjected to LSD post hoc test for multiple comparisons and differences between Means regarded significant at $P<0.05$. The results were expressed as Mean ± SD.

RESULT

Yield of the Ethanol-Chloroform Extracts of Anacardium occidentale Kernel: The three layers were concentrated in a rotatory evaporator and dried in a boiling water bath. The extract yields: for ethanol layer was 0.53%, for the middle layer was 1.48% and for the chloroform layer was 0.74%.

Acute toxicity and lethality (LD_{50}) test: Intraperitoneal administration of up to 5000mg/kg of chloroform-ethanol extract to mice caused no death in the two stages of the test. Thus, the intraperitoneal LD_{50} of chloroform-ethanol extracts in mice was estimated to be greater than 5000mg/kg.

***Phytochemical test*:** The qualitative phytochemical compositions (Table 1) showed relatively moderate presence of bioactive compounds such as flavonoids and reducing sugar in the three layers. The chloroform and middle layers showed relatively moderate presence of alkaloids, saponins, fat and oil. Glycosides and steroids were relatively present in low concentration while ethanol layer showed moderate presence of steroids only and low concentration of glycosides, saponins and alkaloids were apparently present. The bioactive compounds found to be relatively absent in the extracts were tannins and carbohydrate as shown in table 1.

***Effect of chloroform-ethanol extracts on diarrhoea*:** The chloroform-ethanol extracts significantly ($P<0.05$) reduced the number of wet fecal droppings at the tested compared with the control. The inhibition caused by chloroform layer (84mg/kg) and middle layer at both doses was slightly less than that of lomotil (Table 2)

***Effect of chloroform-ethanol extracts on enteropooling*:** $CHCl_3$-ethanol extracts significantly ($P<0.05$) reduced the volume and weight of intestinal content in a non-

dose-related manner. The reduction in the volume and weight caused by the extracts at both doses were significant ($P<0.05$) compared with that by control (Table 3).

***Effect of $CHCl_3$-EtOH extracts on intestinal transit*:** The extracts significantly ($P<0.05$) reduced small intestinal transit of charcoal meal in rats induced by castor oil. The effect of the extracts at both doses was less than that of lomotil (Table 4).

DISCUSSION

Usually the chloroform-ethanol extract yields two layers after partitioning with water provided the filtration is properly done. Surprisingly, in this study partitioning of the chloroform-ethanol extract yielded three layers consistently (n=3). Even in the presence of potassium chloride (KCl) or sodium chloride (NaCl) there were still three layers. Generally, either salt resolves the interface into either or both the upper and lower layer.

In all animals, oral administration of castor oil induced/produced diarrhoea. Evaluation of the effect of ethanol-chloroform extract of kernel of cashew on diarrhoea experimentally induced by castor oil in rats showed that it markedly or significantly ($P<0.05$) reduced the frequency of defecation, number of diarrhoea stools and wetness of the fecal droppings. This result is consistent with the findings of Chitme, et al. (2004) who observed that the castor oil-induced diarrhoea model in rats allows the observation of measurable changes in the number of stools and is a consequence of the action of ricinoleic acid librated from castor oil by lipase enzymes (Chitme, et al., 2004). The freed ricinoleic acid irritates the intestinal mucosa causing inflammation and release of prostaglandins which stimulate gastro-intestinal secretion, motility, epithelial permeability and edema of the intestinal mucosa (Zavala, et al., 1998) thereby preventing the reabsorption of sodium, chloride and water. Active intestinal secretion is driven predominately by net secretion of chloride or bicarbonate, inhibition of net sodium absorption or increase in luminal osmotically active molecules (osmotic pressure) (Shah, 2004) which can all give rise to diarrhoea. The qualitative phytochemical composition of the extracts revealed the presence of flavonoids. Flavonoids are known to modify the production of cyclooxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Moroney, et al., 1998; Otimeyin, et al., 2008). Though several constituents are present in the extracts, it is possible that flavonoids, acting singly or in combination with other constituents produced the observed anti-diarrhoeal effect of *A. occodentale* kernel.

The ethanol, chloroform and middle layers of the extract appeared to act on all parts of the intestine; thus, the layers reduced the intestinal propulsive movement in the charcoal meal treated model. Previous studies conducted by Levy (1982) and Vrushabendra, et al. (2005) showed that activated charcoal avidly absorbs drug and chemicals on the surface of the charcoal particles thereby preventing absorption (Levy, 1982; Vrushabendra, et al., 2005). Pre-treatment with the extracts suppressed the propulsive transit through the gastro-intestinal tract which indicates that the kernel extracts reduced the frequency of stooling in diarrhoea. Delay in gastric motility caused further re-absorption of water from feces and may additionally contribute to reducing its watery texture. It is likely that the layers of the extract inhibited gastro-intestinal hypermotility in diarrhoea induced by castor oil by anticholinegic effect. Anticholinegic agents are known to inhibit gastro-intestinal (GI) hypermotolity (Saralaya, et al., 2010). The above result is supported by the findings of Brown and Taylor (2005) who stated that castor oil-induced gastro-intestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine, a known anticholinegic agent (Brown and Taylor, 2005). The

result of this study showed that the layers of the extract reduced watery texture of diarrhoeal stools as well as GI hypermotility; thus leading to the desired reduction of stooling in diarrhoeal disease.

Studies on gastro-intestinal enteropooling showed that the layers of the extract significantly ($P<0.05$) reduced both the weight and volume of the intestinal contents. These effects, which are direct consequences of reduced water and electrolytes secretion into the small intestine, suggest that the extracts may enhance electrolyte and water absorption from the intestinal lumen. According to Duggan, et al. (2002), electrolyte absorption determines the efficiency of nutrient absorption; this may be attributed to the enhanced electrolyte absorption by both layers of the extract which might encourage the absorption of other intestinal contents. If the volume of the intestinal content is the same with the weight of the intestine then, water will have been the only transported material within the GIT. As the weight of the intestine is greater than the volume of the intestinal content, other substances are secreted into the lumen in addition.

The specific constituent responsible for the anti-diarrhoeal properties of cashew kernel is yet to be identified. None of the several phytochemical constituents identified from the extracts has been reported to possess anti-diarrhoeal properties. Although, the result of the qualitative phytochemical analysis observed in this study showed moderate presence of such bioactive compounds as flavonoids and reducing sugars in the three layers; alkaloids, saponins and fats and oil in the chloroform and middle layers. The bioactive compound that was not detected in the three layers of the extract was tannins. However, the experimental data from this study is insufficient to directly ascribe the anti-diarrhoeal activity to any of phytochemicals present in the three layers of the extract. But flavonoids have been shown to possess anti-diarrhoeal activity attributed to their ability to modify the production of COX-1, COX-2 and LOX (Moroney, et al., 1998); thereby, inhibiting intestinal motility.

Acute toxicity test on the extract in mice established a high LD_{50} value of more than 5000mg/kg body weight which suggests that the kernels may be generally regarded as safe with a remote risk of acute intoxication. The high degree of safety is also consistent with its popular use of the kernel as food.

CONCLUSION

Thus constituents of Cashew (*A. occidentale*) kernel possess anti-diarrhoeal properties by inhibiting hyper-secretion, gastro-intestinal enteropooling, gastro-intestinal motility.

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Table-1: Qualitative phytochemical constituents of the EtOH-$CHCl_3$ extract of *A. occidentale*.

Phytochemical Constituents	Ethanol layer	Chloroform layer	Middle layer
Flavonoid	++	++	++
Alkaloid	+	++	++
Carbohydrate	ND	ND	ND
Saponin	+	++	++
Tannins	ND	ND	ND
Fat and oil	ND	++	++
Reducing sugar	++	++	++
Glycoside	+	+	+
Sterol	++	+	+

- ND= Not Detected, + = Low, ++ = Moderate

Table -2: Effect of ethanol-chloroform extract of *A. occidentale* kernel extract on diarrhoea.

		Wet Fecal Droppings	
Treatments	**Dose (mg/kg b.w)**	**Mean ± SD**	**Inhibition of defecation (%)**
Control	-	2.46 ± 0.62	-
Lomotil	2.5	0	100
Ethanol Layer	21	2.26 ± 0.57	8.1
	84	2.06 ± 0.52	16.3
Chloroform Layer	21	0.86 ± 0.22*	65.0
	84	0.23 ± 0.058*	90.6
Middle Layer	21	0.15 ± 0.038*	93.9
	84	0.10 ± 0.025*	95.9

- n = 4; *P<0.05 compared to control (*one way ANOVA; LSD post hoc*);
- Inhibition of defecation was calculated relative to control; 0 = No wet fecal dropping.

Table 3: Effect of ethanol-chloroform extract of *A. occidentale* kernel extract on enteropooling.

		Intestinal Content	
Treatments	Dose (mg/kg b.w)	Volume (ml)	Weight (g)
Control	-	2.00 ± 0.20	2.80 ± 0.62
Lomotil	2.5	1.33 ± 0.15 (33.5)	3.37 ± 0.25 (15.35)
Ethanol Layer	21	1.53 ± 0.058 (23.50)	2.53 ± 0.21 (9.64)
	84	1.43 ± 0.52 (28.50)	2.67 ± 0.58 (4.64)
Chloroform Layer	21	1.30 ± 0.17* (35.00)	2.73 ± 0.21* (2.50)
	84	1.27 ± 0.46* (36.50)	2.40 ± 0.53* 14.29
Middle Layer	21	1.33 ± 0.29* (33.50)	2.47 ± 0.32* (11.79)
	84	1.44 ± 0.32 (28.00)	2.65 ± 0.36 (30.00)

- n = 4; *P<0.05 compared with the control (*one way ANOVA; LSD post hoc test*);
- Values in parenthesis represent reduction (%) in volume or weight of intestinal content calculated relative to control.

Table-4: Effect of ethanol and chloroform extract of *A. occidentale* kernel extract on small intestinal transit.

Treatment	Dose (mg/kg)	Total length of intestine (cm)	Distance Travel (cm)	Intestinal transit (%)
Control	-	97.75 ± 1.71	77.10 ± 1.04	78.87
Lomotil	2.5	96.50 ± 8.66	46.83 ± 3.37*	48.52
Ethanol Layer	21	87.00 ± 7.39*	61.88 ± 8.87	71.10
	84	95.25 ± 9.50	61.25 ± 2.22	64.30
Chloroform layer	21	90.38 ± 3.20	55.75 ± 2.99*	61.68
	84	102.00 ± 2.83	58.25 ± 3.30*	57.11
Middle Layer	21	82.50 ± 3.42*	40.13 ± 1.03*	49.85
	84	98.25 ± 4.57	50.75 ± 2.99*	51.65

- n = 4; *P<0.05 compared with control (one way ANOVA; LSD post hoc)

