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Antidiarrhoel activity of the saponin and flavonoid fractions of *Anarcadium occidentale* leaves in albino rats

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ABSTRACT

The leaf of *Anacardium occidentale* (family, Anacardiacea) is used traditionally in African folk medicine to manage, control or treat various human ailments, including diarrhoea. In this study, we examined the antidiarrhoea activity of crude aqueous and fractions of *A. occidentale* leave extract on experimentally induced diarrhoea in rats. Crude leave extract (100 to 400 mg/kg p.o) produced a dose dependent and significant protection of rats against castor oil induced diarrhoea. Similar test was conducted on the diethyl ether, aqueous, saponin and flavonoid fractions of *A. occidentale* leave extract in which case the flavonoid and saponins portions showed better antidiarrhoea activity. The active fractions (flavonoid and saponins) were further evaluated using the charcoal meal test and the flavonoid portion showed a 68.5% inhibition of GIT motility while the saponins portion produced a 38% inhibition at doses of 400 mg/kg respectively. Based on the findings, the aqueous leaf extract of this plant may possess anti-diarrhoeal properties and validates its use in traditional medicine for the treatment of diarrhoea.

Keywords: Antidiarrhoea, castor-oil, gastrointestinal motility, albino rats, *Anacardium occidentale*.

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INTRODUCTION

Globally diarrhoea has been estimated to kill about 2.2 million people annually majority of whom are infants and children below the age of 5 years (VenKatesan et al., 2005; Gutierrez, 2008). It involves an increase in the fluidity volume and frequency of bowel movement, increased frequency of bowel sound, wet stools and abdominal pain accompanied by increase secretion and decrease absorption of fluid and thus loss of water and electrolyte (Fontaine, 1988; Field et al., 1989).

Generally, the treatment of diarrhoea is not specific and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movement (Brunton, 1996; Suleiman et al., 2008). In order to overcome the menace of diarrhoea in developing countries especially the discomfort and frequent bowel movement, the World Health Organization (WHO) has introduced a programme for diarrhoea control which involves the use of herbal traditional medicines (WHO, 2004).

Several African medicinal plants have been reported to be useful in the treatment, management and control of diarrhoea, examples include *Terminalia avicennoides* roots (Abdullahi et al., 2008), stem-bark extract of *Annona senegalensis* (Suleiman et al., 2008), *Vitellaria paradoxa (*Abubakar et al., 2013) and *Ziziphus abyssinica* (Chinenye et al., 2013). As part of our broad based search for Africa medicinal plant with antidiarrhoea properties the present study was undertaken to examine the possible usefulness of *Anacardium occidentale* leave extract in the management and control of diarrhoea.

A. occidentale (family Anacardiaceae) popularly called

kanjuu in Hausa and cashew in English) is a multipurpose tree of the Amazon that grows up to 15 m high, it has a thick and tortus bark, and is often found growing wild in drier sandy soil. It is originally native to northern Brazil and is widely grown in tropical climate for its cashew, apple and nuts. The cashew tree, nuts fruits and leaves have been used for centuries by the indigenous tribes of rain forest to treat various ailments such as the fruit juice for influenza, the leave and bark decoction is used for diarrhoea, the seed oil is used to kill external worm on skin. In Brazil, the fruit is taken for syphilis, diuretic, stimulant and aphrodisiac, the bark infusion is used for diabetes, urinary disorders and weaknesses. The North American practitioners use cashew for diabetes, cough, bronchitis, intestinal colic and diarrhoea and as general tonic (Tédong et al., 2006). Phytochemical screening of the plant reveals the presence of the following compounds, tannins, alkaloids, flavonoid, phenols, saponins, vitamins, minerals, protein, carbohydrate, fat and fiber.

Studies carried out on extract of *A. occidentale* includes acute and sub chronic toxicity studies, Antihyperglycaemic and renal protective activities (Tédong et al., 2006), Moluscacidal activity (De Souza et al., 1992). Hypoglycaemic effect was also evaluated by Sokeng et al. (2001) and Kamtchouing et al. (1998).

MATERIALS AND METHODS

Plant material

Fresh leaves of *A. occdientale* (Family, Anacardiacea) were collected around Dambuwa area in Dangeshuni Local Government area of Sokoto State, Nigeria between June and July 2014. The leaves were identified and authenticated by Dr. E. M. Mshelia of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University Sokoto. The fresh leaves were then air dried at room temperature and size reduced into powder using pestle and mortar.

Preparation of the plant extract

Three hundred and fifty grams (350 g) of the dried leave powder was macerated in 2 L of distilled water at room temperature for 24 h, the extractive solvent was filtered and the aqueous soluble extract concentrated to dryness in a hot air oven set at 45°C yielding a 50 g dried extract denoted as the active constituents.

The oven-dried extract was dissolved in distilled water at room temperature and then divided into two portions. One portion of the aqueous extract was used directly to test for antidiarrhoea activity while the other portion was fractionated using the method described by Woo et al. (1980) to obtain saponin and flavonoid rich portions of the extract, as described below.

Fractionation of the extract

The method described by Woo et al. (1980) was followed; it involved defattening initially by addition of N-hexane to the aqueous portion of the extract and allowed to stand overnight. The two different layers formed were collected separately then hydroalcoholic solution (70:30 methanol to water) was added to the water residue. Polar compounds were further removed by dissolving the hydroalcoholic extract in diethyl ether solution and allowed to stand overnight, the two distinct layers formed were also collected separately. Butanol was added to the water residue and the mixture shaken vigorously and allowed to stand overnight. The two distinct layers formed were then separated; the butanolic fraction contains saponins and was divided into two portion. 1% KOH was added to one portion then acidified with concentrated hydrochloric acid to give the flavonoid fraction. The fractions were then dried in an oven at 45°C. And the fractions obtained were then subjected to antidiarrhoea study using castor oil induce model. Below is the scheme showing procedure of extraction and fractionation.

Animals

Wistar rats of both sexes (180 to 200 g) were obtained in different cages from animal house of the Department of Pharmacology, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto. The rats were housed in standard cages and allowed to acclimatise for 1 week before the commencement of the study. Standard commercial chow and water were provided *ad libitium* for the animals. Housing conditions were maintained at 25 ± 20°C at 12 h day/night cycles. They were fasted for at least 18 h prior to the experiments but allowed free access to drinking water. The study was approved by the Animal Research Ethical Committee, Usmanu Danfodiyo University, and Sokoto. The care and handling of the animals were according to the established public health guidelines in Guide for Care and Use of laboratory Animals, 2011.

Castor oil induced diarrhoea in rats

The castor oil-induced diarrhoea was conducted according to the method of Havagiray et al. (2004) as adopted from Chinenye et al. (2013) with slight modification. Thirty rats were divided into five groups containing six rats (n = 6) and fasted for 18 h prior to the experiment. Groups 1 to 3 received oral doses of extract of *A. occidentale* at doses of 100, 200 and 400 mg/kg body weight respectively, while groups 4 and 5 received normal saline (NaCl 0.9%) and Loperamide (5 mg/kg) respectively. After 1 h of drug pre-treatment, each animal was fed orally with 1 ml of castor oil. The animals were kept in separate metabolic cages with a plain sheet of paper placed on the floor to collect their droppings. They were observed every hour for 4 h after castor oil administration. The total number of diarrhoeic faeces was noted. The total diarrhoeal faeces for the control group were considered to be 100%. The results were expressed as a percentage of diarrhoea inhibition.

Percentage of diarrhoea inhibition = (T0 – T1 / T0) * 100

T0 = number of wet faeces in Normal saline group

T1 = number of wet faeces in test group

Castor oil induced diarrhoea in rats (fractions)

The procedure described above was followed in evaluating the fractions of *A. occidentale*, in this case a uniform dose of 400 mg/kg of the different fractions obtained (aqueous, flavonoid, saponin and diethyl ether) were tested for antidiarrhoea activity and result obtained were recorded as shown in Figure 1.

Gastrointestinal motility tests

In this study 20 rats were fasted for 18 h and then divided into five groups (n = 4). Groups 1 and 2 received oral doses of 400 mg/kg of

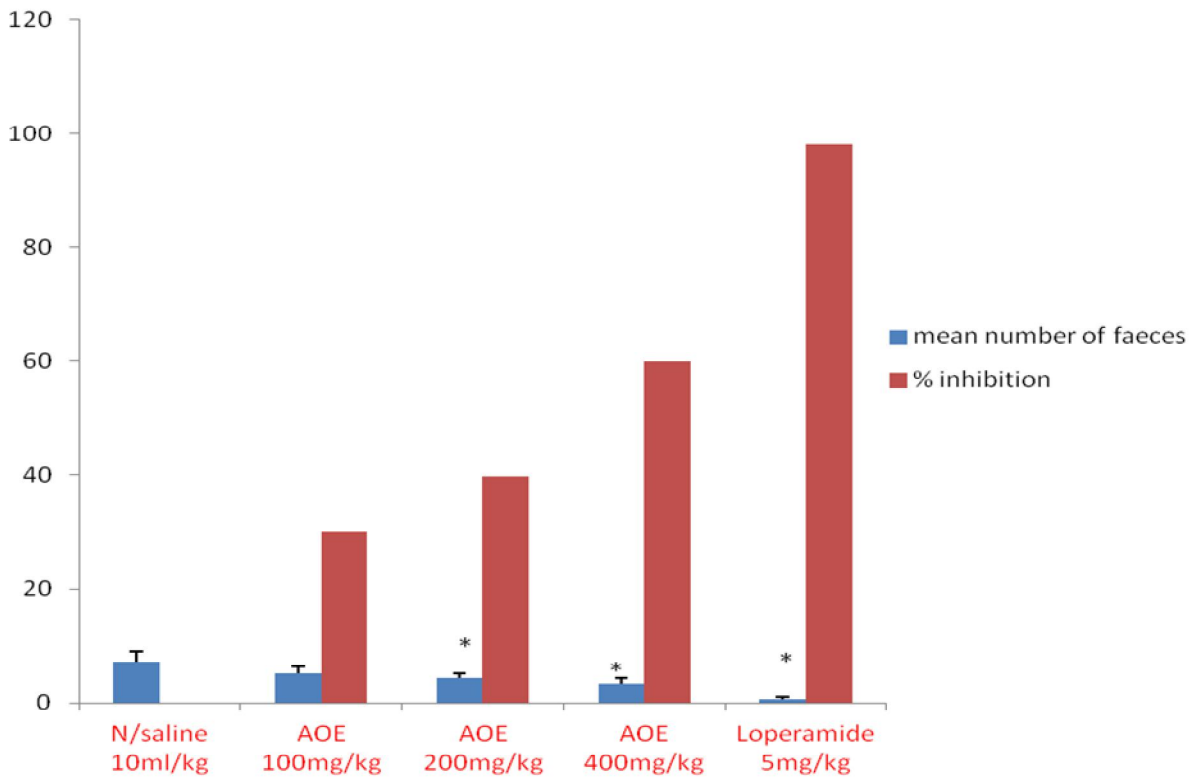

Figure 1. Effect of crude aqueous extract of *Anacardium occidentale* on castor oil induced diarrhoea. AOE = *Anacardium occidentale* extract.

the flavonoid and saponins fractions of the extract. Groups 3 and 4 received Loperamide and distilled water as the positive and negative controls respectively. One ml of charcoal meal (10% charcoal suspension in 5% gum acacia) was administered orally 30 min after the treatment. The rats were sacrificed after 1 h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. The difference was considered significant at $p < 0.05$.

RESULTS

Percentage yield

The percentage yield from the extraction was calculated to be 14.28%

Phytochemical screening

The result of the phytochemical analysis of the fractions of *A. occidentale* is presented in Table 1 and Figure 2.

DISCUSSION

Evaluation of the effect of crude aqueous extract of *A. occidentale* leave on diarrhoea experimentally induced by castor oil in rats showed that it significantly ($p < 0.05$) reduced the frequency of defecation, number of diarrhoea stools and wetness of faecal droppings. The percentage inhibited by the highest dose of *A. occidentale* extract (400 mg/kg p.o) used was however lower when compared to loperamide (5 mg/kg p.o) a standard antidiarrhoea drug widely used in management of diarrhoea disorder. The various fraction of the leaves extract at doses of 400 mg/kg showed different degree of inhibition of diarrhoea experimentally induced by castor oil in rats. Flavonoid fraction have the highest percentage inhibition (100%) which is comparable to loperamide at a dose of 5 mg/kg, saponins fraction also showed a significantly reduction in the number of diarrhoiec stool when compared to the control (Table 2).

Castor oil causes diarrhoea due to its active metabolite recinoleic acid which stimulates peristaltic activity in the small intestine leading to change in the electrolyte permeability of the intestinal mucosa (Galvez et al., 1993) the freed ricinoleic acid librated by lipase enzymes irritate the intestinal mucosa causing inflammation and release of prostaglandins which stimulate gastrointestinal secretion, motility, epithelial permeability and edema of

Table 1. Phytochemical screening of saponin, flavonoid and aqueous fractions of *A. occidentale.*

Phytochemical test	Flavonoid	Saponin	Aqueous
Saponin- Frothing test	-	+	+
Flavonoid			
Ferrric chloride	+	+	+
NaOH	+	+	+
Tannins			
Ferric chloride	-	+	+
Lead acetate	-	+	+
Steroid/triterpenoids			
Salkowski	+	+	+
Lieberman Burchard	+	+	+
Cardiac glycosides			
Keller-killiani	-	-	-
Carbohydrates			
Molisch	+	+	+
Fehling	+	+	+
Alkaloid	-	+	-
Anthraquinone	-	-	+

+ = positive result, - = not detected.

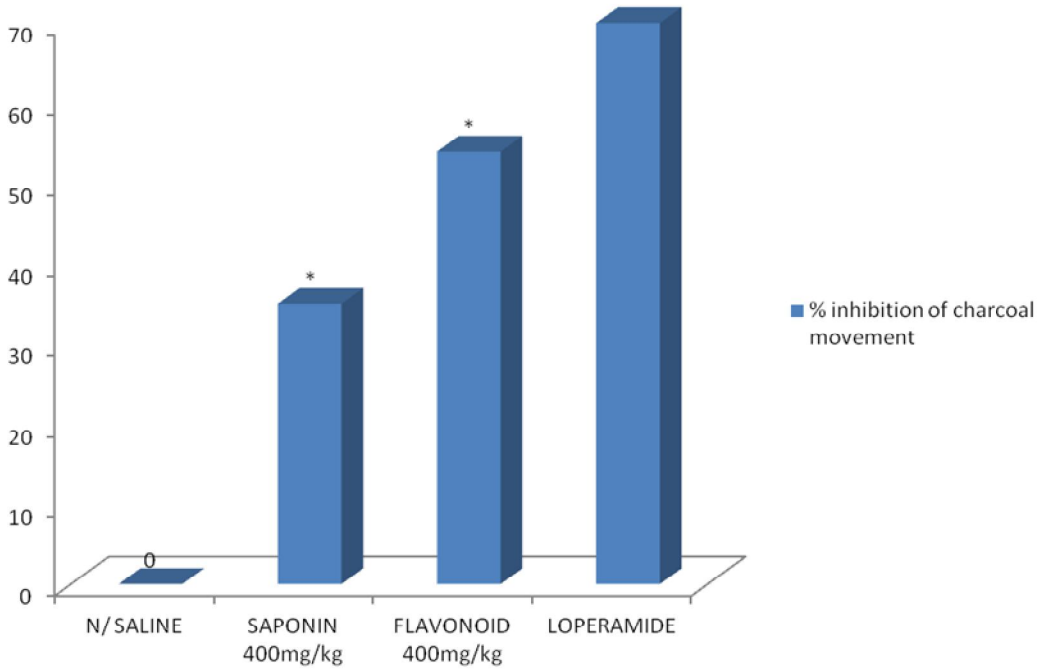

Figure 2. Inhibitory effects of *A. occidentale* saponin and flavonoid portions on gastrointestinal motility. Data presented as mean% ± SEM, n = 6 for all groups, *p < 0.05 compared to the normal saline control group.

Table 2. Percentage protection of the *A .occidentale* leave fractions on castor oil induced diarrhoea.

Fractions	Dose	Mean no. of faeces	% Inhibition
N/saline	10 ml/kg	4.25 ± 0.96	0
Loperamide	5 mg/kg	0 ± 00	100
Aqueous	400 mg/kg	1.25 ± 0.41*	70.5
DEE	400 mg/kg	3.75 ± 0.28	11.78
Saponin	400 mg/kg	0.25 ± 0.21**	94
Flavonoid	400 mg/kg	0 ± 00**	100

Values are mean ± SEM * significant as compared to respective control p< 0.05**.**

intestinal mucosa thereby preventing reabsorption of sodium, chlorine and water thus causing diarrhoea (Zavala et al., 1998).

The remarkable dose related reduction in castor oil induced diarrhoea produced by *A. occidentale* crude extract and that produced by flavonoid and saponin fractions in rat is an indication of the antidiarrhoea efficacy of the extract. Flavonoid fraction (400 mg/kg p.o) have the same efficacy as loperamide (5 mg/kg p.o) a standard antidiarrhoea drug. However a number of investigators have shown that flavonoid, saponins, tannins and triterpenoids possess antidiarrhoea property in various experimental animal models (Dicarlo et al., 1994; Abdullahi et al., 2008; Al-Rehaily et al., 2001).

The administration of the fractions also slowed down the propulsion of charcoal meal through the gastrointestinal tract when compared to the normal saline treated group. There was a significant reduction in the length of the intestine travelled by the charcoal meal when 400 mg/kg of the saponins and flavonoid fractions were administered. The percentage inhibition of intestinal length travelled by charcoal meal in the saponin fraction-treated rats was 38% while that of the flavonoid group was 69.5% when compared to the saline treated group.

Phytochemical analysis of the extract reveals the presence of saponins, flavonoids, tannins and triterpenes while anthraquinones were absent. Moreover, previous research on *A. occidentale* reported that its antidiarrhoea activity may be due to the presence of flavonoids and saponins as reported in this study (Ezeigbo et al., 2013).

CONCLUSION

In conclusion, experimental evidence obtained in the study indicated that *A. occidentale* aqueous leaves extract and fractions may possess constituents that have antidiarrhoea activity. This finding may support the claim of traditional healers about the ethnomedical use of *A. occidentale* leaves extract as natural remedy for the treatment and/or control of diarrhoea in traditional medicine.

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