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Original Article

ANTIDIARRHEAL POTENTIALS OF *SCHWENCKIA AMERICANA* L. (SOLANACEAE): A COMPARATIVE STUDY OF THE AERIAL PART AND ROOT EXTRACTS

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ABSTRACT

Objective: *Schwenckia americana* L. (Solanaceae) is employed as a decoction of either the leaves, roots or the whole plant in the treatment of diarrhoea in most West African countries. Using a comparative study approach, the antidiarrheal potentials of its aerial part and root extracts were evaluated to substantiate its local use in diarrhoea management.

Methods: *In vivo* effects of the extracts on rodent models of diarrhea, enteropooling and gastric motility induced by castor oil were investigated as well as their effects on acetylcholine and histamine-induced guinea pig ileal contractions *in vitro*. Phytochemical analyses of both extracts were also carried out for tentative identification of constituents and quantitative estimation of total saponin and phenolic contents using standard methods.

Results: *S. americana* L. gave a significant (P<0.001) reduction in the number of fecal droppings and fecal watery texture over 4 h. Gastrointestinal motility and the volume of intestinal content were also significantly (P<0.001) reduced. *In vitro* studies revealed potent inhibition of guinea pig ileal contractions induced by acetylcholine and histamine by the extracts. However, the anti-diarrheal potentials in the root extract were higher than the aerial part extract, with the root extract exhibiting complete blockade of acetylcholine and histamine-induced ileal contractions and up to 100% inhibition of diarrheic feces passage at 400 mg/kg. The phytochemical analysis also revealed a higher relative abundance of phenols and saponins in the root extract.

Conclusion: This research has therefore pioneered the establishment of a scientific proof for the acclaimed anti-diarrheal potentials and hence the ethnomedicinal use of this herb. The existence of higher anti-diarrheal potentials in the root has also been established which could possibly be linked to a higher relative abundance of phenols and saponins in this part.

Keywords: *Schwenckia americana,* Anti-diarrheal, Comparative, Aerial parts, Roots, Enteropooling, Gastrointestinal motility, Ileal contractions

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INTRODUCTION

In most tropical and sub-tropical poor and developing countries, acute diarrhea remains an important health threat and a principal cause of infant deaths [1-3]. According to WHO, an approximate of 5 million annual deaths have been linked to diarrhea with about 2.5 million cases occurring in children less than 5 y of age [4-6]. It is a disease condition resulting from altered motility and fluid accumulation in the intestinal lumen often characterized by increased frequency of bowel movement, wet stool and abdominal pain [7, 8]. The use of antimotility agents, antibiotics, electrolyte and fluid replacement therapies are currently the mainstay in acute diarrhea management. Amongst the existing management approaches, however, the antibiotic therapy which targets importantly the microbes has been associated with adverse effects and emerging resistance issues [9].

In many tropical communities, where diarrhoea is a very common ailment, the use of herbal remedies is a common practice in diarrhea management. The need for a continued search for relatively safer effective agents from plant origin for the benefit of man has continued to attract keen research interest. Hence, a number of medicinal plants have been reported to be active against diarrhea containing several phytoconstituents shown to exhibit anti-diarrheal properties.

One of such plants with putative antidiarrheal activity is *Schwenckia americana* L. (Solanaceae). This thin herbaceous weed found in parts of West Africa is a common ingredient in many herbal remedies used in the region. *S. americana* is distributed in almost all geographical regions of Nigeria [10] and is an important ingredient of the popular 'Agbo" mixture used in various diseases conditions. The plant is of particular ethnomedicinal relevance in the treatment of chest pains, respiratory diseases like a cough and asthma [10-12], sexually transmitted diseases, frequent abortions [10, 12], conjunctivitis [13], rheumatic pains/swellings and intestinal infections like neonatal diarrhea [10]. In many areas within the West African region, it was noticed that the aerial parts (leaves and/or stem), the roots and even the whole plant are employed in ethnomedicine. The aerial parts are widely used to treat diseases of babies and small children as the leaf decoction is given to pregnant women when the fetus develops too slowly and also taken by breastfeeding women to prevent diarrhea of the baby [10]. The root decoction is employed as a laxative while the crushed whole plant relieves stomach problems [11]. Some documented preliminary scientific studies are available substantiating the analgesic and anti-inflammatory potentials of this herb [14, 15]. However, there is still no documented research on its anti-diarrheal potentials neither is there any on the particular plant part with this potential. This study has therefore been undertaken with the objective of pioneering the preliminary evaluation of the anti-diarrheal potentials of *S. americana* (root and aerial parts) with the aims of validating its acclaimed anti-diarrheal potentials in ethnomedicine and possibly compare the extent of the existence of this pharmacological potential in the root and aerial parts of the plant.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (200-250 g) and mice (20-30 g) of either sex were obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were housed in steel cages within the Facility and maintained on standard rodent pellets and water *ad libitum*. 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)

and in accordance with the University of Nigeria Ethics Committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria, with the number; NHREC/05/01/2013B.

Chemicals, drugs, reagents and solvents

All chemicals used were of analytical grade and include methanol, dichloromethane, diethyl ether, n-butanol, atropine, histamine, acetylcholine and Folin-Ciocalteu phenol reagent, all purchased from Sigma-Aldrich, Germany. Others include promethazine (Emzor Pharm Ind. Ltd., Nigeria), castor oil, tragacanth, gallic acid, sodium carbonate (Na_2CO_3), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), glucose, calcium chloride ($CaCl_2$), activated charcoal, tween 80 and distilled water.

Equipment/instruments

The equipment used include: electronic weighing balance (Furi; FEJ-600, China), spatula, crucibles, measuring cylinder, separating funnel, milling machine (Lab mill, serial No. 4745, Christy and Norris Ltd., England), water bath, animal weighing balance (B. Bran Scientific and Instruments Co., England), SRI kymograph 7050, rotary evaporator (Buchi, Switzerland),UV-Visible spectro-photo-meter (Easy-Way Medical England 752W, England).

Collection and preparation of plant material

Fresh whole plant of *S. americana* L. was collected from March to June 2014 in Umunnachi, Anambra state. The plant was identified and authenticated by Mr. Ozioko, a taxonomist at the International Centre for Drug Development Nsukka, Enugu State in Nigeria, where a voucher specimen (InterCEDD/066) is maintained. The aerial parts were separated from the roots. The root and aerial parts were thoroughly cleaned, sun-dried and pulverised. About 2 kg each of the root and aerial parts powders were extracted by 48 h cold maceration with intermittent shaking using methanol and methylene chloride: methanol (1:1) respectively. The respective filtrates were concentrated under reduced pressure in a rotary evaporator to obtain the methanol root, RME (72 g, 3.60 %w/w) and methanol/methylene chloride aerial part, MME (87 g, 4.3 %w/w) extracts.

Phytochemical analysis of extracts

The extracts were subjected to qualitative phytochemical analysis for tentantive identification of constituents using the method of Harborne [16] while further quantitative analysis for total phenolic and saponin contents were also carried out using standard methods as follows;

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu assay [17]. Briefly, 0.2 ml of RME and MME (1 mg/ml) were made up to 3 ml with distilled water, mixed thoroughly with 0.5 ml of Folin-Ciocalteu phenol reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate solution. A reagent blank using distilled water was also prepared. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured against the reagent blank at 650 nm and the total phenolic content was evaluated from a gallic acid standard curve.

Determination of total saponin content

Total saponin content was estimated according to the method of Nahapetian and Bassiri [18] with some modifications. Briefly, 10 g each of RME and MME was taken in a flask and 100 ml of 20% ethanol was added to it. The mixture was heated over a hot water bath for a period of 4 h with continuous stirring at 45 °C. The mixture was filtered and the supernatant liquid was separated. The solid residue was re-extracted by mixing it with 100 ml of 25% ethanol, heating at the same temperature and stirred for another 4 h as done previously. The solution was filtered and the extract was mixed with the previously stored extract. The combined extract was concentrated in a rotary evaporator at 40 °C and heated till the volume of the extract was reduced to 40 ml approximately. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to it and shaken vigorously. After the solution settled down, the aqueous layer was carefully separated into another flask and the ether layer was discarded. The extraction process was repeated twice. 30 ml of n-butanol was added. The n-butanol extract was re-extracted twice with 10 ml of 5% sodium chloride. The remaining solution obtained after discarding the sodium chloride layer was evaporated in a water bath to semi-dried form. The sample was then dried in an oven at 40 °C to a constant weight and the saponin content calculated.

Pharmacological tests

Castor oil induced diarrhea test

The effect of the extracts on diarrhea was evaluated in rats using the castor oil-induced diarrhea method [19, 20]. Adult rats (selected without sex discrimination) were divided into eight groups (n = 6). Group I received 2 ml/kg of tween 80 (3% v/v) and served as the control. Animals in group II received atropine (3 mg/kg) and served as the standard while groups III, IV, V and VI, VII, VIII received 100, 200 and 400 mg/kg of MME and RME respectively. The extracts were suspended in tween 80 (3% v/v). One hour after *peroral* administration of treatments, the animals received 1 ml castor oil orally and were individually placed in separate cages lined with white sheets of filter paper for observation of the number and consistency of fecal droppings. The number of both wet and dry droppings was counted hourly for 4 h and the white paper changed after each evaluation. The mean number of 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 and defecation was calculated relative to the control using the relation-

$$\text{Inhibition of defecation (\%)} = \left[\frac{\text{NFeC} - \text{NFeT}}{\text{NFeC}}\right] \times 100$$

Where NFeC= mean number of feces of control group;

NFeT = mean number of feces of the treated group.

The level of reduction (%) in defecation of watery feces was calculated using the relation-

$$\text{Inhibition of diarrheic feces (\%)} = \left[\frac{\text{NDFC} - \text{NDFT}}{\text{NDFC}}\right] \text{x } 100$$

Where NDF_C= mean number of diarrheic feces of control group;

NDF_T= mean a number of diarrheic feces of treated group.

Gastrointestinal motility (charcoal meal transit) test

The effect of MME and RME on gastrointestinal motility was assessed using the castor oil-induced intestinal motility in mice method [21]. Briefly, adult mice of either sex were fasted for 18 h and divided into eight groups (n = 6). Castor oil (0.3 ml) was administered orally to the animals. One hour later, animals in group I received 0.2 ml/kg tween 80 (3% v/v) and served as control, group II received atropine (3 mg/kg) and served as the standard while groups III, IV,V and VI,VII, VIII received MME and RME (100, 200 and 400 mg/kg) respectively. One hour after intraperitoneal administration of treatments, animals received 0.2 ml of charcoal meal (0.5 ml of 10% charcoal suspended in 10% gum acacia) orally. One hour later, the animals were sacrificed by an 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 travelled by the charcoal meal were measured. For each animal, gastrointestinal transit was calculated as the percentage distance travelled by the charcoal meal relative to the total length of the intestine. The inhibitory effect of MME and RME on gastrointestinal transit was calculated relative to the control.

Castor oil-induced enteropooling test

The effect of MME and RME on pooling of enteric contents was assessed using the castor oil-induced enteropooling method [22].

Briefly, adult mice selected without sex discrimination were fasted for 18 h and divided into eight groups (n = 6). Castor oil (1 ml) was orally administered to these animals. One hour later, animals in group I received 0.2 ml/kg of tween 80 (3% v/v) which served as control. Group II received atropine (3 mg/kg) and served as the standard while groups III, IV, V and VI, VII and VIII received MME and RME (100, 200 and 400 mg/kg) respectively suspended in tween 80 (3% v/v). Two hours after intraperitoneal administration of treatments, animals were sacrificed by overdose of ether anaesthesia and the 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 [20]. The level of reduction in the volume of intestinal content was calculated relative to the control. The mean volume of intestinal content of the control were considered as 100%.

***In vitro* effects on isolated guinea pig ileum**

The isolated tissue preparations according to the technique of Perry [23] and Williamson *et al.*, [24] were employed. A male guinea pig (350g) fasted for 18 h, was sacrificed and a segment of the ileum was isolated and dissected into smaller pieces of about 2–3 cm long free of adhering mesentery. The intestinal contents were removed by flushing with tyrode solution of composition: (mM) NaCl 136.8, KCl 2.7, $CaCl_2$ 1.3, $NaHCO_3$ 12.0, $MgCl_2$ 0.5, $NaPO_4$ 0.14 and glucose 5.5g. The tissue was mounted in a 50 ml thermoregulated organ bath containing tyrode solution maintained at 37 °C and aerated with air [25, 26] to obtain a 2 cm long pieces of ileum for the experiment. A piece of guinea pig ileum was tied with the aid of thread at both ends and suspended in a 50 ml organ bath, maintained at 37 °C, containing a tyrode solution [23, 24]. An equilibration period of 1 h was allowed during which the physiological salt solution was changed every 15 min. After the initial equilibration period, responses were established for acetylcholine and histamine (0.8 × 10^{-1}µg/ml) serving as controls. MME and RME at organ bath concentrations of 1×10^{-1}µg/ml, 1µg/ml and 10µg/ml, were added alone for effects. At organ bath concentration ranges of 0.2 ×10^{-1}µg/ml to 2.0 ×10^{-1}µg/ml and 1 ×10^{-1}µg/ml to 10 ×10^{-1}µg/ml respectively co-administered with acetylcholine and histamine (0.8 × 10^{-1}µg/ml), the effects of the root and aerial part extracts of *S. americana* L. were also investigated non-cumulatively. The contact time for each concentration administered was 1 min, after which the tissue was washed three times using physiological salt solution and allowed to rest for 15 min before the next addition. The concentration of the plant extracts at which a complete blockade of response (isometric contractions) occurred were noted. Then promethazine and atropine (0.2× 10^{-1}µg/ml and 1× 10^{-1}µg/ml) were co-administered with 0.8 × 10^{-1}µg/ml organ bath concentrations of histamine and acetylcholine respectively and complete blockades of response also noted. Isometric contractions were recorded as heights of response in cm on kymograph paper using SRI kymograph 7050 [27-29]. Relaxation (%) was calculated using the relation;

$$\text{Relaxation (\%)} = \frac{Ho - Hr}{Ho} * 100$$

Where H_o= original response height, H_r = height of relaxation response. The antihistamine effects and the anticholinergic effects of the extracts at different doses were compared with promethazine and atropine respectively.

Statistical analysis

Results obtained were subjected to one-way analysis of variance (ANOVA) using Graph pad Prism version 5.03, followed by Dunnet post hoc test and $P<0.05$ was considered significant. The results are expressed as Mean+SEM.

RESULTS

Phytochemical constituents of extracts

Qualitative phytochemical analysis show that both extracts tested positive to alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids, reducing sugars and terpenoids (table 1). However there was some disparity in the relative abundance of some phyto-constituents which was further confirmed from the quantitative analysis which revealed a relatively higher concentration of phenols and saponins in RME (645.161, 1.745 g/100g) than in MME (580.645, 1.376 g/100 g).

Table 1: Phytochemical constituents of *S. americana* L. extracts

Phytochemical constituents	Relative presence	
	MME	RME
Tannins	++	+
Reducing Sugars	++	+
Glycosides	+++	+
Phenols	++	+++
Terpenoids	+	++
Flavonoids	++	+++
Alkaloids	+++	++
Steroids	++	+
Saponins	+	++

RME = root extract; MME = aerial parts extract; +++= Present in abundant amount; ++= Present in moderate concentration; += Present in trace amount; -= Likely not present.

Table 2: Effect of extracts on castor oil induced diarrhea

Treatment	Dose (mg/kg)	NFe	NDF	Mean stool weight (g)	Inhibition of defecation (%)	Inhibition of diarrheic droppings (%)
Control	--	1 3.50±1.84	6.50±2.05	1.57	--	--
MME	100	1 4.83±1.14	2.83±1.14	1.01	9.9	55.3
	200	7.50±1.77	4.83±1.97	0.88	44.4	23.7
	400	5.67±1.63*	3.17±1.14	0.36	58.0	49.9
RME	100	8.00±2.15	3.67±1.36	0.58	40.7	42.0
	200	5.17±1.87*	0.50±0.34**	0.39	61.7	92.0
	400	2.67±1.52**	0.00±0.0**	0.18	80.3	100
Atropine	3 3	9.17±2.01	4.17±0.95	0.96	44.4	34.1

Values are mean±SEM., n=6, *,** Significant difference from control ($P<0.05$ and $P<0.001$) respectively, RME = root extract, MME = aerial part extract, NDF= mean number of diarrheic feces; NFe = mean number of feces

Effect of extracts on castor oil induced diarrhea

Pre-treatment of animals with an extract from *S. americana* aerial part and root (MME and RME) significantly (P<0.05) reduced diarrhea induced by castor oil and inhibited defecation within 1 h (table 2) compared to the control.

The methanol root extract was more active than the aerial parts extract. RME exhibited a dose-dependent effect giving the highest percentage inhibition of diarrheic droppings (100 %) at 400 mg/kg dose (fig. 1). MME, however, displayed a non-dose dependent inhibition and less antidiarrheal activity compared to RME. RME also gave the highest percentage inhibition of defecation (80.3%) and the least mean weight of feces (0.18 g) among all the treatment groups.

Effects of extracts on gastrointestinal motility in rats

MME and RME retarded intestinal charcoal meal propulsion in rats compared to the control for an observation period of 2 h. RME showed a dose-dependent and higher inhibitory effect than MME and atropine. RME at 400 mg/kgexhibited the highest (69.1%, 95.1%) while MME at 200 mg/kg produced the least inhibitory and reductive effects (9.4%, 62.7%) on intestinal transit and volume of intestinal contents respectively. The percentage inhibitory effects on intestinal motility by RME at 200 and 400 mg/kg (48.9 and 69.1% respectively) were higher than that of the standard drug, atropine sulphate (48.2%) as depicted in fig. 1. At all doses tested, RME gave a higher inhibitory effect on enteropooling (80,83,95.1%) than the reference drug, atropine even at 6 mg/kg (77.5%).

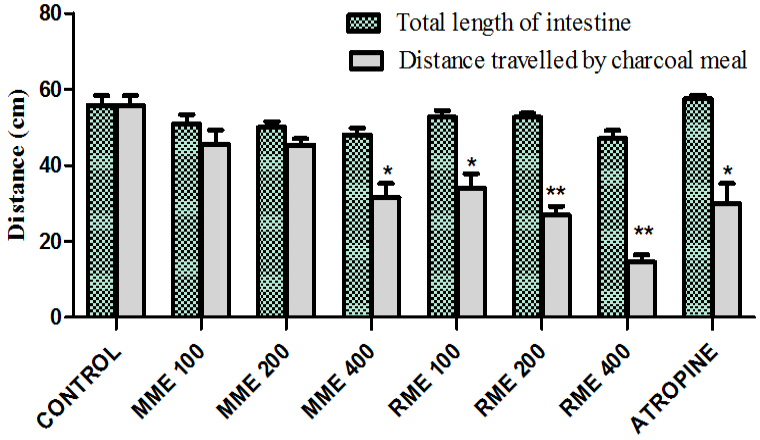

Fig. 1: Effect of extracts on gastrointestinal motility, values are mean±SEM., n=6, *, Significant difference from control (P<0.05 and P<0.001) respectively, RME = root extract, MME = aerial part extract**

Effect of extracts on enteropooling

The tested extracts showed a significant reduction in intestinal volume with RME exhibiting a higher and dose dependent effect unlike MME. RME at 400 mg/kg exhibited the highest (95.1%) while MME at 200 mg/kg produced the least reduction (62.7%) in the volume of intestinal contents respectively. At all doses tested, RME gave a higher inhibitory effect on enteropooling (80%, 83%, 95.1%) than the reference drug, atropine even at 6 mg/kg(77.5%) as depicted in fig. 2.

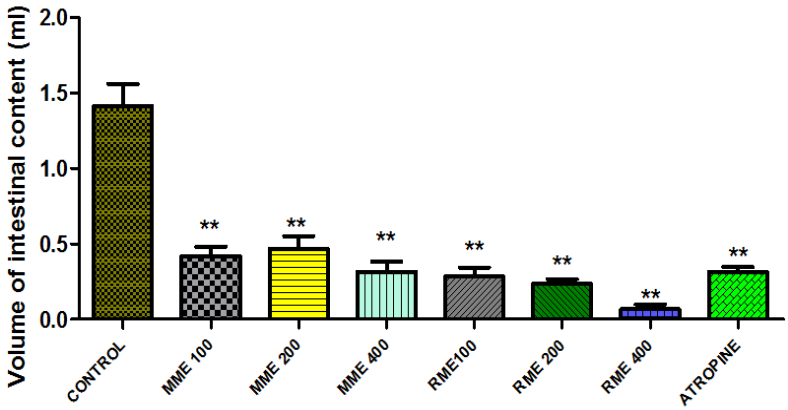

Fig. 2: Effect of extracts on passage of diarrheic feces, values are mean±SEM., n=6, **Significant difference from control (P<0.001), RME = root extract, MME = aerial part extract

Effect of extracts on guinea pig ileal contractions *in vitro*

Atropine and promethazine at 0.2× 10-¹µg/ml and 1× 10-¹µg/ml respectively completely blocked ileal contractions induced by acetylcholine and histamine at 8 × 10-¹µg/ml respectively. RME exhibited dose-related inhibitory effects, achieving a complete blockade at 2.0 ×10-¹µg/ml and 10 ×10-¹µg/ml for acetylcholine and histamine (table 3 and 4). MME also exhibit similar inhibitory effects as RME on histamine-induced contractions. However the inhibition observed with acetylcholine-induced ileal contraction was non-dose related and less than that seen with RME (table 3 and 4).

Table 3: Effects of extracts on histamine-induced guinea pig ileal contractions *in vitro*

Treatment	Height of response (cm)	Relaxation (%)
8×10^{-2} µg/ml Histamine	2.65	-
8×10^{-2} µg/ml Histamine+0.8 $\times 10^{-1}$µg/ml MME	1.20	54.72
8×10^{-2} µg/ml Histamine+1 $\times 10^{-1}$µg/ml MME	0.60	77.36
8×10^{-2} µg/ml Histamine+2 $\times 10^{-1}$µg/ml MME	0.30	88.68
8×10^{-2} µg/ml Histamine+4 $\times 10^{-1}$µg/ml MME	0.10	96.23
8×10^{-2} µg/ml Histamine+8 $\times 10^{-1}$µg/ml MME	0.00	100
8×10^{-2} µg/ml Histamine+10 $\times 10^{-1}$µg/m MME	0.00	100
8×10^{-2} µg/ml Histamine+1 $\times 10^{-1}$µg/ml RME	1.55	41.51
8×10^{-2} µg/ml Histamine+2 $\times 10^{-1}$µg/ml RME	0.35	86.79
8×10^{-2} µg/ml Histamine+4 $\times 10^{-1}$µg/ml RME	0.15	94.34
8×10^{-2} µg/ml Histamine+8 $\times 10^{-1}$µg/ml RME	0.05	98.11
8×10^{-2} µg/ml Histamine+10 $\times 10^{-1}$µg/ml RME	0.00	100

Values are mean heights of response obtained in triplicates, MME = aerial parts extract; RME = root extract. The experiment was performed in three times for confirmation of results

Table 4: Effects of extracts on acetylcholine-induced guinea pig ileal contractions *in vitro*

Treatment	Height of response (cm)	Relaxation (%)
8×10^{-2} µg/ml ACh	3.25	-
8×10^{-2} µg/ml ACh+0.2 $\times 10^{-1}$µg/ml MME	2.79	14.15
8×10^{-2} µg/ml ACh+0.4 $\times 10^{-1}$µg/ml MME	1.95	40.00
8×10^{-2} µg/ml ACh+0.6 $\times 10^{-1}$µg/ml MME	1.60	50.77
8×10^{-2} µg/ml ACh+0.8$\times 10^{-1}$µg/ml MME	1.40	56.92
8×10^{-2} µg/ml ACh+1.0 $\times 10^{-1}$µg/ml MME	1.37	57.85
8×10^{-2} µg/ml ACh+2.0 $\times 10^{-1}$µg/ml MME	2.07	36.31
8×10^{-2} µg/ml ACh+0.2 $\times 10^{-1}$µg/ml RME	2.35	27.69
8×10^{-2} µg/ml ACh+0.4 $\times 10^{-1}$µg/ml RME	1.45	55.38
8×10^{-2} µg/ml ACh+0.8 $\times 10^{-1}$µg/ml RME	1.05	67.69
8×10^{-2} µg/ml ACh+1.6 $\times 10^{-1}$µg/ml RME	0.95	70.77
8×10^{-2} µg/ml ACh+2.0 $\times 10^{-1}$µg/ml RME	0.00	100

Values are mean heights of response obtained in triplicates, MME = aerial parts extract; RME = root extract. ACh=Acetylcholine.

DISCUSSION

Diarrhea results from hyperperistalsis of the small intestine or colon with large amounts of Na^+, K^+ and water being washed out of the colon and small intestine in diarrheic stools [30]. These alterations in gastrointestinal motility and fluid accumulation in the intestinal lumen often seen in diarrheic conditions are a consequence of innumerable pathologies like dehydration, hypovolaemia and eventually shock and cardiovascular collapse [30, 31]. It may be caused by an increased secretion of electrolytes (secretory diarrhea), an enhanced ingestion of osmotic substances (osmotic diarrhea), or the presence of a virulent microorganism (infectious diarrhea) [32-34].

The extracts significantly inhibited the severity and frequency of castor oil-induced diarrhea in the tested Swiss albino mice. The gastric contents as well as watery texture of diarrheic stools were significantly reduced. Castor oil-induced diarrhea is described to be an appropriate model that characterizes secretory diarrhea [35]. Though several mechanisms have been proposed to explain the diarrheal effect of castor oil, castor oil-induced diarrhea has been largely attributed to the irritating and inflammatory action of its hydrolytic product, ricinoleic acid on the intestinal mucosa leading to prostaglandins release and increased permeability of mucosal cells which provokes secretion and changes in electrolyte transport and hence diarrhea [36, 37]. Hence, the antidiarrheal activity of the plant against the experimentally-induced diarrhea by castor oil may be attributed to such anti-secretory action.

In the evaluation of the effect on gastrointestinal motility and transit, the extracts caused retardation in the intestinal charcoal meal propulsion in rats. The standard drug used, atropine sulphate is known to inhibit intestinal transit probably due to its anticholinergic effect [38]. Anticholinergic agents are known to inhibit gastrointestinal hypermotility. According to Akah 1996 [39], drugs which inhibit intestinal motility can also possess anti-diarrheal activity. Castor oil-induced gastrointestinal hypermotility has also been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine, a known anticholinergic agent [40]. It is therefore likely that the extract inhibition of gastrointestinal hypermotility and the observed reduction in intestinal propulsive movement of the charcoal meal may have been mediated through anticholinergic effect.

The extracts also exhibited significant inhibition of ileal contractions induced by acetylcholine and histamine *in vitro*. Histamine and acetylcholine are important endogenous spasmogens and agents that inhibit their contractions may have good antispasmodic potentials. However, this antagonism is non-specific and could either be through the receptor site such as the muscarinic and histaminic receptors or through other musculotropic route such as influx or out flux of calcium ions [41, 42]. The root extract exhibited a more potent dose-dependent inhibition of contractions induced by the spasmogens suggesting that it may possess higher antispasmodic and anti-diarrheal potentials than aerial part extract. Thus the antispasmodic activity of these extracts has further highlighted the potential anti-diarrheal activity of the plant.

Most anti-diarrheal therapies act through reduction in propulsive movement and/or antisecretory effects. The anti-diarrheal activity of some medicinal plants have been linked to phytoconstituents such as tannins [43-45], alkaloids [46], saponins [47], phenols [44], flavonoids, steroids and terpenoids [4,48]. Anti-diarrheal effects of flavonoids have also been attributed to inhibition of hydro-electrolytic secretion [49, 50]; inhibition of intestinal motility and contractions induced by spasmogenes [51, 52]. Tannins are documented to form protein tannates which inhibit intestinal secretion while terpenoids have been reported to reduce hypersecretion in isolated rat aorta probably via calcium channel blockade [53]. Phytochemical analyses of RME and MME revealed the presence of phenols, terpenoids, tannins, flavonoids, saponins and terpenoids. The anti-diarrheal effects of the studied herb may therefore be attributed to these bioactive constituents. *Datura*

stramonium, a plant from the Solanaceae family has also been documented to contain some of these phytoconstituents and exhibit similar anticholinergic property (54-56). However, the specific constituent(s) responsible is yet to be identified since our current experimental data is insufficient to directly ascribe the observed anti-diarrheal activity to any of them. The higher relative abundance of phenols and saponins in the root extract may account for the observed higher anti-diarrheal potentials in the underground part than in the aerial part [44, 57].

CONCLUSION

The anti-diarrheal activities of *Schwenkia americana* L. is being reported herein for the first time validating its ethnomedicinal use in the treatment of diarrhea. This potential was found to be higher in the root than the aerial part of the plant which could be linked to disparity in relative abundance of some bioactive constituents. Though the exact mechanism(s) involved are yet to be established, the observed anti-diarrheal activity may have been mediated via antispasmodic and anti-secretory effects. Further research to elucidate the specific mechanism(s) of action and isolate the bioactive constituent(s) responsible for the anti-diarrheal activity is still ongoing.

AUTHORS CONTRIBUTIONS

The work was designed by Nwabunike IA while all the authors contributed equally in performing the experiment and preparation of the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflict of interests

REFERENCES

1. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrheal activity of *Calotropis gigantean* in experimental animals. J Pharmacol Pharm Sci 2004;7:70-5.
2. Heinrich M, Heneka B, Ankli A, Rimple H, Sticher O, Kostiza T. Spasmolytic and anti-diarrheal properties of the yucatec mayan medicinal plant *Casimora tetrameria*. J Pharm Pharmacol 2005;57 Suppl 9:1081-5.
3. Abdullahi IA, Azbo MO, Amos S, Gamaniel KS, Wambebe C. Anti-diarrheal activity of the aqueous extract of *Terminalia avicinnoides* root. Phytother Res 2001;51:431-4.
4. Havagiray R, Ramesh C, Sadhna K. Study of anti-diarrheal activity of *Calotropis gigantean* r. b. r. in experimental animals. J Pharm Pharm Sci 2004;7:70-5.
5. Balogun SO, Tanayen JK, Ajayi AM, Ibrahim A, Ezeonwumelu JOC, Oyewale AA, *et al.* Preliminary evaluation of anti-diarrheal, ulcer-protective and acute toxicity of aqueous ethanolic stem bark extract of *Ficus trichopoda* in experimental rodents. Asian J Med Sci 2011;3 Suppl 1:37-42.
6. Ahmadu AA, Zezi AU, Yaro AH. The anti-diarrheal activity of the leaf extracts of *Daniellia oliveri* Hutch and Dalz (Fabaceae) and *Ficus sycomorus* Miq (Moraceae). Afr J Tradit Complementary Altern Med 2007;4 Suppl 4:524-8.
7. Farthing MJG. Novel targets for the control of secretory diarrhea. Gut 2002;50:15-8.
8. Ezekwesili CN, Obiora KA, Ugwu OP. Evaluation of anti-diarrheal property of crude aqueous extract of *Ocimum gratissium* L. (Labiatae) in rats. Biokemistri 2004;16:122-31.
9. Soberon JR, Sgariglia MA, Sampietro DA, Quiroga EN, Vattuone. Antibacterial activities of plant extracts from northwestern Argentina. J Appl Microbiol 2007;102:1450-61.
10. Iwu MM. Handbook of African medicinal plants. United States: CRC Press; 1993.
11. Schmelzer GH, Gurib-Fakim A. Editors. Plant resources of tropical Africa 11. Medicinal plants 1. Netherlands: PROTA Foundation/Backhuys Publishers/CTA; 2008. p. 498.
12. Kibungu Kembelo OA. Quelquel plantes medecinales du Bas-Congo et leurs usages. London: DFID; 2003. p. 157-60.
13. Mbuta KKA, Mwima K, Bitengeli M, Y'okolo I, Kavuna M, Mamdanga M, *et al.* Plantes medicinales de traditions-province de la Equateur, R. D. Congo. Kinshasha: Insitut de Recherche en Sciences de la Sante; 2012. p. 305. Available from: www.ethnopharmacologia.org>pdf>bi. [Last accessed on 10 Sep 2017].
14. Jimoh AO, Chika A, Umara MT, Adebisi I, Abdullahi N. Analgesic effects and anti-inflammatory properties of the crude methanolic extract of *Schwenckia americana* Linn (Solanaceae). J Ethnopharmacol 2011;137:543-6.
15. Nwabunike IA, Ezike AC, Nwodo NJ, Udegbunam SO, Okoli CO. Bioactivity-guided studies on the anti-inflammatory activity of extract of aerial parts of *Schwenckia americana* L. (Solanaceae). J Med Plant Res 2014;8 Suppl 22:794-801.
16. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; 1988.
17. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol 2002;37:178-82.
18. Nithya TG, Jayanthi J, Ragunathan MG. Antioxidant activity, total phenol, flavonoid, alkaloid, tannin, and saponin contents of leaf extracts of *Salvinia molesta* D. S. Mitchell. Asian J Pharm Clin Res 2016;9 Suppl 1:200-3.
19. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ. Delay of castor-oil induced diarrhea in rats: a new way to evaluate prostaglandin synthesis. J Pharmacol 1978;30:41-5.
20. Lakshminarayana M, Shivkumar H, Rimaben P, Bhargava VK. Anti-diarrheal activity of leaf extract of *Moringa oleifera* in experimentally induced diarrhea in rats. Int J Phytomed 2011;3 Suppl 1:68-74.
21. Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and castor oil-induced diarrhea. Am Soc Pharmacol Exp Ther 1994;268:291-5.
22. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay: a test for diarrhea produced by prostaglandins. Prostaglandins 1976;11:809-28.
23. Di Carlo GD, Mascolo N, Izzo AA, Capasso F. Effects of quercetin on the gastrointestinal tract in rats and mice. Phytother Res 1994;8:42-5.
24. Perry WLM. Pharmacological experiments on isolated preparations. London: Churchill Livingstone Press; 1996.
25. De la Puerta R, Herrera MD. Spasmolytic action of essential oil of *Achillea ageratum* L. in rats. Phytother Res 1995;9:150-2.
26. Galvez J, Duarte J, de Medina S, Jimenez J, Zarzuelo A. Inhibitory effects of quercetin on the guinea pig ileum contractions. Phytother Res 1996;10:66-9.
27. Onyeto CA, Akah PA, Nworu CS, Okoye TC, Okorie NA, Mbaoji FN, *et al.* Anti-plasmodial and antioxidant activities of methanol extract of the fresh leaf of *Lophira lanceolata* (Ochnaceae). Afr J Biotech 2014;13 Suppl 16:1731-8.
28. Amos S, Okwusaba FK, Gamaniel K, Akah PA, Wambebe C. Inhibitory effects of the extracts of *Parefta crassipes* leaves on gastrointestinal and uterine smooth muscle preparations isolated from rabbits, guinea pigs and rats. J Ethnopharmacol 1998;61 Suppl 3:209-13.
29. Agunu A, Yusuf S, Andrew GO, Zezi AU, Abduaham EM. Evaluation of medicinal plants used in diarrhea treatment in Nigeria. J Ethnopharmacol 2005;101:27-30.
30. Ezekwesili JO, Nkemdilim UU, Okeke CU. Mechanism of anti-diarrheal effect of ethanolic extract of *Psidium guajava* leaves. Biokemistri 2010;22 Suppl 2:85-90.
31. Teke GN, Lunga P, Wabo HK, Kuiate JR, Dzoyem JP. *In vivo* evaluation of anti-diarrheal activity of methanol extract, fractions and isolated compounds from *E. abyssinica* stem Bark. Pharmacologia 2012;3:583-90.
32. Hughes S, Higgs NB, Turnberg LA. Anti-diarrheal activity of loperamide: Studies of its influence on ion transport across rabbit ileal mucosa *in vitro*. Gut 1982;23:974-9.
33. Field M. Intestinal ion transport and the pathophysiology of diarrhea. J Clin Invest 2003;111:931-43.
34. Brijesh S, Daswani PG, Tetali P, Rojatkar SR, Antia NH, Birdi TJ. Studies on *Pongamia pinnata* (L.) pierre leaves: understanding the mechanism(s) of action in infectious diarrhea. J Zhejiang Univ Sci B 2006;7:665-4.
35. Niemegeers CJE, Awouters F, Janssen PAJ. The castor oil test in rats: an *in vivo* method to evaluate anti-propulsive and

anti-secretory activity of anti-diarrheals. Drug Dev Res 1984; 4:223-7.

36. Mohd AK, Naeem AK, Iqbal AQ, Ghufran A, Shadab Z. Protective effect of *Arque-Ajeeb* on acute experimental diarrhea in rats. BMC Complementary Altern Med 2004;4:8-8.
37. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhea: Modes of action and effects on intestinal function. Phytother. Res 2006;20:717-4.
38. Mascolo N, Izzo AA, Barbato F, Capasso F. Inhibitors of nitric oxide synthetase prevent castor oil induced diarrhea in the rat. Br J Pharmacol 1993;108:861-4.
39. Akah PA. Anti-diarrheal activity of *Kigelia afriana* in experimental animals. J Herbs, Spices and Med Plants 1996; 4:31-8.
40. Brown JH, Taylor P. Muscarinic receptor agonists and antagonist. In: Hardman JG, Limbird LE, editors. Goodman and Gilman's the pharmacological basis of therapeutics. 9th ed. New York: MacGraw Hill; 1996. p141-60.
41. Offiah VN, Akah PA, Isizoh AO. Spasmolytic activity of *Cissampelous mucromata* leaf extract. Phythother Res 1996;10:322-4.
42. Hassan HS, Haruna AK, Hassan AS, Aguru A, Musa KY. Phytochemical an antispasmodic studies of the fruits of *Solanum gilo*. Nig. J Nat Prod And Med 2006;10:45-6.
43. Murugesh T, Ghosh L, Mukherjee K, Das J, Pal M, Saha BP. Evaluation of anti-diarrheal profile of *Jussiaea suffruticosa* linn extract in rats. Phytother Res 2000;14:381-3.
44. Kamgang R, Fankem GO, Ngo TJ, Gon SH, Fonkoua MC. Antimicrobial and antidiarrheal effects of four Cameroon medicinal plants: *Dichrocephala integrifolia, Dioscorea preusii, Melenis minutiflor,* and *Tricalysia okelensis*. Int J Curr Pharm Res 2015;7 Suppl 1:21-4.
45. Banerjee S, Bandyopadhyay A. *Buchanania lanzanspreng*: a veritable storehouse of phytomedicines. Asian J Pharm Clin Res 2015;8 Suppl 5:18-22.
46. Ghani A. Introduction to pharmacognosy. Vol. 45. Zaria: Ahmadu Bello University Press Ltd; 1990. p. 187-97.
47. Ojewole JA, Awe EO, Nyinawumuntu A. Anti-diarrheal activity of *Hypoxia hemerocallidea* fisch and CA Mey (Hypoxidaceae) Corm ('African potato') aqueous extract in rodents. Phytother Res 2009;23:965-71.
48. Bahekar SE, Kale RS. Anti-diarrheal activity of ethanolic extract of *Manihot esculenta* crantz leaves in wistar rats. J Ayurveda Integrative Med 2015;6 Suppl 1:35-40.
49. Di Carlo G, Autore G, Izzo AA, Maibline P, Mascolo N, Viola P, *et al.* Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure activity relationship. J Pharm Pharmacol 1993;45:1054-9.
50. Rao VSN, Santos FA, Sobreika TT, Souza MF, Melo LL, Silveira ER. Investigation on the gastro-protective and anti-diarrheal properties of ternatin, a tetramenthoxyflavone from *Egletes viscose*. Planta Med 1997;63:146-9.
51. Macauder PJ. Flavonoids affect acetylcholine, prostaglandin E and antigen motivated muscle contraction. Prog Clin Biol Res 1986;213:489-92.
52. Mata R, Rojas A, Acevedo L, Estrada S, ZCalzada F, Rojas I, *et al.* Smooth muscle relaxing flavonoids and terpenoids from *Cinya filaginoids*. Planta Med 1997:63:31-5.
53. Claeson P, Zygmunt P, Högestätt ED. Calcium antagonistic properties of the sesquiterpene T-cadinol: a comparison with nimodipine in the isolated rat aorta. Pharmacol Toxicol 1991;69 Suppl 3:173-7.
54. Friedman M. Analysis of biologically active compounds in potatoes (*Solanum tuberosum)* tomatoes (*Lycopersicon esculentu*), and Jimson weed (*Datura stramonium*) seeds. J Chromatogr A 2004;1054:143-55.
55. Artekin V, Sehmoglu MA, Altinkaynak S. A combination of unusual presentations of *Datura stramonium* intoxication in a child. (Rhabdomylysis and fulminant hepatitius). Emerg Med 2005;28:227-8.
56. Devi MR, Bawari M, Paul SB, Sharma GD. Characterization of the toxic effects induced by *Datura stramonium* L. leaves on mice: a behavioral, biochemical and ultrastructural approach. Asian J Pharm Clin Res 2012;5 Suppl 3:143-6.
57. Abubakar K, Abubakar MR, Ugwah-Oguejiofor JC, Muhammad AA, Usman M, Mshelia HE. Anti-diarrheal activity of the saponin and flavonoid fractions of *Anarcadium occidentale* leaves in albino rats. Adv Med Plant Res 2015;3 Suppl 1:23-8.