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Anti-diarrheal Potential of Ethanolic Crude Extract of *Gouania longipetala* Leaves in Rats

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ABSTRACT: This study explored the anti-diarrheal properties of *Gouania longipetala* crude extract in a rat model, aiming to provide scientific validation for its traditional use as a remedy for diarrhea. The extract was evaluated using different doses (200, 400, 600 and 800 mg/kg body weight) orally for anti-diarrhea activity in albino rats induced for diarrhea with castor oil, charcoal meal transit time leading to enteropooling. The activities of the crude extract at different doses of up to 800 mg/kg body weight were compared with that of the standard drug, loperamide (0.5 mg/kg). Data were analyzed using, statistical package for the Social Sciences, Version 18.0. In the *in vivo* anti-diarrhea studies, the crude extract at all doses used showed significant ($P<0.05$) antidiarrheal activity, evidenced by delay in the onset of diarrhea of up to 56.00 ± 4.36 mins, compared with the control 58.60 ± 3.65. The extract also significantly decreased the distance travelled by the charcoal meal in a dose-dependent manner, with the highest activity of 59.4 ± 2.70^{b} observed for 800 mg/kg of the extract. This favorably compared with that of loperamide with 50.2 ± 1.64^{a} decrease. These results represent 33.17 ± 1.88^{e} % and 33.17 ± 2.41^{f} % inhibitions respectively ($P>0.05$). The enteropooling study showed reduction in the intraluminal fluid accumulation in all groups in a dose-dependent manner, with highest reduction observed in the group treated with 800 mg/kg b.w. of the extract (from 13.30 ± 1.69^{dg} to $5.90 \pm 1.49^{a,b}$ reduction), compared with loperamide (5.18 ± 1.54^{a} reduction). The findings of this study demonstrate that *Gouania longipetala* exhibits significant anti-diarrheal activity ($P<0.05$), validating its traditional use and suggesting its potential as a natural source for the development of novel anti-diarrheal agents.

INTRODUCTION

Diarrhea, is the passage of three or more loose, liquid or unformed stools per day, or more frequently than is normal in an unchecked or uncontrollable frequency for an affected animal or individual. Diarrhea is a major cause of mortality and morbidity among children worldwide [1,2]. It is predominantly a symptom of gastrointestinal infection- a clinical condition referred to as gastroenteritis- and are elicited by a number of bacterial, viral and parasitic organisms. Poor hygiene are implicated in the infection and are widely spread through contaminated food or drinking water, or from person to person [3]. Besides the infection-associated causes of diarrhea, a number of non-infectious causes include; lactose intolerance, irritable bowel syndrome, non-celiac gluten sensitivity [4], celiac disease, inflammatory bowel disease (e.g., ulcerative colitis), hyperthyroidism, bile acid diarrhea [5] and some number of medications such as purgatives [6].

Severe diarrhea leads to dehydration, and may pose a life-threatening situation, especially in young children and the malnourished or immune-compromised persons. According to WHO report (2017), diarrhea is the second leading cause of death in children under five years old, and has on yearly basis accounts for the deaths of approximately 525, 000 children. Diarrhea can persist for several days, and rid the affected individual's body of fluid (water and salts) that are necessary for survival [7]. As such, these fluids and electrolytes (sodium, chloride, potassium and bicarbonate) when lost through liquid stools, vomits, urine and sweat, need to be urgently replaced. Dehydration being one of the most severe threats implicated in diarrhea, occurs when these losses are not immediately replenished. For most people, serious dehydration and fluid loss

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were the main causes of diarrhea- related deaths. However, other factors such as septic bacterial infections are steadily becoming more prevalent, accounting for an increasing proportion of all diarrhea-associated deaths [8]. Children who are malnourished or have impaired immunity as well as individuals with HIV infection are most at risk of life-threatening diarrhea [9].

Diarrheal diseases predominantly affect children in rural settings, where treatment often relies on herbal medicines. The use of herbal medicines over the past few decades has gained acceptance and prominence due to less safety concern, ease of access, and affordability. These qualities offered herbal medicines as a viable alternative to conventional medications that are costly with some adverse effects. This experiment was proposed having in mind the invaluable importance of medicinal plants to global healthcare.

MATERIALS AND METHODS

Plant material collection and authentication

The plant material collection and the extract preparation were carried out as earlier reported [2]. Fresh leaves of *Gouania longipetala* were collected from a bush in Nsukka, Nsukka Local Government Area of Enugu State, Nigeria and were identified at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike in the month of February, 2021. Dried sample of the material was assigned voucher number MOUAU/ZEB/HERB/21/006 and preserved in the herbarium of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The plant was confirmed present on http://www.theplantlist.org

Extract preparation

Cold maceration technique was adopted in the preparation of leaf extract. Briefly, fresh leaves were dried under shade for 14 days, after which they were pulverized to fine powder using a manual blender. For each round of extraction, 200 grams of the powdered sample was macerated in 1.5 liters of ethanol (BDH, UK) as solvent and shaken vigorously intermittently every two hours for a period of 48 hours. The solution was filtered using a Whatman filter paper. The collected filtrate was dried in a laboratory oven at 40 ºC to obtain a brown pasty extract which weighed 8.89 g.

Animals

Laboratory animals were used for the research after approval was obtained from the Animal Ethics Committee on use of laboratory animals of the University. They were housed in standard cages and maintained under standard laboratory conditions in accordance with the "NIH guideline for the care and use of Laboratory animals" (NIH Publication No. 85; rev.1985). The male albino rats weighing 120-160 grams were used for the different segments of the study. The animals were kept in the animal house (Michael Okpara University of Agriculture, Umudike, Nigeria), allowed to acclimatize for two (2) weeks, and were used, following the approval of institutional animal ethical committee. The animals were fed with normal rat feed (Chikun Finisher) and water *ad libitum* but were starved for 12 hours before commencement of each experiment.

In vivo evaluation of the effect of *Gouania longipetala* extract on charcoal meal transit in rats

The method used by Ijioma *et al*.[10] was adopted. Twenty-five (25) adult male albino rats assigned to 5 groups of 5 rats each were used. The rats were fasted for 12 hours prior to commencement of the experiment but were allowed free access to water. Group 1 received no treatment and served as the control; group 2 was administered loperamide (0.5 mg/kg body weight), while groups 3-5 received 200, 400 and 800 mg/kg body weight of the extract respectively, as acute toxicity (LD50) value for the extract was found to be >5000 mg/kg body weight in rats [2]. All treatments were carried out via the oral route. Thirty minutes (30 mins) after treatments, all animals received 1 ml of charcoal meal (10 % charcoal suspended in 10 % gum acacia) orally. In further 30 minutes, the animals were sacrificed by cervical dislocation and the small intestine carefully harvested and its full length measured from the pyloric sphincter to the ileocecal junction. For each animal, the distance travelled by the charcoal meal was also measured and expressed as a percentage of the full length using the equation below:

$$Gastrointestinal\ transit\ (\%) = \frac{Distance\ moved\ by\ charcoal\ meal}{Whole\ length\ of\ small\ intestine} \times 100$$

The inhibitory effect of the extract on gastrointestinal transit was calculated relative to the control as:

$$\%\ inhibition = \frac{Gastrointestinal\ transit\ of\ control - Gastrointestinal\ transit\ of\ test}{Gastrointestinal\ transit\ of\ control} \times 100$$

Effect of *Gouania longipetala* extract on castor oil-induced diarrhea in rats

Principle

In this study, castor oil was used to induce diarrhea. Castor oil has a laxative effect. This effect is mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by the intestinal lipase. The liberated ricinoleic acid causes irritation and inflammation of the intestinal mucosa leading to the release of prostaglandins and nitric oxide which stimulate gastrointestinal secretion, motility, epithelial permeability and oedema of the intestinal mucosa.

Procedure

Twenty-five (25) adult male albino rats assigned to 5 groups of 5 rats each were used. The rats were fasted for 18 hours prior to commencement of the experiment but were allowed free access to water. Group 1 received no treatment and served as the control; group 2 was administered loperamide (0.5 mg/kg body weight) while groups 3, 4 and 5 received 200, 400 and 800

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mg/kg body weight of the extract respectively. All treatments were given via the oral route. Thirty minutes after treatments, animals received 1 ml of castor oil orally and were placed individually in a cage with the bottom lined with weighed absorbent paper and diarrhea episode was observed for a period of 3 hours. The parameters recorded included the onset of diarrheal stool (latent period), the number of both wet and dry stools and weight of the wet stools. All these were measured every 1 hour and the paper changed after each evaluation. The percentage of rats that responded to diarrhea in each group was calculated. The mean number of stools passed by the treated groups was compared with that of the control and the mean number of diarrhea feces pooled by the control group was considered as 100 %.

The percentage inhibition of wet feces and frequency of stool caused by extract was calculated relative to the control using the relation:

$$Inhibition\ of\ defecation\ (\%) = \left[\frac{N_C - N_T}{N_C}\right] \times 100$$

Where: N_C = Number of feces of control group
N_T = Number of feces of treated group.

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

$$Inhibition\ of\ diarrhea\ feces\ (\%) = \left[\frac{N_C - N_T}{N_C}\right] \times 100$$

Where:
N_C = Number of diarrhea feces of control group;
N_T = Number of diarrhea feces of treated group

Effect of crude extract of *Gouania longipetala* on castor oil-induced fluid accumulation

Thirty adult albino rats divided into six groups of 5 rats each were used. All animals were fasted for 18 hours, with access to water. Animals in respective groups received treatments specified for their group. Group 1 received no treatment and served as the normal control, group 2 also received no treatment and was the negative control, groups 3- 5 received 200, 400 and 800 mg/kg body weight of the extract respectively while group 6 received loperamide (0.5 mg/kg body weight). All treatments were carried out via the oral route. Thirty minutes after treatments, animals in group 2 to group 6 received 1 ml of castor oil orally. One hour after castor oil administration the animals were sacrificed and the small intestine removed from pylorus to caecum, after ligating the ends, it was weighed. Intestinal content was milked into a graduated measuring cylinder, the volume was measured and the intestine re-weighed. The difference between full and empty intestine was calculated. Also, blood was collected from the heart of the animals and serum was separated for electrolyte analysis.

Weight of intestinal content (g) = W_1- W_2
Where:
W_1 = Weight of full intestine
W_2 = Weight of empty intestine

The level of reduction (%) in volume and weight of intestinal content was calculated as follows:

$$Reduction\ in\ volume\ (\%) = \left[\frac{V_C - V_T}{V_C}\right] \times 100$$

Where:
V_C = mean Volume of intestinal content of control group
V_T = meanVolume of intestinal content of treated group.

$$Reduction\ in\ weight\ (\%) = \left[\frac{W_C - W_T}{W_C}\right] \times 100$$

Where:
W_C = mean Weight of intestinal content of control group

W_T = mean Weight of intestinal content of treated group.

Statistical analysis
The software package used for data analyses was SPSS Version 20.0 (IBM SPSS Inc, Chicago, IL) and level of significance was calculated by One Way Analysis of Variance.

RESULTS

Measurement of charcoal meal transit time
The effects of the ethanol extract of leaves of *Gouania longipetala* at the selected doses on charcoal meal transit time, by employing the charcoal meal marker diet model, using loperamide as the standard antidiarrheal agent was evaluated. The results are as represented on the **Table 1**.

The measurements, presented on **Table 1**, revealed a dose-dependent inhibition of the charcoal plug, by significantly ($p<0.05$) decreasing the distance travelled by charcoal meal. However, treatment with castor oil enhanced intestinal transit time, and increased the distance traveled by the marker in the control group. The administration of the crude extract at 200 mg/kg b.w significantly ($p<0.05$) lowered the distance traveled by the control group from 87.2 ± 3.11^{d} cm to 73.6 ± 4.04^{c} cm, representing 14.84 ± 1.48^{b} % inhibition. Treatment with 400 mg/kg b.w of the extract also reduced significantly the distance travelled by the charcoal plug of normal control from 87.2 ± 3.11^{d} cm to 72.2 ± 4.71^{c} cm which represents 17.54 ± 1.91^{c} % inhibition. Similarly, at 600 mg/kg and 800 mg/kg b.w treatments, the gastrointestinal distance traveled by the charcoal meal in the experimental animals significantly ($p<0.05$) lessened from the normal control value 87.2 ± 3.11^{d} cm to 63.6 ± 3.58^{b}, 59.4 ± 2.70^{b} cm, respectively. These results represent 26.41 ± 2.19^{d} % and 33.17 ± 1.88^{e} % inhibitions respectively. The ethanol extract of *Gouania longipetala* at 800 mg/kg b.w

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produced the highest percentage inhibition when compared to all other lower doses, and this value is comparable to the value obtained upon treatment with the standard anti-diarrheal agent, loperamide. In addition, under similar experimental conditions, the percentage decrease in charcoal meal intestinal transit time produced by the reference drug, loperamide was 33.17 ± 2.41^{f} % at a dose of 0.5mg/kg; as it caused a significant ($p<0.05$) reduction of the normal control value from 87.2 ± 3.11^{d} cm to 50.2 ± 1.64^{a} cm.

The results obtained with the various doses of the extract were suggestive of either similarity or identity of the active pharmaceutical ingredient(s) with loperamide, the standard or reference drug.

Castor oil-induced diarrhea

In this study, the effects of the different concentrations of the ethanol extract of *Gouania longipetala* in castor oil-induced diarrhea in albino rats using loperamide as positive control were investigated and the findings of this evaluation are as shown in **Table 2** and **Figure 1**.

In the controls, copious diarrhea was evident in 100 % of rats after the first hour following the oral administration of castor oil, as there was zero record of dry stool. Oral administration of loperamide in a dose of 0.5 mg/kg b.w exerted a significant ($p < 0.05$) anti-diarrhea effects against castor oil-induced diarrhea for up to 4 hours post-administration. In the castor oil-induced diarrhea model (**Table 2**), the ethanol extract of *Gouania longipetala* at all the selected doses (200, 400, 600 and 800) mg/kg b.w, significantly ($p<0.05$) delayed the time for onset of diarrhea when compared with control. Time obtained for these doses were 37.00 ± 4.95^{b}, 44.60 ± 4.16^{c}, 49.60 ± 3.72^{c}, and 56.00 ± 4.36^{d} minutes respectively in dose-dependent fashion. In addition, at all the selected doses, the ethanol extract of *Gouania longipetala* at 200 mg/kg b.w produced the least time of onset, while the maximum protracted time of diarrhea onset was expressed by the extract at 800 mg/kg b.w, which yielded comparable effect with the standard drug, loperamide (0.5 mg/kg) body weight (58.60 ± 3.65^{d}) minutes, as the outcome were not statistically significant.

The results of this investigation further revealed that the mean frequency of wet fecal droppings decreased with increase in dose of the ethanolic extract of *Gouania longipetala*, with the mean frequency of defecation being lower in the group that was treated with 800 mg/Kg b.w and higher but with significant drop in rate of wet fecal egestions in the groups that were treated with 600 mg/kg, 400 mg/Kg and 200 mg/Kg b.w, respectively as shown in **Table 2**. The mean frequency of wet fecal droppings for the rats treated with 800 mg/kg b.w was however comparable to the loperamide-treated group which was (0.6 ± 0.21^{a}), as the values showed no statistical difference. However, the negative control group treated with 1.5 ml distilled water had the highest mean frequency of feces (**Table 2**), implying, no inhibition of diarrhea in this group.

The extract exhibited significant anti-diarrhea effects against castor oil-induced diarrhea in the experimental animals. There was a statistically significant reduction ($p<0.05$) in the number of wet defecation by 80.8 % at 800 mg/kg b.w when compared with the negative control rats. Also, there was a significant difference ($p>0.05$) in percentage reduction by (22.9, 50.2, and 66.1) % of wet feces in the 200, 400 and 600 mg/kg b.w for the ethanol extract-treated groups respectively, when compared with the negative control group. The ethanol extract of *Gouania longipetala*, showed dose-related inhibition with respect to the significant reduction in percentage diarrhea episodes as shown in **Figure 1**. The activity of the extract at 800 mg/Kg b.w was comparable to that of loperamide at 0.5 mg/kg b.w treated group which yielded 82.93 % inhibition, since there was no significant difference ($p>0.05$) in the percentage value of fecal droppings between these two groups.

Castor oil-induced enteropooling

The anti-enteropooling efficacy of the various doses of *Gouania longipetala* ethanol extract was assessed in a castor oil-induced rat model, using loperamide as a reference anti-enteropooling agent, to evaluate its potential in reducing intestinal fluid accumulation. The findings of the assays are as shown in **Table 3**.

From the results of the gastrointestinal enteropooling test, the ethanol extract of *Gouania longipetala* leaves at different doses and the reference drug, loperamide (0.5 mg/kg b.w) reduced the volume of intra-luminal fluid accumulation and weight of the intestinal contents significantly ($p<0.05$) in a dose-dependent manner in the animals studied when compared with the normal control group. The oral administration of the crude ethanol extract at different doses (200, 400, and 600 mg/kg b.w) significantly ($p<0.5$) lowered the weights of the intestinal contents as well as the intra-luminal fluids in the experimental rats from 13.30 ± 1.69^{d} g to (11.02 ± 1.20^{c}, 9.68 ± 0.86^{c} and 6.98 ± 0.76^{b}) g, respectively in a dose-related manner. These values respectively represent 16.72 ± 0.55^{b}, 26.89 ± 1.62^{c}, and 47.44 ± 1.74^{d} % inhibitions of volumes and/or weight of intra-luminal contents. The values were statistically significant ($p<0.05$). While the crude extract at 200 mg/kg b.w exhibited the least, though significant ($p<0.5$) percentage inhibition, the maximum and most significant ($p<0.5$) percentage inhibition of the volume and/or weight of intra-luminal contents was observed at 800 mg/kg b.w of the extract by reducing it from 13.30 ± 1.69^{d} g to $5.90\pm1.49^{a,b}$ g, which represents 56.23 ± 2.50^{e} % inhibition. There were, however, statistically significant differences in the volumes of intestinal fluids and weights of intestinal contents of the animal groups when all doses of the ethanol extract of *Gouania longipetala* were compared with the standard antidiarrheal agent, loperamide.

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Table 1. Effect of the ethanol extract of the leaves of *Gouania longipetala* on charcoal meal test intestinal transit time.

Treatment groups	Length of intestin	Distance travelle	% movement	% Inhibition
Control	93.0±2.92^{a}	87.2±3.11^{d}	93.78±2.50^{d}	0.00±0.00^{a}
200 mg/kg GL extract	91.0±1.73^{a}	73.6±4.04^{c}	79.59±2.56^{c}	14.84±1.48^{b}
400 mg/kg GL extract	94.0±2.55^{a}	72.2±4.71^{c}	76.98±6.11^{c}	17.54±1.91^{c}
600 mg/kg GL extract	94.4±1.52^{a}	63.6±3.58^{b}	67.36±3.13^{b}	26.41±2.19^{d}
800 mg/kg GL extract	94.2±3.03^{a}	59.4±2.70^{b}	63.04±1.25^{b}	33.17±1.88^{e}
Loperamide 0.5 mg/kg	93.0±4.53^{a}	50.2±1.64^{a}	54.54±3.48^{a}	33.17±2.41^{f}

Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly ($p<0.05$) different from any paired mean within each column. The table shows significant dose dependent inhibition of intestinal transit following treatment with graded doses of the extract and correlates with its antidiarrhoeal activity.

Table 2. Effect of the ethanol extract of *Gouania longipetala* on castor oil induced diarrhea.

	Time for onset of diarrhea (mins)	Number of wet stool after 1hr	Number of wet stool after 2 hrs	Number of wet stools after 3 hrs	Total number of wet stool after 3 hrs	Number of dry stool after 1 hr	Number of dry stool after 2hrs	Number of dry stool after 3 hrs	Weight of wet stool after 3 hrs
Control	23.80±3.70^{a}	4.6±1.14^{d}	4.6±0.89^{c}	3.8±0.08^{d}	13.0±1.58^{e}	0.6±0.05^{a}	0.0±0.00^{a}	0.0±0.00^{a}	8.4±0.62^{c}
200 mg/kg of G.l Extract	37.00±4.95^{b}	4.2±0.84^{d}	3.8±0.84^{c}	2.0±0.07^{c}	10.0±0.71^{d}	0.2±0.01^{a}	0.6±0.02^{a,b}	0.8±0.03^{b,c}	4.9±0.31^{d}
400 mg/kg of G.l Extract	44.60±4.16^{c}	3.0±0.71^{c}	2.0±0.71^{b}	1.2±0.08^{b,c}	6.2±1.30^{c}	0.8±0.04^{a}	0.4±0.03^{a,b}	0.6±0.03^{b,c}	2.9±0.34^{c}
600 mg/kg of G.l Extract	49.60±3.72^{c}	2.2±0.45^{b,c}	1.4±0.55^{a,b}	0.6±0.05^{a,b}	4.2±0.84^{b}	0.6±0.05^{a}	0.8±0.02^{c}	0.4±0.02^{a,b}	1.6±0.40^{b}
800 mg/kg of G.l Extract	56.00±4.36^{d}	1.8±0.34^{a,b}	1.0±0.71^{a}	0.2±0.04^{a}	3.0±0.71^{a,b}	0.6±0.04^{a}	0.4±0.01^{a,b}	1.0±0.02^{c}	0.8±0.22^{a}
0.5 mg/kg of loperamide	58.60±3.65^{d}	1.2±0.23^{a}	0.8±0.45^{a}	0.2±0.05^{a}	2.2±0.84^{a}	1.0±0.03^{a}	0.0±0.00^{a}	0.8±0.03^{b,c}	0.6±0.21^{a}

Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly ($p<0.05$) different from any paired mean within each column. Results here show significant antidiarrheal effect of the extract at different doses administered. The dose dependent fall in number of wet faeces following treatment with the extract attests to its antidiarrheal effect. The effect of the extract also compared favorably with that of loperamide, the standard agent used.

Table 3. Effects of ethanol extract of leaves of *Gouania longipetala* on castor oil induced enteropooling in experimental albino rats. Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly ($p<0.05$) different from any paired mean within each column. The results contained in the table show dose dependent fall in the weight of intestinal contents following treatment with the extract and are indicative of antidiarrhoeal activity via osmotic mechanism.

Treatments	Weight of full intestine (g)	Weight of empty intestine (g)	Weight of intestine content (g)	Percentage inhibition (%)
Control	22.20±2.28^{a}	8.90±1.05^{b,c}	13.30±1.69^{d}	0.00±0.00^{a}
200 mg/kg of G.l Extract	20.00±1.41^{c}	8.98±0.23^{c}	11.02±1.20^{c}	16.72±0.55^{b}
400 mg/kg of G.l Extract	18.00±0.71^{b}	8.32±0.30^{a,b,c}	9.68±0.86^{c}	26.89±1.62^{c}
600 mg/kg of G.l Extract	14.80±0.84^{a}	7.82±0.48^{a}	6.98±0.76^{b}	47.44±1.74^{d}
800 mg/kg of G.l Extract	13.80±0.87^{a}	8.14±0.40^{a,b}	5.90±1.49^{a,b}	56.23±2.50^{e}
0.5 mg/kg of loperamide	13.60±1.14^{a}	8.42±0.53^{a,b,c}	5.18±1.54^{a}	62.62±2.39^{f}

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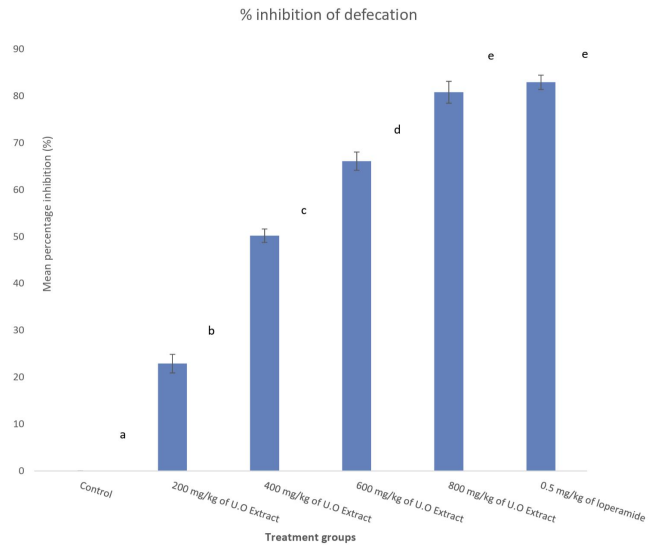

Figure 1. Showing inhibition of stooling frequency in rats following treatment with graded doses of ethanol extract of *Gouania longipetala* and also the standard drug (Loperamide). The data represent the percentage inhibition of wet stool output on groups treated with the extract and standard drug and indicates significant reduction in stooling frequency following treatment with both agents. Bars are present as mean ± standard deviation (n = 5) and bars with different superscripts are significantly ($p<0.05$) different from any paired mean.

DISCUSSION

Diarrhea is evidenced by fecal urgency and incontinence due to an imbalance between intestinal absorption and secretion mechanisms often accompanied by hypermotility, resulting in excessive loss of water and electrolytes in the feces [11]. The study of the effect of ethanol extract of leaves of *Gouania longipetala* on experimental diarrhea induced by castor oil in rats showed a significant reduction in the number, the mass and frequency of diarrhea, an inhibition of the peristaltic index, a decrease in the volume of intestinal contents and the intestinal secretions of the electrolytes. Depending on its etiology, diarrhea may be characterized by increased secretion of electrolytes (secretory diarrhea), increased luminal osmolality (osmotic diarrhea), decreased electrolyte absorption, and/or increased intestinal motility, all of, which are responsible for reduced transit time. Many antidiarrheal agents can therefore act by increasing the transit time by inhibiting gastrointestinal motility, inhibiting intestinal secretions, and/or increasing the intestinal absorption of water and electrolytes. Laxatives such as castor oil induce diarrhea by increased motility and/or gastrointestinal secretions. Castor oil elicits this physiological effect via its active ingredient, ricinoleic acid [12]. Ricinoleic acid triggers the production of nitric oxide and activates adenylate cyclase in epithelial cells, ultimately leading to the generation of prostaglandins that induce diarrhea [12, 13]. Similarly, ricinoleic acid can also stimulates epithelial cells following above referred process [13]. Also, ricinoleic acid modifies the permeability of the intestinal mucosa to electrolytes by inhibiting the intestinal Na^+/K^+-ATPase activity [14], and stimulating the biosynthesis and release of the endogenous prostaglandins responsible for diarrhea [15]. The effects of *Gouania longipetala* would be justified as it could be attributable to the presence of flavonoids, alkaloids and tannins [16]. The anti-diarrhea activity of flavonoids has earlier been ascribed to their ability to inhibit peristaltic activity and hydro-electrolyte secretion, which increase in diarrhea. In addition, flavonoids possess antioxidant properties which have inhibitory effects on several enzymes, including those involved in the arachdonic acid metabolism [17]. Therefore, it is possible that the anti-secretory, anti-inflammatory and antioxidant properties of flavonoids may have contributed and could be significantly responsible for the anti-diarrheal activity of *Gouania longipetala* extract. The findings from this study agree with other studies which showed that the ethanol extracts of many plant parts could reduce stool frequency in a dose-dependent manner [18,19].

In the enteropooling studies, intraluminal fluid accumulation was determined by castor oil-induced enteropooling. Castor-oil and its active ingredient, ricinoleic acid, induced changes in mucosal fluid and electrolyte transport that opiate in hypersecretory response and fluid accumulation in the intestine. Also, liberation of ricinoleic acid from castor oil leads to release of prostaglandins, which stimulate motility and secretion [20]. The result of our study showed that the reference drug loperamide (0.5 mg/kg) and the extract in all the doses used, significantly ($p<0.05$) lowered the volume of intestinal fluid accumulation in experimental animals in a dose-dependent manner with the extract at 800 mg/kg having a better activity than the other tested doses, but less comparable to the effect of the standard test drug, loperamide. Loperamide, which is one of the most efficacious and widely used anti-diarrheal drug; as demonstrated in this study, effectively antagonized diarrhea induced by castor oil. The therapeutic effect of loperamide is believed to be due to its anti-motility and anti-secretory properties. The mechanisms of anti-secretory action of loperamide have been discussed with reference to (1) opiate agonism, 2) blocking of calcium channels, and 3) inhibition of calmodulin [21]. From this study, it is likely that the ethanol extract of *Gouania longipetala* produced the observed effects on the animal model, following similar mechanisms.

Administration of castor oil to experimental animals stimulated small intestinal transit as shown by the over 90 % of the small intestine traveled by the charcoal plug in the normal control experimental rats. Oral administration of the ethanol extract of *Gouania longipetala* caused a dose-dependent and significant ($p<0.5$) reduction in the percentage of the intestinal transit with the extract at the dose of 800 mg/kg b.w being as effective as the reference drug loperamide

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and more efficacious than the lower doses of the extract. This physiological activity exerted by this extract may be due to the ability of the extract to suppress intestinal motility. This reduction in percentage distance traveled could be used to establish the intestinal smooth muscle relaxation. The property of reducing intestinal contractions (and consequently, intestinal transit) is demonstrated by most antidiarrheal drugs [22], and this property was shown by the ethanol extract of the leaves of *Gouania longipetala*, further demonstrating its anti-diarhea properties. In addition, the inhibition of intestinal transit by the extract was similar to that of loperamide (0.5 mg/kg) bw. Loperaimide is widely believed to slow intestinal transit, due to its anticholinergic effect which blocks the muscarinic receptor [23]. This extract, like loperamide, may also be acting as a muscarinic antagonist by preventing the mobilization of Ca^{2+} ions responsible for muscle contraction [24,25], by blocking the muscarinic receptors responsible for the formation of Inositol phosphate-3 (IP-3), thus, eliciting the increase in intracellular calcium [26]. It could also, may have acted by blocking the membrane calcium channels. Overall, the physiological effects, may have been due to the possible inhibition of intestinal peristalsis, increasing the intestinal transit time [27]. The effect of ethanol extract of *Gouania longipetala* on intestinal transit could have resulted from the capacity of the extract's antidiarrheal activity on muscarinic receptor function and/or possibly other mechanisms that would lead to the inhibition of intracellular calcium mobilization such as inhibition of IP-3 and ricinoleic acid-induced prostaglandin in castor oil. Herein, further studies will be required, testing this hypothesis.

CONCLUSION

In conclusion, in this study, the crude ethanol extract of the leaves of *Gouania longipetala* were tested for their anti-diarrheal potentials in castor oil-induced diarrhea, using albino rats as a model.

At all doses (200-800 mg/kg b.w), the extract exhibited significant ($p<0.5$) anti-diarrheal effect but in a dose-dependent manner, and 82.93 % inhibition was observed for the highest dosage of 800 mg/kg b.w and loperamide. The extract delayed the time for the onset of diarrhea, inhibited defecation, significantly inhibited diarrheal episodes, reduced intestinal weights during enteropooling and transit time of charcoal plug, as well as electrolyte losses. The anti-diarrheal effects of *Gouania longipetala* can be attributed to the presence of bioactive compounds, such as flavonoids, alkaloids, and tannins, contributing to its therapeutic value and properties [2].

This study validates the traditional use of *Gouania longipetala* in the treatment of diarrheal and other human related ailments. However, further studies are necessary to fully elucidate the underlying mechanisms of action, including its potential interactions with opioid receptors, calcium channels, calmodulin, and muscarinic receptors, so as to fully harness its therapeutic potential.

DECLARATIONS

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript. The Journal and Publisher will not be responsible for any copyright infringement or plagiarism issues.

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AUTHORS' CONTRIBUTIONS

Nwachukwu, K.C: Conceptualization, Methodology, Experimentations, Original draft preparation, Visualization and investigation and **Okoh M.P**: Conceptualization, Methodology, Supervision, Data Investigation/validation, Writing reviewing and editing.

CONFLICT OF INTEREST

The authors of this manuscript declare no conflict of interest.

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DATA AVAILABILITY

All the key information is available in the manuscript.

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