Anti-diarrhoeal effects of *Garcinia kola* (Heckel-Holl) seed methanolic extract and its fractions in animal models

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Abstract

This study investigated the *In vitro* spasmolytic and *In vivo* anti-diarrhoeal effects of *Garcinia kola* seed extract/fractions. Extraction was done by maceration in 70% methanol, serially partitioned in ethyl acetate and n-hexane. Qualitative phytochemical screening was carried out on the crude extract/fractions. The *In vitro* spasmolytic effect of the extract and fractions at different concentrations (0.5×10^3, 0.2×10^3, 0.1×10^3, 0.6×10^2 mg/ml) were investigated against spontaneous and acetylcholine-induced contractions in isolated rabbit jejunum as well as histamine-induced contractions using isolated guinea pig ileum. The *In vivo* anti-diarrhoeal effect of the extract was evaluated using three diarrhoeal models: castor oil-induced diarrhoea, charcoal meal gastrointestinal transit time and castor oil-induced enteropooling. In each model, 25 mice were randomly divided into five groups of 5 mice each. Group I served as the untreated control, while group II was a positive control. Groups III-V were administered 125, 250 and 500 mg/kg of the crude methanol extract, respectively. The crude extract, ethyl acetate and aqueous fractions at 0.5×10^3 mg/ml respectively exhibited 14.4%, 12.9% and 12.2% spasmolytic activities against acetylcholine-induced rabbit jejunum contractions. Histamine-induced guinea pig ileum contractions were inhibited by crude extract (6.2%), ethyl acetate (6.2%), aqueous fraction (7.2%) at 0.6×10^2 mg/ml. For castor oil-induced diarrhoea, the crude extract at 500 mg/kg produced a significant ($p < 0.05$) decrease in the diarrhoeal index and faecal weight with a percentage inhibition of 70.4% compared with controls. Similarly, the crude extract (500 mg/kg) significantly ($p < 0.05$) decreased the charcoal meal gastrointestinal transit time with a percentage inhibition of 33.9% and elicited significant ($p < 0.05$) intraluminal fluid reduction (9.1%) in castor oil-induced enter pooling test when compared with the untreated group. In conclusion, the anti-motility

and anti-secretory activities of the crude extract were attributed to the phytochemical constituents present.

Introduction

Diarrhoea is the alteration in the overall absorption of water and electrolytes to increase the secretion of fluid in the gastrointestinal (GI) tract (Stefano, 2018). Intestinal infection is a major factor that causes diarrhoea symptoms and death globally (Hodges & Gill, 2010). Although diarrhoea remains a remediable condition, it accounts for 82% of deaths of children under-five years old in Africa and South Asia (CDC, 2012; Gupta, 2012; O'Reilly *et al.*, 2012). The mortality rate in this age group in Africa has decreased by nearly 4% per year but diarrhoeal cases remain unacceptably high (Arif & Naheed, 2012). Diarrhoea is also often seen in small animal practice and ruminants and it is associated with a large number of differential diagnoses (McCann & Simpson, 2006).

Acute diarrhoea is usually self-limiting after a short course (Stefano, 2018), while chronic diarrhoea can be classified as watery, malabsorptive (fatty) and inflammatory (infectious or non-infectious) or the combination (Juckett & Trivedi, 2011; Corinaldesi *et al.*, 2012). The treatment of diarrhoea is aimed symptomatically at replacing lost fluid and electrolytes with simultaneous antibiotic administration (Riddle *et al*., 2017). The use of fluid and electrolytes replacement therapy, intestinal protectants and motility modifiers in diarrhoea are not indicated in diarrhoea of infectious origin. The extensive use of antibiotics in veterinary practice for the treatment of diarrhoea of any cause has led to abuse and eventual resistance to antibiotics in animals (Khan & Scott, 2010). Undesired effects have been observed with the use of synthetic opioid drugs (diphenoxylate and loperamide), such as severe constipation, dependence and reduced gastrointestinal transit, which can lead to increased absorption of bacterial toxins in infectious diarrhoea (Suleiman *et al.*, 2017). The cellular and molecular mechanisms of diarrhoea involve altered movement of ions that can occur either through transporters or the lateral spaces between cells, which are regulated by tight junctions. In this regard, some transporters seem to be tightly coupled with water movement, including sodium-dependent glucose transporter (SGLT1), Na^+/H^+ exchanger isoform and the apical Cl^-/HCO_3^- exchanger (Hodges & Gill, 2010). The classical secretory diarrhoea is due to cAMP-dependent activation of transmembrane conductance regulator (TCR), a Cl^- channel. Alternately, changes in Ca_2^+ levels increase the activity of the calcium activated chloride channel (CACC). In some cases, the increase in Cl^- secretion is paired with a decrease in Na^+ absorption. In addition, the direct reduction of water transport proteins, such as aquaporins, results in less fluid absorption (Hodges & Gill, 2010). Enteric pathogens can either directly modulate epithelial ion transport processes and barrier function or do so indirectly through inflammation, neuropeptides or loss of absorptive surface. For example, pathogens such as the intestinal parasite Giardia cause loss of brush border absorptive surface and diffuse shortening of villi (Connor *et al.*, 2020; McMurry *et al.*, 2021). Similarly, enteropathogenic *Escherichia coli* (EPEC) cause effacement of microvilli, which decreases the surface area for nutrient absorption and causes increased osmolarity of the intestinal contents and malabsorption (McMurry *et al.*, 2021). However, recent evidence suggests that the rapid onset of diarrhoea induced by EPEC could result from direct effects on intestinal epithelial ion transport processes. Several invasive pathogens, including *Shigella* sp., and *Salmonella* sp., cause inflammatory diarrhoea characterised by fever and polymorphonucleocytes (PMNs) in the stool (Brown & Cumming, 2020). PMNs regulate absorption through cytokine secretion but also have a more direct role through the secretion of a precursor to adenosine, a secretagogue that activates TCR. *Clostridium difficile* and rotavirus infection also work indirectly through modulation of ion transport after cytokine secretion and activation of enteric nerves via neuropeptides (Brown & Cumming, 2020; Miyabe *et al.*, 2020).

Garcinia kola-Heckel is an angiosperm belonging to the family *Guttiferae*. It is referred to as bitter kola in English, "Aku ilu" in Igbo language, "Orogbo" in Yoruba language and "Namijin goro" in Hausa language (Adesuyi *et al.*, 2012). The seed of *Garcinia kola* is chewed traditionally as anti-sialagogue. It is used in traditional hospitality, cultural and social ceremonies in the Southern part of Nigeria (Buba *et al.*, 2016). The traditional use of the various parts of the plant is enormous. Extracts of the plant have been traditionally used for ailments such as laryngitis, liver

diseases, diarrhoea and cough. They are also used as an antidote for snake bites, while the stem bark serves as a purgative (Buba *et al.*, 2016). In traditional medicine, the powdered bark is applied and used to treat malignant tumours, the sap is used for curing parasitic skin diseases, and the latex or gum is used against gonorrhoea infection and applied topically on fresh wounds to prevent bacteria contamination (Buba *et al.*, 2016).

The crude methanol extract of the various parts of *Garcinia kola* has been shown to contain bioactive components such as alkaloids, saponins, tannins, flavonoids, glycosides, sterols, phlorotannins, anthraquinones, and phenols (Ezeanya & Daniel, 2013; Nmaju *et al.*, 2014). The most studied constituent of *Garcinia kola* seeds is the kolaviron biflavonoid complex (Maňourová *et al.*, 2019). Other secondary metabolites of the plant recorded are garcinia biflavonoid (GB)-1a-glucoside, GB-1a, GB-1, GB-2, kolaflavonone, benzophenone, xanthone, coumarin, apigenin, quercetin, garcinoic acid, garcinianin (Buba *et al.*, 2016).

Several studies on different *Garcinia kola* extracts have demonstrated its antimicrobial (Uzondu *et al.*, 2014; Idowu *et al.*, 2020), anti-fungal (Dah-Nouvlessounon *et al.*, 2015), hepatoprotective (Ajayi *et al.*, 2018), analgesic and anti-inflammatory (Olaleye *et al.*, 2000), anti-ulcerative and proton pump inhibitors (Onasanwo *et al.*, 2011), antispasmodic (Udia *et al.*, 2009), anti-emetic (Ahmed *et al.*, 2013) and antidiabetic (Smith *et al.*, 2012) effects. Evaluating the antidiarrhoeal activity of the plant in an animal model does not only validate its traditional use but could be a potentially safe and effective treatment regimen for diarrhoea in both animals and humans.

Materials and Methods
Collection of plant material and identification
Garcinia kola seeds weighing 4 kg were harvested and purchased in the month of April 2019 from a local farmer in Ututu, Arochukwu, Abia State. The seeds were identified and authenticated at the Herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. Voucher number 02600 was assigned and kept for future reference.

Preparation of seed samples, extraction and partitioning
The seeds of *Garcinia kola* were prepared as described by Udenze *et al.* (2012). The *Garcinia kola* seeds were washed and air-dried for 48 hours after removing the testa. Each seed was cut into small pellets and dried in an electric oven for 12 hours at $40℃$. The dried seed pellets were grounded to a fine powder using a manual grinder and then sieved. The powder obtained was extracted using 70% methanol. Extraction of 1.3 kg of the pulverised *Garcinia kola* seed was by maceration in 3.9 litres of 70% methanol. The mixture was allowed to stand for 48 hours at room temperature, and the liquid extract was filtered using Cytiva's Whatman™ 1001-085 Grade 1 qualitative filter paper (diameter: 8.5 cm and pore size:11μm). This process was repeated on the marc and resultant extracts were pooled together and concentrated *in vacuo* using a rotary evaporator at a temperature of $40℃$. The crude methanol extract was dissolved with 0.1% of 10% dimethylsulphoxide (DMSO) due to the insoluble nature of the extract in water. The extract in DMSO was further dissolved in distilled water to form an aqueous methanol fraction which was serially partitioned using a separating funnel with n-hexane and ethyl-acetate to obtain n-hexane and ethyl acetate fractions, respectively. The n-hexane and ethyl acetate fractions were concentrated *in vacuo* at $69℃$, respectively, using a rotary evaporator as described by Suleiman *et al.* (2017). The crude extract and fractions were weighed and stored in labelled plastic containers at $4℃$.

Experimental animals
Five adult New Zealand rabbits weighing from 1.5 kg - 2.0 kg and five guinea pigs with weight range 243 – 329 g were sourced from and kept in the Department of Veterinary Pharmacology and Toxicology Animal House, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Ninety albino mice of both sexes weighing 20.3 – 30.9 g were purchased from the Department of Pharmacology and Therapeutics Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. All animals were allowed to acclimatise in cages for two weeks at room temperature, fed with commercial laboratory animal feed (Chikun Feed®: Crude Protein, Fat, Fiber, Calcium, Phosphorous, Metabolized Energy) prior to the commencement of the experiment and water provided *ad libitum*. All experiments were done in accordance with the approved use of animals with the number ABUCAUC/2020/49 assigned by the Ahmadu Bello University Committee on Animal Use and Care.

Phytochemical analysis
The crude methanol extract and fractions of *Garcinia kola* were subjected to phytochemical analysis using standard methods to test for alkaloids, tannins,

flavonoids, saponins, anthraquinones, steroids and cardiac glycosides (Evans, 2009).

Test for alkaloids (Mayer's Test): Two drops of Mayer's reagent were added to 3 ml of each fraction and the crude extract. A cream precipitate indicated the presence of alkaloids.

Test for tannins (Lead acetate Test): Three drops of lead acetate solution were added to 3 ml of each fraction and the crude extract. A coloured precipitate indicated the presence of tannins.

Test for flavonoids (Shinoda Test): For every 3 ml of each fraction and the crude extract, 2 ml of 50% methanol were added. Thereafter, metallic magnesium chips and a few drops of concentrated hydrochloric acid were added carefully down the side of the test tube. The appearance of red, orange or pink colour indicates the presence of flavonoids.

Test for saponins (Frothing Test): Ten millilitres of distilled water were added to 3 ml of each fraction and extracted and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and observed for 30 minutes. A honeycomb froth that persisted for 15 minutes indicated the presence of saponins.

Test for cardiac glycosides (Keller-Killiani Test): Three millilitres of each fraction and the crude extract was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride solution. The ferric chloride solution was transferred into a dry test tube and 1 ml of concentrated sulphuric acid was added carefully down the side of the test tube to form a lower layer at the bottom. The interphase was observed carefully for the colour of the ring. A pale green colour in the upper acetic acid layer indicated the presence of cardiac glycoside.

Test for sterols (Salkowski Test): To 3 ml of the crude extract or each fraction, 3 drops of concentrated sulphuric acid were added at the side of the test tube. Colour changes at the interphase of the extract and sulphuric acid were noted for an hour. The Cherry red colour indicated the presence of sterols.

Test for anthracenes (Bontrager' Test): To 3 ml of the crude extract or each fraction placed in a dry test tube, 5 ml of chloroform was added and shaken for about 5 minutes. It was filtered and the filtrate was shaken with an equal volume of 10% ammonia solution. The bright pink colour observed in the aqueous layer indicated the presence of free anthraquinones.

Drugs and chemical sourcing
Acetylcholine (Sigma-Aldrich Inc., St. Louis, USA), histamine (Sigma-Aldrich Inc., St. Louis, USA), loperamide (Lemotil®-Zahidi Enterprise, Chinch Bunder Mumbai), Dimethyl sulphoxide (10% DMSO), castor oil (Bell, Sons and Co LTD, Southport PR9, 9AL England), carboxymethylcellulose, chloroform, 10% formalin and activated charcoal.

Median lethal dose
The median lethal dose (LD50) was determined using the method described by Lorke (1983). The test was carried out in two phases. All the animals were fasted for 12 hours prior to oral administration of the crude methanol extract of *Garcinia kola*. In phase one, nine apparently healthy mice were randomly divided into three groups of three mice each. Mice in groups one, two and three received crude methanol extract orally at 10 mg/kg, 100 mg/kg and 1000 mg/kg, respectively. The mice were observed over a period of 48 hours for signs of toxicity and mortality. In the second phase, three mice were randomly assigned into three groups of one mouse each. Animals in groups one, two and three were treated with crude methanol extract orally at 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, respectively. Similarly, the animals were observed for 48 hours for any signs of toxicity or mortality. Results obtained were recorded accordingly.

Experimental design
In vitro studies: The *In vitro* studies were conducted using isolated rabbit jejunum and guinea-pig ileum.

Effect of crude extract and fractions of Garcinia kola on isolated rabbit jejunum: Five rabbits were deprived of food but not water for 12 hours before the experiment. Each animal used was sacrificed humanely by cervical dislocation. The jejunum was dissected from the abdominal region, and the mesentery was trimmed off. The entire length was placed in the petri dish and 2 cm length was dissected and placed immediately in a container containing Tyrode's solution (NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $NaHCO_3$ 1.0, $MgCl_2$ 0.1, $NaHPO_4$ 0.05 and glucose 1.0 g/L). The solution was kept at a temperature of 37 ± 1℃. Each tissue was suspended in an organ bath containing Tyrode's solution and aerated with air (Udia *et al.*, 2009).

Different concentrations of acetylcholine 2×10^{-3} mg/ml, 4×10^{-3} mg/ml, 6×10^{-3} mg/ml and 8×10^{-3} mg/ml were tested on the isolated rabbit jejunum. The concentration that produced maximum tissue response was taken as the working concentration against different concentrations of the crude extract and fractions. The contact time for each tested fraction on the tissue was 60 seconds which was followed by washing the tissue three times. Each tissue preparation was allowed to equilibrate for a period of 45 minutes under a resting tension of 1.5 g. Changes in tension produced by the test agent were recorded with an isometric force transducer coupled to Powerlab®. Maximum tissue response of acetylcholine-induced contractions and percentage inhibitions were recorded for various fractions and crude extract. IC50 was obtained by regression using a straight-line equation (y = ax + b), and 50% was substituted on the Y-axis.

Effect of crude extract and fractions of Garcinia kola on isolated guinea pig ileum: Five guinea pigs were deprived of food but not water for 12 hours before the experiment. Each animal used was sacrificed by cervical dislocation. The ileum was dissected from the abdominal region, and the mesentery was trimmed off. The entire length was placed in a petri dish and 2 cm length was dissected and placed immediately in a container containing Tyrode's solution (NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $NaHCO_3$ 1.0, $MgCl_2$ 0.1, $NaHPO_4$ 0.05 and glucose 1.0 g/L). The solution was kept at a temperature of $37 \pm 1°C$. Each tissue was suspended in an organ bath containing Tyrode's solution and aerated with air (Udia *et al.*, 2009).

Histamine concentrations of 8.0×10^{-4} mg/ml, 1.6×10^{-3} mg/ml, 2.4×10^{-3} mg/ml, 3.2×10^{-3} mg/ml were tested on the isolated guinea-pig ileum. The concentration (8.0×10^{-4} mg/ml) which produced maximum tissue response was taken as the working concentration against different concentrations of the crude extract and fractions. The contact time for each tested fraction on the tissue was 60 seconds which was followed by washing of the tissue three times. Each tissue preparation was allowed to equilibrate for a period of 45 minutes under a resting tension of 1.5 g. Changes in tension produced by the test agent were recorded with an isometric force transducer coupled to Powerlab®. Maximum tissue response of histamine-induced contractions and percentage inhibitions were recorded for various fractions and crude extract. IC50 was obtained by regression analysis using a straight-line equation (y = ax + b) and 50% was substituted on the Y-axis.

In vivo studies

Effect of Garcinia kola extract on castor oil induced diarrhoea: Twenty-five mice were randomly selected and divided into five groups of five each. The mice were deprived of food but not water 12 hours prior to the start of the experiment. Mice in group one received distilled water (5 ml/kg) and served as the untreated control group. Animals in group two were treated with loperamide (5 mg/kg) and served as the treated group. Mice in groups three, four and five were administered 125, 250 and 500 mg/kg of the crude methanol extract of *Garcinia kola,* respectively. The doses chosen for the *In vivo* studies were one-tenth (1/10), one-twentieth (1/20) and one-fortieth (1/40) of the median lethal dose (LD50) of the extract at 5000 mg/kg. All treatments were administered orally. One hour after dosage, castor oil (2 ml/kg) was administered orally. The mice were placed individually in a cage over a white, clean filter paper where faeces were collected after induction of diarrhoea (Suleiman *et al.*, 2017). The filter paper was changed intermittently and the net stool weight was recorded over a period of 5 hours. The severity of diarrhoea was graded as follows; Grade 0 = normal pelleted faeces, Grade 1 = discrete soft-formed faeces, Grade 2 = soft-formed faeces, Grade 3 = soft-watery faeces and Grade 4 = watery stool with little solid matter. The diarrhoeal index was calculated by multiplying the number of mice in each grade by the number of grades divided by the number of mice in each group as described by (Vogel & Vogel, 2013).

Effect of Garcinia kola extract on gastrointestinal transit time of charcoal meal: Five groups of five mice each were randomly selected. They were starved of feed but not water 12 hours before the experiment. Animals in group one were given distilled water (5 ml/kg) and served as the untreated group. Animals in group two were the treated control group administered with loperamide (5 mg/kg). Groups three, four and five were administered 125, 250 and 500 mg/kg of the crude methanol *Garcinia kola* extract, respectively. All treatments were administered orally. After treatment, about 0.3 ml of an aqueous suspension of 5% charcoal in 2% carboxymethylcellulose solution were administered orally 60 minutes later. Two hours later, the mice were sacrificed in a chloroform chamber (Suleiman *et al.*, 2017). The intestine from the cardia to anus was carefully dissected out of the cut abdomen. The intestine was immersed immediately in formalin to arrest peristalsis. The distance traversed by the meal was measured in each mouse. The peristaltic index

and percentage inhibition were calculated as described by Vogel & Vogel (2013) and Igboeli *et al.* (2015).

[Peristaltic Index = (Distance travelled by charcoal meal ÷ Total length of small intestine) × 100].

Effect of Garcinia kola extract on castor oil-induced enteropooling: Twenty- five mice were allocated into five groups of five each and were deprived of food and water for 12 hours before the experiment. Mice in group one (untreated control) received distilled water (5 ml/kg), while those in group two (treated control) received loperamide (5 mg/kg). Animals in groups three, four and five received 125, 250 and 500 mg/kg of the crude methanol extract, respectively. All treatments were administered orally one hour before the oral administration of castor oil (2 ml/kg). Following castor oil administration, the mice were sacrificed by cervical dislocation, and the small intestine of each mouse was removed after tying the ends with threads and weighed. The intestinal content of each mouse was collected in a graduated cylinder, and the volume was measured. Thereafter, it was weighed again, and the difference between the initial and final weights of the intestines was recorded. The percentage reduction of intestinal secretion (volume and weight) was calculated relative to the negative control using the formula described by Suleiman *et al.* (2017).

[Percentage inhibition = Control-Test ÷ Control × 100]

Data analysis
Data obtained were expressed as mean ± standard error of the mean (SEM). Diarrhoea score was analysed by Kruskal-Wallis test, followed by Dunn's test to detect the significant difference among groups. Results of the *In vitro* and *In vivo* studies were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. GraphPad Prism version 8.0.2 for Windows (GraphPad software San Diego, California, USA) was used. Values of $p \leq 0.05$ were considered significant.

Result
Extraction and partitioning
On complete extraction, 1.3 kg of pulverised *G. kola* seed yielded 9.62% w/w of black semi-solid non-polar crude methanol extract. The following percentage yields of aqueous (32.9% w/w), ethyl acetate (62.2% w/w) and n-hexane (4.9% w/w) fractions were obtained after partitioning 82 g of the crude methanol extract.

Phytochemical screening
The crude extract, ethyl acetate, aqueous and n-hexane fractions showed the presence of flavonoids, alkaloids, tannins, anthraquinones and carbohydrates. Cardiac glycosides were only present in the crude methanol extract. Saponin was absent in the n-hexane fraction. The extract and fractions showed the absence of steroids.

In vitro studies
Effect of the crude extract and fractions of *Garcinia kola* on isolated rabbit jejunum: The crude methanol extract elicited inhibition of spontaneous contractions in a concentration-dependent manner. However, ethyl acetate fraction significantly ($p < 0.05$) decreased spontaneous contractions at concentrations of 0.1×10^3 - 0.6×10^2 mg/ml while the aqueous fractions decreased spontaneous contractions at concentrations of 0.2×10^3 - 0.1×10^3 mg/ml. (Figure 1). Acetylcholine-induced contractions of the jejunum were inhibited significantly ($p < 0.05$) by crude methanol extract and fractions in a biphasic concentration-dependent manner (Figure 2). The crude extract with an IC50 of 16.8 mg/ml showed a better spasmolytic activity when compared with the ethyl acetate fraction (EAF) (IC50 of 72.3 mg/ml) and aqueous fraction (AF) (IC50 of 41.9 mg/ml) (Figure 3).

Effect of the crude extract and fractions of *Garcinia kola* on isolated guinea pig ileum: The lowest concentration (0.6×10^2 mg/ml) of both crude extract and fractions of *Garcinia kola* in the presence of histamine possessed spasmolytic activity while highest concentration (0.5×10^3 mg/ml) possessed spasmodic activity (Figure 4). The percentage inhibitions of crude extract (IC50 = -25.3 mg/ml), EAF (IC50 = -65.2 mg/ml) and AF (IC50 = -47.9 mg/ml) at 0.6×10^2 mg/ml are 6.2%, 6.4% and 7.2% respectively (Figure 5).

Median lethal dose: Oral administration of the crude methanol *Garcinia kola* extract at a dose between 10 mg/kg and 5000 mg/kg produced no toxic signs or mortality. The median lethal dose was therefore considered as ≥ 5000 mg/kg.

In vivo studies
Effect of *Garcinia kola* extract on castor oil-induced diarrhoea: The crude methanol extract at 500 mg/kg showed significant ($p < 0.05$) cessation of the

diarrhoeal episode in castor oil-induced diarrhoea in mice. Spasmodic action of the crude extract was observed at 125 mg/kg (Figure. 6). The faecal weight recorded showed significant ($p < 0.05$) percentage inhibition (70.36%) of the crude extract at 500 mg/kg when compared with the untreated group (Table 1).

Effect of crude methanol extract on gastrointestinal transit time: The crude methanol extract of *Garcinia kola* at 500 mg/kg reduced significantly ($p < 0.05$) the distance travelled by the charcoal meal from the cardia to the anus (8.46 ± 2.37 cm) when compared with the untreated group (15.30 ± 3.16 cm).
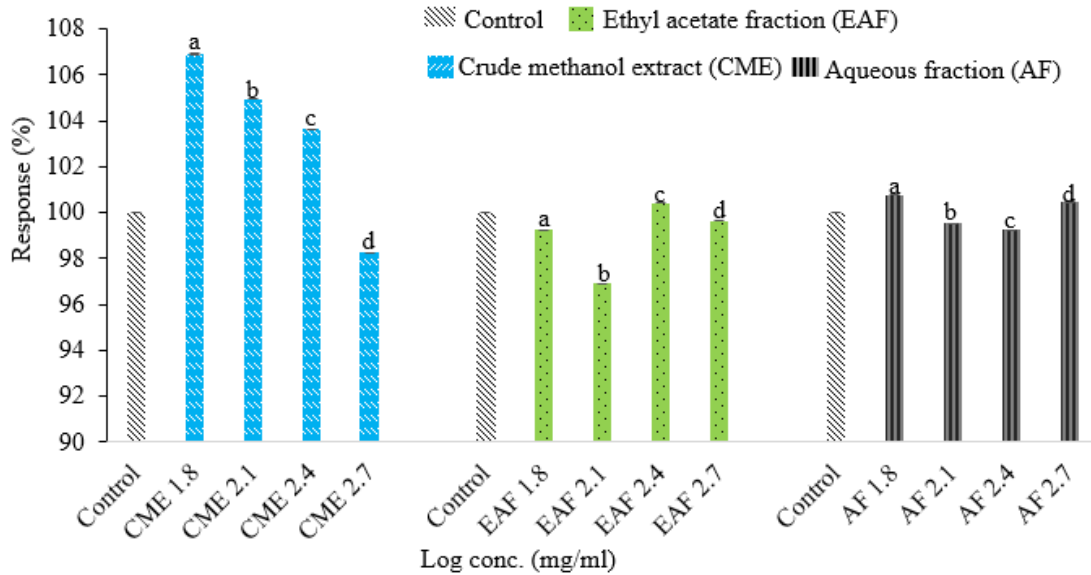

Figure 1. Effect of different concentrations of the crude extract and fractions of *G. kola* on spontaneous contractions of rabbit jejunum. a, b, c, d Means with different letters are significantly different ($p < 0.05$) when compared to control
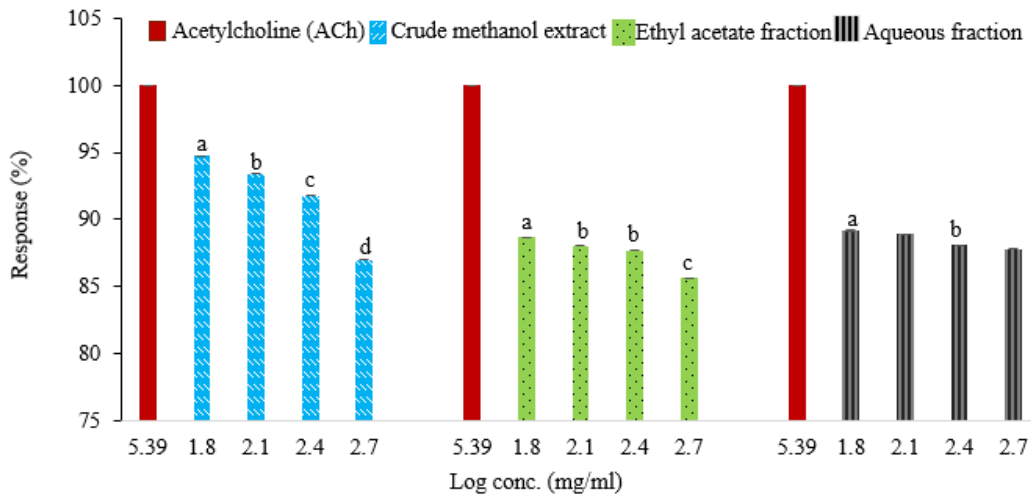

Figure 2. Effect of different concentrations of the crude extract and fractions of *G. kola* on acetylcholine-induced contractions of rabbit jejunum. a, b, c, d, Means with different letters are significantly different ($p < 0.05$) when compared to control (Acetylcholine)
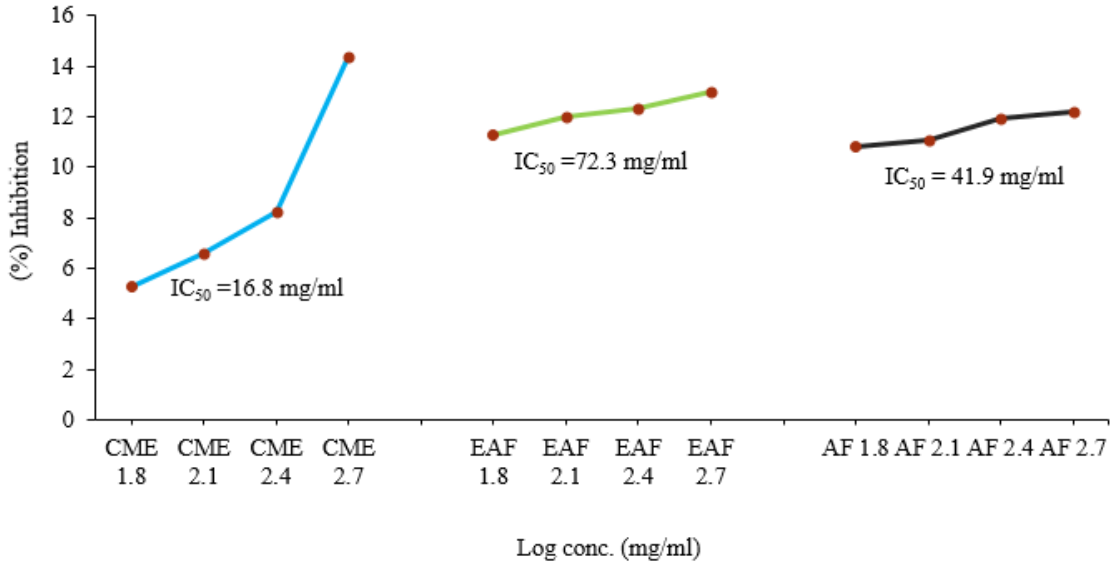
Figure 3. Inhibition curves of the crude extract and fractions of *G. kola* on acetylcholine-induced contractions of rabbit jejunum. Key: CME = Crude methanol extract, AF = Aqueous fraction, EAF = Ethyl acetate fraction
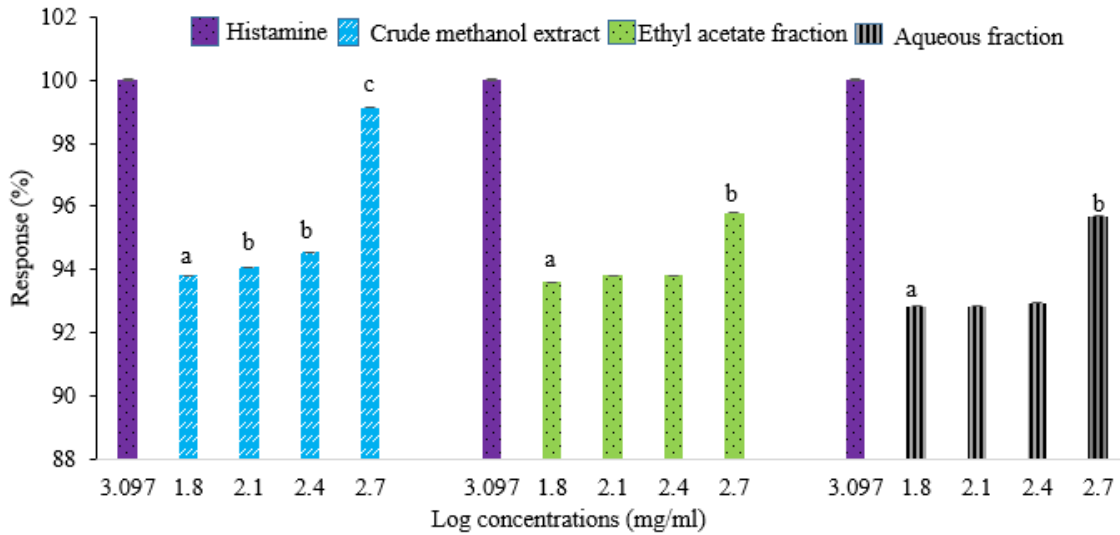

Figure 4. Effect of different concentrations of the crude extract and fractions of *G. kola* on histamine-induced contractions of guinea pig ileum. a, b, c, Means with different letters are significantly different ($p < 0.05$) when compared to control (Histamine)

Table 1: Effect of the crude methanol extract of *G. kola* on castor oil-induced diarrhoea in mice (n=5)

Treatment	Faecal weight within 5 hours (g)	Percentage inhibition (%)
Distilled water (5 ml/kg)	0.13 ± 0.03^a	-
Loperamide (5 mg/kg)	0.06 ± 0.01^b	52.77
Crude methanol extract (125 mg/kg)	0.14 ± 0.02^a	9.04
Crude methanol extract (250 mg/kg)	0.11 ± 0.03^a	18.76
Crude methanol extract (500 mg/kg)	0.03 ± 0.00^c	70.36

[a,b,c] Means with different superscript letters along column are significantly ($p < 0.05$) different

8

There was a significant difference (p < 0.05) in the peristaltic index of the group that received crude methanol extract at 500 mg/kg when compared with the untreated group and other treatment groups (Table 2).

Effect of crude methanol extract on castor oil-induced enteropooling

The extract of *Garcinia kola* at 500 mg/kg significantly inhibited (p < 0.05) the volume of intestinal content (0.26 ± 0.03 ml) when compared with the untreated group and the other treatment groups. However, high percentage inhibition of loperamide at 5 mg/kg (18%) was observed when compared with the untreated group and the groups treated with crude extract at 125, 250 and 500 mg/kg (Table 3).

Discussion

Phytotherapy used as single-herb preparation and also as combination therapy play an important role in the treatment of gastrointestinal disorders (Kelber *et al.*, 2017). The cause of diarrhoea is multifactorial, and this has led to an irrational combination of herbs, anti-infective agents and mucosal protectants. The
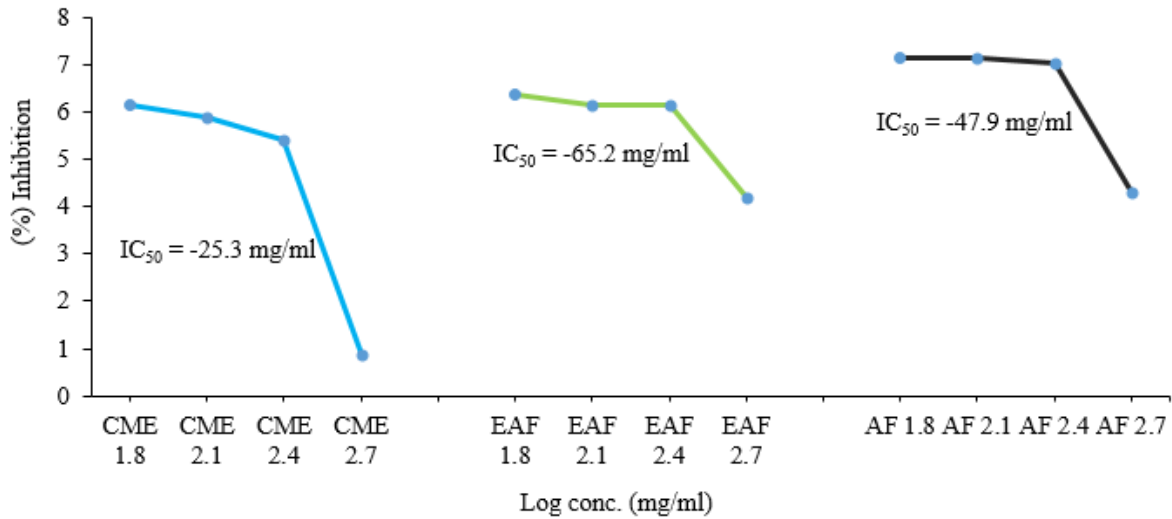

Figure 5. Inhibition curves of the crude extract and fractions of *G. kola* on histamine-induced contractions of Guinea pig ileum. Key: CME = Crude methanol extract, AF = Aqueous fraction, EAF = Ethyl acetate fraction
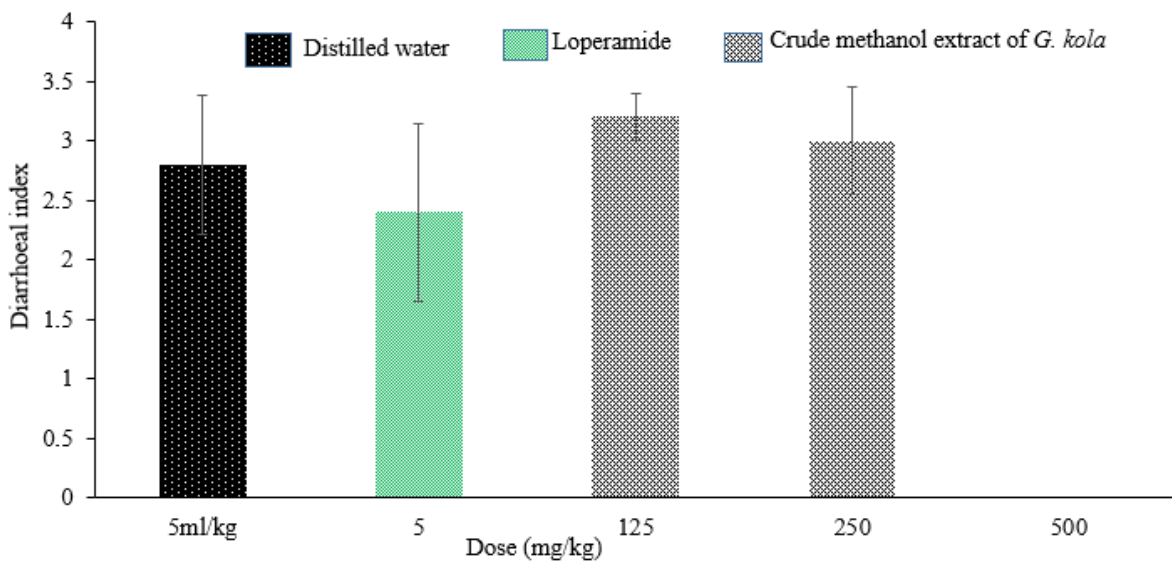

Figure 6. Effect of the crude methanol extract of *G. kola* on castor oil-induced diarrhoea in mice

Table 2: Effect of the crude methanol extract of *G. kola* on gastrointestinal transit time in mice (n=5)

Treatment	Mean intestinal length of small intestine (cm)	Mean charcoal meal distance (cm)	Peristaltic index	Percentage inhibition (%)
Distilled water (5 ml/kg)	35.20 ± 2.33[a]	15.30 ± 3.16[a]	44.56 ± 9.70[a]	-
Loperamide (5 mg/kg)	32.10 ± 2.50[a]	12.80 ± 1.77[b]	40.05 ± 5.28[b]	16.34
Crude methanol extract (125 mg/kg)	37.40 ± 2.23[b]	19.78 ± 1.60[c]	52.72 ± 1.94[c]	-29.28
Crude methanol extract (250 mg/kg)	32.14 ± 1.98[a]	16.04 ± 0.74[a]	50.71 ± 3.80[c]	-4.84
Crude methanol extract (500 mg/kg)	33.30 ± 1.59[a]	8.46 ± 2.37[d]	25.16 ± 6.67[d]	44.71

[a,b,c,d] Means with different superscript letters along column are significantly ($p < 0.05$) different

Table 3: Effect of the crude methanol extract of *G. kola* on castor oil-induced enteropooling in mice (n=5)

Treatment (mg/kg)	Weight of small intestine (g)	Percentage inhibition (%)	Volume intestinal content (ml)	Percentage inhibition (%)
Distilled water (5 ml/kg)	0.808 ± 0.16[a]	-	0.352 ± 0.07[a]	-
Loperamide (5)	0.774 ± 0.11[a]	4.21	0.286 ± 0.07[b]	18.75
Crude methanol extract (125)	0.872 ± 0.20[a]	-7.92	0.37 ± 0.06[a]	-5.11
Crude methanol extract (250)	0.894 ± 0.17[a]	-10.64	0.344 ± 0.06[a]	2.27
Crude methanol extract (500)	0.64 ± 0.05[b]	20.79	0.26 ± 0.03[b]	26.14

[a,b] Means with different superscript letters along column are significantly ($p < 0.05$) different

combination therapy to combat diarrhoea will eventually lead to abuse and resistance (Khan & Scott, 2010). Hence, the aim of the study was to explore an alternate, safe and effective regimen for the treatment of diarrhoea in animals and humans.

Methanol was preferred in this study for extraction because of its low boiling point (64.7℃.) and ease of evaporation at a lower temperature when using rotary vapour. Aqueous methanol tends to penetrate plant tissues and extract better than water and other solvents (Altemimi *et al.*, 2017). It has been found to be efficient in plant extraction and gives a higher yield when compared to ethanol (Do *et al.*, 2014). The phytochemical analysis of the CME of *Garcinia kola* showed that water-soluble metabolites (e.g., alkaloids and carbohydrates) were present in the non-polar fraction (n-HF). The emulsifying property of saponin found in the extract can reduce the surface tension of a non-polar solvent, hence the detection of polar constituents in a non-polar solvent. According to Kregiel *et al.* (2017), the hydrophilic sugar molecules and the hydrophobic aglycone backbone structure of saponins give saponin its soap-like property.

The results obtained from the *In vitro* studies showed the spasmodic and spasmolytic activities of the extracts and fractions of *Garcinia kola* in the presence of different spasmogens. The spasmodic activity observed in this study is due to the presence of anthraquinone found in the extract and fractions. Anthraquinones have been reported to increase peristalsis and are also used as laxatives (Lombardi *et al.*, 2020). The *In vitro* acetylcholine-induced contractions on isolated rabbit jejunum showed that the CME, EAF and AF of *Garcinia kola* seed exhibited biphasic spasmodic and spasmolytic activities in a concentration-dependent manner. Hormesis is a biphasic dose-response phenomenon that is characterised by low dose stimulation and high dose inhibition (Calabrese & Mattson, 2017). The inhibitory effect of the extract and fractions in acetylcholine-induced contractions suggests that *Garcinia kola* seed could act as a muscarinic receptor antagonist or calcium influx inhibitor. A similar observation by Udia *et al.* (2009) was postulated to be as a result of a direct action of *Garcinia kola* seeds extract on intestinal smooth muscle not as a partial agonist or depolarising blockage action.

Spontaneous contractions were inhibited by CME in a concentration-dependent manner, whereas EAF and AF inhibited contractions at varying concentrations. *Garcinia kola* seeds extract, and fractions were observed to have a modulatory effect on the intestinal smooth muscle in the presence or absence

of spasmogens. *Garcinia kola* seeds fractions possessed a non-specific mode of action on gastrointestinal smooth muscle.

Evaluating the effect of CME and fractions on histamine-induced contractions exhibited significant inhibition with low concentration, while increased contractions were observed with high concentration. This observation agrees with previous reports that histamine exerts a varying effect on smooth muscle cells depending on the animal species, and it is also involved in modulating intestinal motility (Kim *et al.*, 2011; Fabisiak *et al.*, 2017). The EAF significantly reduced histamine-induced contraction when compared with the CME and AF, which further demonstrates the non-specific way *Garcinia kola* interacts with intestinal smooth muscle.

The assessment of the lethal dose (LD50) is a major parameter in measuring acute toxicity and also an initial procedure for general screening of chemical and pharmacological agents for toxicity. The use of fewer animals in acute toxicity studies are also important for ethical reasons (Chinedu *et al.*, 2013). The median lethal dose of crude *Garcinia kola* seed extract at ≥ 5000 mg/ml in this study is relatively safe. The doses chosen for the *In vivo* studies were one-tenth (125 mg/kg), one-twentieth (250 mg/kg) and one-fortieth (500 mg/kg) of the median lethal dose (LD50) of the extract at 5000 mg/kg. The decrease in the diarrhoeal index of the crude methanol extract at 500 mg/kg, when compared to loperamide, could be due to flavonoids, saponins and tannins present in the extract; these phytochemical constituents have been reported to possess anti-diarrhoeal properties. Bioflavonoids of *Garcinia kola* significantly reduced intestinal motility (Odukanmi *et al.*, 2018) and act as prostaglandin and autacoid inhibitors (Derebe *et al.*, 2018). Saponins exert an inhibitory effect on histamine release (Mekonnen *et al.*, 2018), hence, the inhibitory effect on intestinal motility.

The decrease in intestinal transit time and reduced intraluminal fluid accumulation by the crude extract of *Garcinia kola* seed (500 mg/kg) are comparable to the opiate anti-diarrhoeal agent; -loperamide. Loperamide acts on the µ (mu)-opiate receptors and possesses both anti-secretory and anti-motility actions on the gastrointestinal tract (Baumer, 2017). The anti-motility and anti-secretory anti-diarrhoeal effects of the crude extract at 500 mg/kg may be ascribed to the presence of tannin. Phenolics and tannins are reported to inhibit release of autacoids and prostaglandins, thereby inhibiting motility and secretion induced by castor oil (Ayele *et al.*, 2021). Tannins are known to increase intestinal mucosa

tone, reduce secretion and intestinal motility (Derebe *et al.*, 2018). Furthermore, it will be reasonable to assume that the extract and fractions of *G. kola* exert their anti-motility and anti-secretory anti-diarrhoeal effects through these aforementioned effects.

In conclusion, the results of both the *In vitro* and *In vivo* experiments revealed that *Garcinia kola* seed methanol extract and fractions possess the potential for anti-diarrhoeal phytotherapy. CME showed significant effects when compared to the fractions, and this could result from fractionation where compounds that will normally produce additive or synergistic effects are separated. The CME, EAF and AF of *Garcinia kola* seed attenuated acetylcholine and histamine-induced contractions on isolated rabbit jejunum and guinea pig ileum. The spontaneous contractions were also inhibited by the extract and fractions of *Garcinia kola* seed. The spasmolytic and anti-diarrhoeal effects were attributed to one or more phytochemicals in the extract and fractions. The mechanism of action in relation to the dose of the extract and fractions is unclear and non-specific. This study was limited by lack of quantitative phytochemical screening which would have helped to quantify the active principles present.

Conflict of interest
The authors declare that there is no conflict of interest.

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