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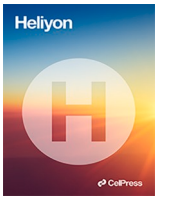

Research article

Antidiarrheal activities of methanolic crude extract and solvent fractions of the root of *Verbascum sinaiticum* Benth. (Scrophulariaceae) in mice

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ABSTRACT

Background: In Ethiopian traditional medicine, *V. sinaiticum* is one of the most often utilized medicinal herbs for the treatment of diarrhea. Therefore, this study was conducted to validate the use of the plant for the treatment of diarrhea in the traditional medical practice of Ethiopia.

Methods: Castor oil-induced diarrhea, enteropooling, and intestinal motility test models in mice were used to evaluate the antidiarrheal properties of the 80% methanol crude extract and the solvent fractions of the root component of *V. sinaiticum*. The effects of the crude extract and the fractions on time for onset, frequency, weight, and water content of diarrheal feces, intestinal fluid accumulation, and intestinal transit of charcoal meal were evaluated and compared with the corresponding results in the negative control.

Results: The crude extract (CE), aqueous fraction (AQF), and ethyl acetate fraction (EAF) at 400 mg/kg ($p < 0.001$) significantly delayed the onset of diarrhea. Besides, the CE and AQF at 200 and 400 mg/kg ($p < 0.001$) of the doses, and EAF at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) significantly decreased the frequency of diarrheal stools. Furthermore, CE, AQF, and EAF at their three serial doses ($p < 0.001$), significantly reduced the weights of the fresh diarrheal stools as compared to the negative control. The CE and AQF at 100 ($p < 0.01$), and 200 and 400 mg/kg ($p < 0.001$) of their doses and EAF at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) significantly decreased the fluid contents of diarrheal stools compared to the negative control. In the enteropooling test, the CE at 100 ($p < 0.05$), and 200 and 400 mg/kg ($p < 0.001$), AQF at 200 ($P < 0.05$) and 400 mg/kg ($p < 0.01$), and EAF at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) significantly decreased the weights of intestinal contents compared to the negative control. Additionally, the CE at 100 and 200 ($p < 0.05$) and 400 mg/kg ($p < 0.001$), AQF at 100 ($p < 0.05$), 200 ($p < 0.01$), and 400 mg/kg ($p < 0.001$) of the doses, and EAF at 400 mg/kg ($p < 0.05$), produced significant reductions in the volumes of intestinal contents. In the intestinal motility test model, the CE, AQF, and EAF at all their serial doses ($p < 0.001$), significantly suppressed the intestinal transit of charcoal meal and peristaltic index compared to the negative control.

Conclusion: Overall, the results of this study showed that the crude extract and the solvent fractions of the root parts of *V. sinaiticum* had considerable *in vivo* antidiarrheal activities. Besides, the crude extract, especially at 400 mg/kg, produced the highest effect followed by the aqueous fraction at the same dose. This might indicate that the bioactive compounds responsible for the

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effects are more of hydrophilic in nature. Moreover, the antidiarrheal index values were increased with the doses of the extract and the fractions, suggesting that the treatments might have dose-dependent antidiarrheal effects. Additionally, the extract was shown to be free of observable acute toxic effects. Thus, this study corroborates the use the root parts of *V. sinaiticum* to treat diarrhea in the traditional settings. Furthermore, the findings of this study are encouraging and may be used as the basis to conduct further studies in the area including chemical characterization and molecular based mechanism of actions of the plant for its confirmed antidiarrheal effects.

1. Introduction

Diarrhea is defined as defecation of three or more loose or liquid stools in a day [1]. It comes about as a result of an imbalance between the bowel's secretory and absorptive processes [2]. Based on WHO criteria, it can be classified into three types: as acute, persistent, and chronic diarrhea [3]. Acute diarrhea is the passage of stool with increased water content, volume, or frequency that lasts less than 2 weeks [4]. Pathogens such as *V. cholerae* or *E. coli*, as well as *rotavirus* are common causes of acute watery diarrhea (2). Persistent diarrhea is defined as diarrhea that lasts at least 14 days and may include blood, and chronic if it lasts more than 4 weeks in duration. Children who are malnourished or who have other illnesses, such as AIDS, are more likely to have chronic diarrhea [5].

Diarrhea is the second leading cause of pediatric mortality following pneumonia, with an estimated 688 million morbidities and 499,000 deaths globally among children under the age of five. Sub-Saharan African and South Asian countries account for 90% of all diarrhea related deaths in the world [6]. A review study also reported that about 1.6 million people died from diarrheal diseases globally in 2017 and one-third of them were children under five years old [7]. Evidence from demographic and health surveys of 34 sub-Saharan countries also reported that there was a significant clustering of diarrheal disease among under-five children across the communities and the overall prevalence of diarrhea in this age group was 15.3% [8]. Furthermore, a meta-analysis study showed that in three east African countries, the prevalence of diarrheal diseases in children of less than five years of old was 27% from 2012 to 2017 and varied from 11% to 54% between different studies [9]. Ethiopia, like sub-Saharan African nations, has a high level of morbidity and mortality due to acute diarrhea. The Ethiopian Demographic and Health Survey (EDHS) conducted in 2016 reported a 12.0% prevalence of diarrhea in the population [10].

Many cases of sudden onset of diarrhea are self-limiting requiring no intervention. In severe cases, however, excessive fluid loss and electrolyte imbalance are the main concerns, especially in infants, children, and elderly patients, necessitating either non-pharmacologic treatments, such as oral rehydration therapy (ORT) and zinc supplements, or pharmacological treatments or both [11]. Agents that suppress secretion and/or motility of the intestine are used in the symptomatic treatment of diarrhea. Of them, opioid drugs and their derivatives are being widely used in the management of diarrhea. Opioid drugs including diphenoxylate, loperamide, and difenoxin are commonly used opioids for this purpose. There are also many other drugs having antimotility or antisecretory effects on the intestine and used in treating diarrhea [12,13]. Antimicrobials are used for the treatment of infectious diarrhea and can reduce its severity and duration [14]. The majority of the enteropathogens causing persistent diarrhea are treatable with antimicrobial drugs [15].

The current drugs used for treatment of diarrhea have many problems, like drug resistance, drug-drug interaction, and adverse effects [16]. Because of this, investigation of alternative medications derived from natural products is mandatory. Approximately 80% of the population in developing countries such as Ethiopia depends on traditional medicines for primary healthcare [17]. In particular, the use of medicinal plants to treat gastrointestinal disorders such as diarrhea and dysentery occupied a major place in the traditional medicine of the Ethiopian community [18]. There are a variety of medicinal plants used for the treatment of diarrheal diseases in Ethiopia. *Verbascum sinaiticum, Cordia africana*, *Rumex nepalensis*, *Zehneria scabra*, *Verbena officinalis*, *Amaranthus caudatus*, *Calpurnia aurea*, and *Coffea arabica* are some of the most commonly used medicinal plants [19].

V. sinaiticum ('kutitina' or 'yeahiya jero' in Amharic) is one of the medicinal plants used to treat diarrheal diseases [20–22]. The root and the leaf parts of the plant are used for the treatment of diarrheal diseases. The leaf part is crushed, homogenized in water and drunk. Similarly, the root is crushed and drunk with water or the juice of the root is taken orally [19]. The herb is also utilized for the treatment of other ailments including hepatitis [23], mental illness, amnesia, tapeworm infestation, syphilis, gonorrhea, relapsing fever, rheumatic pain, elephantiasis, wound, and measles in Ethiopian traditional medicine [24]. In addition, the plant has experimentally verified antibacterial, antitrypanosomal, hepatoprotective, and anti-proliferative activities [25–28].

V. sinaiticum is among the most commonly used medicinal plants to treat diarrhea in the traditional medicine of Ethiopia [19]. However, the traditional claim of the plant for this use is not determined yet using scientific methods. Therefore, this study was conducted to validate the use of the plant to treat diarrhea in the traditional medical practice of Ethiopia. In addition, the findings of this study may initiate the research community in the field of pharmaceutical science to further investigate the chemical constituents of the plant for its antidiarrheal activity and their mechanism of action.

2. Materials and methods

2.1. Chemicals, drugs, and reagents

Distilled water, absolute methanol 99.9% (Hrego Chemical Ethiopia PLC), loperamide hydrochloride (Medochemie Ltd, Cyprus), atropine sulfate (Humanwell Pharmaceutical PLC, Ethiopia), castor oil (Amman Pharmaceutical Industries, Jordan), ethyl acetate (Alpha Chemika, India), activated charcoal (SD Fine Chemicals Limited, India), Tween 80 (Atlas Chemical Industries, India) were chemicals, drugs or reagents used. Additional lab reagents and chemicals were also used in the phytochemical screening test.

2.2. Instruments, apparatuses, and supplies

Digital electrical balance (Abron Exports, India), hot air oven (Medit Medizintechnik Vertriebs-GmbH, Germany), rotary evaporator (Yamato Scientific CO. Ltd., Japan), Whatman filter paper N°1 (Schleicher & Schuell Microscience GmbH, Germany), surgical blade (SteriLance Medical Inc., China), oral gavage, gloves, gauze bandage, absorbent cotton, and syringes with needles were also used in this study.

2.3. Collection of the plant material and authentication

The roots of *V. sinaiticum* were collected from Tara Gedam Monastery Forest which is located in South Gondar Zone, Amhara National Regional State, Ethiopia. In the mean while the plant specimen showing its full feature was collected for identification. Authentication of the plant specimen was done by a botanist at the Department of Biology, College of Natural and Computational Sciences, University of Gondar and the voucher number (SA01) was deposited there for future reference.

2.4. Extraction procedure

The collected roots were thoroughly washed with distilled water to remove dirt, soil, and any other foreign materials. The cleaned roots were chopped into smaller pieces manually and dried under shade at room temperature. The dried material was then grinded to coarse powder using mortar and pestle and extracted by cold maceration using 80% methanol as a solvent. Three flasks were taken and 650 g of the powder was soaked in a liter of 80% methanol in each flask and kept for 72 h at room temperature with ocassional shaking. Then the extract in each flask was filtered by using muslin cloth and Whatman grade N°1 filter paper and the marc was re-extracted and filtered two times in the same fashion by using fresh 80% methanol. The methanol part of the filtrates was evaporated using a rotary evaporator set at 40 °C. The residue was then put in deep freezer at −20 °C and the aqueous portion was removed using a lyophilizer. The dried crude extract from each flask was combined and stored in a closed container and placed in a deep freezer until used for intended experiment.

2.5. Fractionation

Sixty five g of 80% methanol crude extract was taken and successively fractionated using n-hexane, ethyl acetate, and distilled water. First, the crude extract was suspended in 390 ml of distilled water and an equal volume of n-hexane was added. The mixture was then shaken well in a separatory funnel and the n-hexane phase was separated. The aqueous residue was fractionated twice more using the same volume of n-hexane and separated similarly. The n-hexane portions from the three separate fractionation processes were combined. In the same fashion, the aqueous residue was fractionated in three rounds using 390 ml of ethyl acetate in each round and the ethyl acetate portions were combined. The n-hexane and ethyl acetate portions were concentrated by using a rotary evaporator. Then the ethyl acetate concentrate was stored in a tight container. But the n-hexane concentrate was found insignificant (all most null) and excluded from further consideration. The aqueous residue was also lyophilized and the dried aqueous fraction was stored in a tightly closed container. Finally, the dried ethyl acetate and aqueous fractions were placed in a deep freezer set at −20 °C until used.

2.6. Experimental animals

A total of 239 healthy Swiss albino mice of either sex weighing 20–30 g and aged 6–8 weeks were used in the study. The mice were bred under standard conditions. They were housed in plastic cages with softwood shavings as bedding, in the animal house of Department of Pharmacology, University of Gondar with a 12:12 dark-to-light period, at room temperature, and with free access to clean water and pelletized food *ad libitum*. All mice were acclimatized to the working laboratory environment one week prior to the experiment [29].

2.7. Animal grouping and dosing

The animals were randomly assigned to different groups for evaluation of the activities of the 80% methanol crude extract and the solvent fractions on castor oil-induced diarrhea, enteropooling, and charcoal meal transit models.

In the evaluation of the effects of the crude extract, five groups each containing six mice were used for each model and dosed as follows.

Group 1: received 10 ml/kg of 2% Tween 80 (negative control).

Group 2: received 3 mg/kg of loperamide (in castor oil-induced diarrhea and enteropooling models) and 1 mg/kg of atropine (in gastrointestinal motility model) (positive control).

Groups 3, 4, and 5: received 100, 200, and 400 mg/kg of 80% methanol extract.

In each of the three models, eight groups each containing six mice were also used in the evaluation of the activities of the solvent fractions and received the treatments as follows.

Group 1: received 10 ml/kg of 2% Tween 80 (negative control).

Group 2: received 3 mg/kg of loperamide (in castor oil-induced diarrhea and enteropooling models) and 1 mg/kg of atropine (in gastrointestinal motility model) (positive control).

Groups 3, 4, and 5: received 100, 200, and 400 mg/kg of the aqueous fraction, respectively.

Groups 6, 7, and 8: treated with 100, 200, and 400 mg/kg of the ethyl acetate fraction, respectively.

2.8. Acute oral toxicity test

The acute toxicity of 80% methanol extract of *V. sinaiticum* roots was assessed according to OECD criteria for chemical testing [30]. For the experiment, five female Swiss albino mice were chosen at random. First, a limit test dose of 2000 mg/kg body weight of the extract was administered to a single animal, which was then monitored for 24 h. Next, the limit dose was given to each of the remaining four animals because the first animal was still survived after a 24-h follow-up. The mice were closely observed for any signs of toxicity in the first 4 h, and then occasionally for the next 24 h. Thereafter, the mice were kept for up to 14 days with daily follow-up for the occurrence of any signs of morbidity or mortality.

2.9. Antidiarrheal activity determination

2.9.1. Castor oil-induced diarrhea in mice

This was done in accordance with the method employed by Shoba and Thomas [31]. Thirty mice were divided into five groups at random, and each group was prevented access to food for 18 h. Each mouse was then put into a cage with a non-wetting paper sheet floored, which was changed every hour. Following this, each group received the crude extract, either of the fractions, standard drug, or the vehicle as narrated in the grouping and dosing section above. One hour after receiving the treatments, each animal in each group received 0.5 ml of castor oil orally. Following the castor oil delivery, each group's total number and weight of diarrheal drops were determined over the course of a 4-h observation period. The time for the onset of diarrhea in each animal was also determined as the interval between the castor oil delivery and the appearance of the first diarrheal feces. Then, the percentage inhibition of diarrhea from the negative control group was determined using the following formula.

$$\%\ \text{inhibition of diarrhea} = \frac{\text{Mean number of diarrheal stools of (negative control} - \text{treated) group}}{\text{Mean number of diarrheal stools of control group}} \times 100$$

This procedure was used in testing of the effect of the crude extract of the plant and the aqueous and ethyl acetate fractions of the crude extract.

2.9.2. Castor oil-induced enteropooling

The effects of the 80% methanol extract and the solvent fractions on intraluminal fluid buildup were assessed using a method used by Sharma et al. [32]. The mice were starved for 18 h and divided into groups. Then each group was housed in a cage, and given the treatment as described in the grouping and dosing section above. Each animal received 0.5 ml of castor oil an hour after receiving the vehicle (2% Tween 80), the crude extract, the aqueous or ethyl acetate fraction, and was sacrificed an hour later. The abdomen of each animal was then opened and the small intestine was ligated at the pyloric sphincter and the ileocecal junction and dissected out. Immediately after dissection, the intestine was weighed and its contents were collected by milking into a graduated tube and reweighed. The difference in the weights of the intestine before and after milking was noted. Then, using the following formulas, the percentage of decrease in intestinal secretion (in terms of weight and volume) was determined.

$$\%\ \text{reduction in volume of intestinal content} = \frac{\text{MVICC} - \text{MVICT}}{\text{MVICC}} \times 100$$

where, MVICC – mean volume of intestinal content (ml) of the negative control group, MVICT – mean volume of intestinal content (ml) of the treated group,

$$\%\ \text{reduction in weight of intestinal content} = \frac{\text{MWICC} - \text{MWICT}}{\text{MWICC}} \times 100$$

where, MWICC – mean weight of intestinal content (g) in the negative control, MWICT – mean weight of intestinal content (g) in the treated group.

2.9.3. Gastrointestinal motility test

The effects of the crude extract and the solvent fractions on gastrointestinal motility (transit) were examined in mice using the

technique developed by Than et al. [33]. The mice were chosen at random, fasted for 18 h, divided into groups, and subjected to the corresponding treatments as described above in the grouping and dosing section. After an hour of treatment, 0.5 ml of castor oil was administered orally to each mouse. Then, each animal received 1 ml of a 5% activated charcoal suspension in 2% Tween-80 orally, 1 h after castor oil administration. After 30 min of charcoal meal, each mouse was sacrificed and the small intestine was promptly dissected out from the pylorus to the caecum and placed lengthwise on a white paper sheet. Both the overall length of the intestine and the intestinal length traveled by the charcoal meal from the pylorus were measured. Finally, the peristaltic index (PI) and the percentage inhibition of the intestinal transit were calculated as follows for each animal.

$$\text{Peristalysis index (PI)} = \frac{\text{Intestinal length travelled by the charcoal meal}}{\text{Total length of the small intestine}} \times 100$$

$$\%\ \text{inhibition of intestinal transit} = \frac{\text{Mean intestinal length moved by charcoal in (control} - \text{treatment) group}}{\text{Mean intestinal length moved by charcoal in control group}} \times 100$$

2.9.4. *In vivo antidiarrheal index (ADI)*

The *in vivo* anti-diarrheal index (ADI *in vivo*) was then expressed according to the formula developed by Than et al. [33].

$$\text{ADI } \textit{in vivo} = \sqrt[3]{\text{D freq} \times \text{G meq} \times \text{P freq}}$$

where, *D freq* is the delay in diarrheal onset (as % of control), *G meq* is the gut meal travel reduction (as % of control), and *P freq* is the reduction in the number of diarrheal stools (as % of control).

$$\text{D freq} = \frac{\text{Mean time of onset of diarrhea in the (treated} - \text{negtative contrrol) group}}{\text{Mean time of onset of diarrhea in the negative control group}} \times 100$$

2.9.5. *Preliminary phytochemical screening*

The 80% methanol extract and the fractions were all subjected to qualitative phytochemical screening tests according to established testing protocols [34].

2.10. *Ethical clearance*

The proposal of the study was presented to the Animal Ethics Review Committee of the Department of Pharmacology, University of Gondar, and an ethical approval letter was obtained from the Department of Pharmacology on behalf of the committee (Reference No. SoP 4/101/2013). Moreover, the animals were handed according to the guideline for the care and handling of laboratory animals [35].

2.11. *Statistical analysis*

The results were analyzed using SPSS software version 23 and expressed as mean ± standard error of the mean (SEM). The comparisons between group means were made using One-way Analysis of Variance (ANOVA) followed by Tukey HSD Post-hoc test. The differences between the group means were considered statistically significant at p-value < 0.05.

3. Results

3.1. *Percentage yields*

A total of 203 g of dried crude extract was obtained from 1950 g of the coarse powder of the plant material. Accordingly, the percentage yield of the CE was 10.41%. From 65 g of the crude extract fractionated, 38 g (58.46%) and 16 g (24.62%) of AQF and EAF were obtained, respectively. The n-hexane concentrate was extremely small and beyond the sensitivity of the digital balance which was

Table 1
Results of phytochemical screening test of the 80% methanolic extract and the solvent fractions.

Secondary metabolite	Crude extract	AQF	EAF
Anthraquinones	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	−	−	−
Tannins	+	+	+
Terpenoids	+	+	+
Saponins	+	+	−
Steroids	−	−	−
Phenols	+	+	+

AQF: aqueous fraction, EAF: ethyl acetate fraction, +present, −absent.

being used at the time.

3.2. Preliminary phytochemical screening

Preliminary phytochemical screening of the 80% methanol extract of the roots *V. sinaiticum* indicated the presence of anthraquinones, alkaloids, flavonoids, terpenoids, tannins, saponins, and phenols. However, steroids and glycosides were absent in 80% methanol crude extract of the plant. All of the phytochemicals detected in the methanolic extract were also detected in the AQF. Saponins were absent while others were similarly detected in the EAF (Table 1).

3.3. Acute oral toxicity test

Over the course of the 14-day observation period, the limit dose, 2000 mg/kg, of the 80% methanol crude extract of *V. sinaiticum* roots did not result any significant toxicity or mortality. Furthermore, it was demonstrated that neither food nor liquid intake was decreased during the observation period.

3.4. The antidiarrheal activity of 80% methanol extract

3.4.1. Effects on castor oil-induced diarrhea

The crude extract of the plant significantly delayed the onset of diarrhea at 400 mg/kg ($p < 0.001$), relative to the negative control. The frequency of diarrheal drops was significantly decreased ($p < 0.001$) in groups received 200 and 400 mg/kg doses of the crude extract as compared to the negative control. The 100, 200, and 400 mg/kg of the extract treatments reduced diarrhea by 6.00, 41.8, and 50.76%, respectively. The three serial doses of the extract significantly reduced ($p < 0.001$) the weight of diarrheal stool compared to the negative control. Similarly, compared to the negative control, the water content of the fresh diarrheal stool was significantly decreased in groups received 100 ($p < 0.01$), 200, and 400 mg/kg ($p < 0.001$) doses of the extract. The effects of the highest dose of the crude extract were comparable with those of the standard drug in all parameters measured. The standard drug produced significant effects ($p < 0.001$) on the time for onset of diarrhea, frequency of diarrheal feces, and weights and water contents of the fresh diarrheal drops compared to the negative control (Table 2).

3.4.2. Effects on castor oil-induced enteropooling

In the enteropooling assay, the 80% methanol extract of the root of *V. sinaiticum* demonstrated a significant reduction in the weight of intestinal contents at 100 ($p < 0.05$), 200 ($p < 0.001$), and 400 mg/kg ($p < 0.001$) of the doses. The percentage inhibitions in the weights of intestinal contents were found to be, 28.13, 45.31, and 43.75% at 100, 200, and 400 mg/kg doses of the extract in their order and 46.88% by the standard drug (Fig. 1(a) and (b)). The extract also significantly reduced the volume of intestinal contents at 100, 200 ($p < 0.05$), and 400 mg/kg ($p < 0.001$) doses as compared to the negative control. The percentage reductions in the volume of intestinal contents from that of the negative control were 35.37, 36.59, and 59.76%, at 100, 200, and 400 mg/kg doses of the crude extract, respectively. The standard drug produced 60.98% reduction in the volume of intestinal contents from the negative control (Fig. 1(c) and (d)).

3.4.3. Effects on intestinal motility

The crude extract significantly reduced the intestinal transit of charcoal meal at all tested doses ($p < 0.001$) compared to the negative control. Compared to the lowest dose, the middle and the highest doses of the crude extract significantly reduced gastrointestinal transit of charcoal meal ($p < 0.001$) (Fig. 2 (a)). The three serial doses of the extract also showed significant reduction ($p < 0.001$) in peristaltic index compared to the negative control (Fig. 2 (b)). The percentage reductions in gastrointestinal transit were 44.52, 61.81, and 68.10% at 100, 200, and 400 mg/kg doses of the extract, respectively. The highest percentage reduction was produced by atropine, 70.03% (Fig. 2 (c)).

Table 2
Effect of 80% methanol extract of the root of *V. sinaiticum* on castor oil-induced diarrhea in mice.

Group (treatment)	Dose (mg/kg)	Onset of diarrhea (min)	Frequency of diarrheal stool	% inhibition of diarrhea	Weight of diarrheal stool (g)	Water content of diarrheal stool (g)
Group 1 (2% Tween 80)	–	61.50 ± 9.22	11.17 ± 0.60	–	1.72 ± 0.05	0.82 ± 0.06
Group 2 (Loperamide)	3	185.33 ± 19.29^{a3b3c1}	4.67 ± 0.76^{a3b3}	58.19	0.38 ± 0.05^{a3b3}	0.23 ± 0.03^{a3}
Group 3 (CE)	100	72.17 ± 8.43	10.50 ± 0.56	6.00	0.99 ± 0.09^{a3}	0.43 ± 0.08^{a2}
Group 4 (CE)	200	120.50 ± 24.32	6.50 ± 0.43^{a3b3}	41.81	0.60 ± 0.06^{a3b2}	0.31 ± 0.07^{a3}
Group 5 (CE)	400	198.33 ± 8.12^{a3b3c1}	5.50 ± 0.62^{a3b3}	50.76	0.44 ± 0.05^{a3b3}	0.30 ± 0.05^{a3}

Results are expressed as mean ± SEM (n = 6). ^{a}compared to Group 1(negative control), ^{b}compared to Group 3, ^{c}compared to Group 4, $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, CE: crude extract, Group 1: mice received 10 ml/kg of 2% Tween 80 in water and designated as negative control.

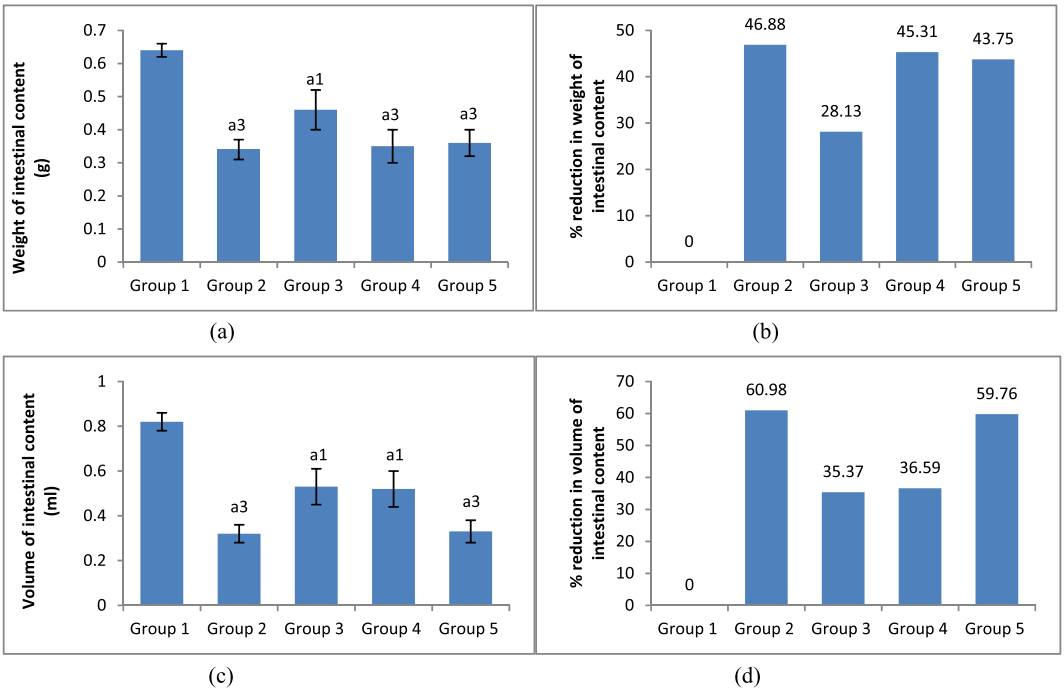

Fig. 1. Effect of 80% methanol extract of the root of *V. sinaiticum* on castor oil-induced enteropooling in mice. (a) effect on weight of intestinal content, (b) % reduction in weight of intestinal content, (c) effect on volume of intestinal content, (d) % reduction in volume of intestinal content. Results are expressed as mean ± SEM (n = 6). [a]compared to Group 1(negative control), [1]$p < 0.05$, [3]$p < 0.001$. Group 1: mice received 10 ml/kg of 2% Tween 80 in distilled water (negative control), Group 2: mice treated with 3 mg/kg of loperamide (positive control), Group 3, 4, and 5: mice received 100, 200, and 400 mg/kg of the crude extract, respectively.

3.4.4. *In vivo antidiarrheal index (ADI)*

The *in vivo* antidiarrheal indices of the crude extract were 16.67, 63.27, and 91.62 at the doses of 100, 200, and 400 mg/kg, respectively (Table 3).

3.5. *Antidiarrheal activities of the solvent fractions*

3.5.1. *Effects on castor oil-induced diarrhea*

The AQF and EAF significantly delayed ($p < 0.001$) the onset of diarrhea at 400 mg/kg. Significant reductions in the frequency of diarrheal feces were produced by the AQF at 200 and 400 mg/kg ($p < 0.001$) and EAF at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) doses compared to the negative control. The percentage inhibitions of diarrhea were 8.57, 35.73, and 45.78% in groups treated with 100, 200, and 400 mg/kg of AQF and 8.57, 28.62, and 32.90% in groups received 100, 200, and 400 mg/kg of EAF, respectively. The highest percentage inhibition of diarrhea, 64.27%, was produced by loperamide. Furthermore, both the AQF and EAF at all of their serial doses ($p < 0.001$) showed significant reduction in the weight of fresh diarrheal stools compared to the negative control. Significant reductions on the fluid content of diarrheal stool were also produced by AQF at 100 ($p < 0.01$), 200, and 400 mg/kg ($p < 0.001$) and EAF at 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) compared to the negative control. The highest doses of AQF and EAF showed comparable effects to the standard drug in all parameters measured in this model. The standard drug significantly ($p < 0.001$) delay the time of diarrhea onset and decreased the number, weight, and fluid content of diarrheal feces as compared to the negative control (Table 4).

3.5.2. *Effects on castor oil-induced enteropooling*

The AQF significantly reduced the weight of the intestinal contents at 200 ($P < 0.05$) and 400 mg/kg ($p < 0.01$) and the volume of the intestinal content at 100 ($p < 0.05$), 200 ($p < 0.01$), and 400 mg/kg ($p < 0.001$) of the doses, respectively, compared to the negative control. The percent reductions in the weight of intestinal contents were 26.98, 36.51, and 42.86% at 100, 200, and 400 mg/kg doses of this fraction, respectively. Similarly, this fraction produced 30.77, 42.31, and 50.00% reductions in the volume of intestinal contents at 100, 200, and 400 mg/kg of the doses, respectively. Significant reductions in the weight of the intestinal contents were also produced by 200 ($p < 0.01$) and 400 mg/kg ($p < 0.001$) doses of the EAF, while only 400 mg/kg of the fraction significantly decreased ($p < 0.05$) the volume of intestinal contents compared to the negative control. This fraction produced of 25.40, 36.51, and 42.86% reductions in the weight of intestinal contents at 100, 200, and 400 mg/kg of its doses, respectively. The fraction also produced 29.49, 39.74, and 48.72% reductions in the volume of intestinal contents at 100, 200, and 400 mg/kg of the doses, respectively. The standard

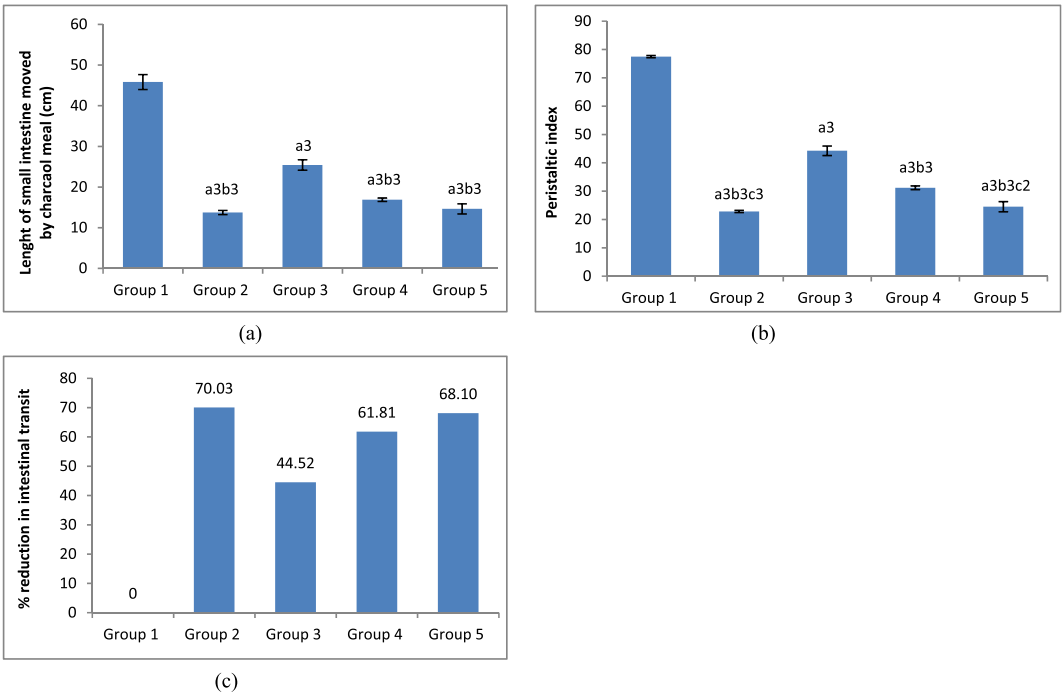

Fig. 2. Effect of 80% methanol extract of the root of *V. sinaiticum* on gastrointestinal transit in mice. (a) effect on length of small intestine moved by charcoal meal (intestinal transit), (b) effect on peristaltic index, (c) % reduction in intestinal transit. Results are expressed as mean ± SEM (n = 6). [a]compared to Group 1(negative control), [b]compared to Group 3, [c]compared to Group 4, [2]$p < 0.01$, [3]$p < 0.001$. Group 1: mice received 10 ml/kg of 2% Tween 80 in water (negative control), Group 2: mice treated with 1 mg/kg of atropine (positive control), Group 3, 4, and 5: mice received 100, 200, and 400 mg/kg of the crude extract, respectively.

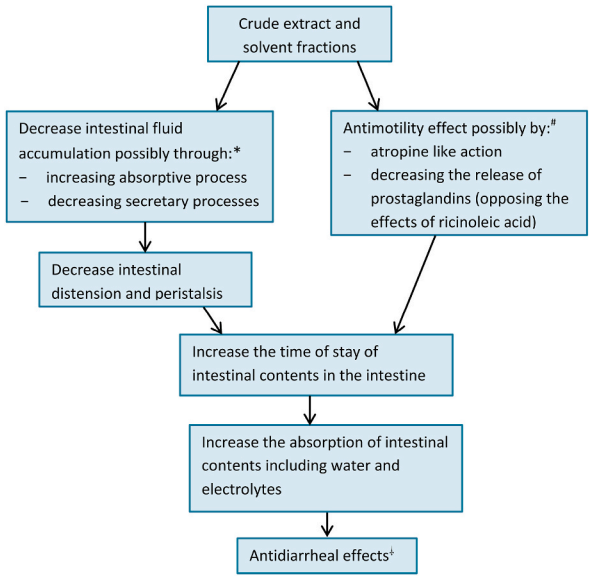

Fig. 3. Diagrammatic representations of proposed antidiarrheal effects of the crude extract and the solvent fractions. *evidence from effects on castor oil-induced enteropooling, ♯evidence from effects on gastrointestinal motility, †evidence from effects on castor oil-induced diarrhea.

drug, loperamide 3 mg/kg, showed a significant reduction in the weight ($p < 0.01$) and volume ($p < 0.01$) of intestinal fluid accumulation relative to the negative control. It decreased the weight and volume of intestinal fluid by 44.44 and 70.03%, respectively, relative to the negative control. There was no statistically significant difference between the effects of all doses of AQF and EAF. Similarly, there was no statistically significant difference between the effects of the standard drug and the solvent fractions on

Table 3
In vivo antidiarrheal index of 80% methanol extract of the root of *V. sinaiticum*.

Group (treatment)	Dose (mg/kg)	Delay in onset of diarrhea (D freq)	Reduction in intestinal length traveled by charcoal meal (G meq)	Reduction in diarrheal stools (P freq)	Antidiarrheal index (ADI)
Group 1 (negative control)	–	–	–	–	–
Group 2 (positive control)	–	201.35	70.03	58.19	93.62
Group 3 (CE)	100	17.35	44.52	6.00	16.67
Group 4 (CE)	200	95.93	63.16	41.81	63.27
Group 5 (CE)	400	222.49	68.09	50.76	91.62

Negative control: a group of mice that received 10 ml/kg of 2% Tween 80. Positive control: a group of mice received loperamide (3 mg/kg) in castor oil-induced diarrhea model and atropine (1 mg/kg) in gastrointestinal motility test model.

Table 4
Effects of the solvent fractions of the crude extract of the root of *V. sinaiticum* on castor oil-induced diarrhea in mice.

Group (treatment)	Dose (mg/kg)	Onset of diarrhea (min)	Frequency of diarrheal stool	% inhibition of diarrhea	Weight of diarrheal stool (g)	Fluid content of diarrheal stool
Group 1 (2% Tween 80)	–	61.67 ± 4.92	11.67 ± 0.49	–	1.70 ± 0.03	0.82 ± 0.06
Group 2 (loperamide)	3	181.67 ± 19.15^{a3}	4.17 ± 0.75^{a3}	64.27	0.36 ± 0.04^{a3}	0.21 ± 0.04^{a3}
Group 3 (AQF)	100	73.67 ± 4.58^{b3}	10.67 ± 0.76^{b3d2}	8.57	1.05 ± 0.09^{a3b3}	0.50 ± 0.09^{a2b1}
Group 4 (AQF)	200	112.17 ± 23.31^{b1}	7.50 ± 0.43^{a3b2c2}	35.73	0.65 ± 0.05^{a3b2c3}	0.40 ± 0.02^{a3}
Group 5 (AQF)	400	172.33 ± 7.25^{a3c3}	6.33 ± 0.56^{a3c3}	45.78	0.52 ± 0.05^{a3b3}	0.40 ± 0.07^{a3}
Group 6 (EAF)	100	65.50 ± 5.47^{b3}	10.67 ± 0.61^{b3}	8.57	1.08 ± 0.06^{a3b3}	0.65 ± 0.05^{b3}
Group 7 (EAF)	200	107.17 ± 23.31^{b2}	8.33 ± 0.50^{a2b3}	28.62	0.70 ± 0.05^{a3b3e3}	0.45 ± 0.09^{a2}
Group 8 (EAF)	400	168.50 ± 8.25^{a3e3f1}	7.83 ± 0.54^{a3b2e1}	32.90	0.54 ± 0.06^{a3e3}	0.36 ± 0.09^{a3e1}

Results are expressed as mean ± SEM (n = 6). [a]compared to Group 1(negative control), [b]compared to Group 2 (positive control), [c]compared to Group 3 (100 mg/kg AQF), [d]compared to Group 4 (200 mg/kg AQF), [e]compared to Group 6 (100 mg/kg EAF), [f]compared to Group 7 (200 mg/kg EAF), $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, AQF: aqueous fraction, EAF: ethyl acetate fraction.

intestinal fluid accumulation (Table 5).

3.5.3. Effects on intestinal motility

All the serial test doses of both of the solvent fractions of the root of *V. sinaiticum* significantly decreased ($p < 0.001$) the intestinal transit of charcoal and peristaltic index compared to the negative control. The 200 and 400 mg/kg doses of both fractions showed significant reductions ($p < 0.001$) in the intestinal transit of charcoal meal and peristaltic index compared to 100 mg/kg. The 100, 200, and 400 mg/kg doses of the AQF produced 40.05, 49.65, and 58.32% reductions in the gastrointestinal transit of the charcoal meal, respectively. The EAF inhibited intestinal transit of charcoal meal by 30.76, 49.69, and 59.11% at the doses of 100, 200, and 400 mg/kg, respectively. The standard drug, atropine 1 mg/kg, significantly reduced ($p < 0.001$) intestinal transit and peristaltic index compared to the negative control. The effect of the standard drug against gastrointestinal transit and peristaltic index was also significantly greater than those of the three serial doses of each fraction. The highest percent reduction in the gastrointestinal transit was produced by atropine, which was 69.59% (Table 6).

Table 5
Effects of the solvent fractions of the crude extract of the root of *V. sinaiticum* on castor oil-induced enteropooling in mice.

Group (treatment)	Dose (mg/kg)	Weight of intestinal content (g)	% reduction in weight of intestinal content	Volume of intestinal content (ml)	% reduction in volume of intestinal content
Group 1 (2% Tween 80)	–	0.63 ± 0.06	–	0.78 ± 0.04	–
Group 2 (loperamide)	3	0.35 ± 0.02^{a2}	44.44	0.32 ± 0.04^{a3}	73.02
Group 3 (AQF)	100	0.46 ± 0.02	26.98	0.54 ± 0.07^{a1}	30.77
Group 4 (AQF)	200	0.40 ± 0.08^{a1}	36.51	0.45 ± 0.09^{a2}	42.31
Group 5 (AQF)	400	0.36 ± 0.05^{a2}	42.86	0.39 ± 0.03^{a3}	50.00
Group 6 (EAF)	100	0.47 ± 0.05	25.40	0.55 ± 0.09	29.49
Group 7 (EAF)	200	0.40 ± 0.03^{a2}	36.51	0.47 ± 0.14	39.74
Group 8 (EAF)	400	0.36 ± 0.02^{a3}	42.86	0.40 ± 0.04^{a1}	48.72

Results are expressed as mean ± SEM (n = 6). [a]compared to Group 1(negative control), [b]compared to Group 2 (positive control, $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, AQF: aqueous fraction, EAF: ethyl acetate fraction.

Table 6
Effect of the solvent fractions of the crude extract of the root of *V. sinaiticum* on gastrointestinal transit in mice.

Group (treatment)	Dose (mg/kg)	Length of small intestine (cm)	Length moved by the charcoal meal (cm)	Peristaltic index	% inhibition in intestinal transit
Group 1 (2% Tween 80)	–	60.62 ± 1.69	45.32 ± 1.84	74.62 ± 1.06	–
Group 2 (Atropine)	1	60.05 ± 1.77	13.78 ± 0.80^{a3}	22.91 ± 0.98^{a3}	69.59
Group 3 (AQF)	100	58.40 ± 1.60	27.17 ± 0.80^{a3b3}	46.53 ± 0.48^{a3b3}	40.05
Group 4 (AQF)	200	55.85 ± 1.51	22.82 ± 0.33^{a3b3c1}	40.95 ± 0.79^{a3b3c3}	49.65
Group 5 (AQF)	400	59.37 ± 0.49	18.89 ± 0.33^{a3b2c3}	31.82 ± 0.61^{a3b3c3d3}	58.32
Group 6 (EAF)	100	61.07 ± 0.79	31.38 ± 0.81^{a3b3}	51.37 ± 0.95^{a3b3}	30.76
Group 7 (EAF)	200	56.13 ± 0.70	22.80 ± 0.94^{a3b3e3}	40.61 ± 1.58^{a3b3e3}	49.69
Group 8 (EAF)	400	58.35 ± 0.85	18.53 ± 0.42^{a3b1e3}	31.77 ± 0.71^{a3b3e3f3}	59.11

Results are expressed as mean ± SEM (n = 6). [a]compared to Group 1(negative control), [b]compared to Group 2 (positive control), [c]compared to Group 3 (100 mg/kg AQF), [d]compared to Group 4 (200 mg/kg AQF), [e]compared to Group 6 (100 mg/kg EAF), [f]compared to Group 7 (200 mg/kg EAF), $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$, AQF: aqueous fraction, EAF: ethyl acetate fraction.

3.5.4. *In vivo* antidiarrheal index (ADI)

The *in vivo* antidiarrheal indices of AQF were 18.83, 52.57, and 78.25, while that of EAF were 11.79, 47.17, and 69.58 at the doses of 100, 200, and 400 mg/kg, respectively. The highest antidiarrheal index, 95.47%, was shown by atropine (Table 7).

4. Discussion

According to published ethnobotanical study reports from Ethiopia [20–22], either the roots or leaves of *V. sinaiticum* are used to alleviate diarrheal diseases in the traditional medical practice. However, no prior investigation has been done to verify this assertion. Therefore, the reports were used as the foundation for the current experimental work. The antidiarrheal effects of various plants have been scientifically validated through analyzing their effects on different animal models. In light of this, castor oil-induced diarrhea, enteropooling, and gastrointestinal motility models in mice were used in this work to assess the antidiarrheal effects of the crude extract of the plant and the solvent fractions. The number and characteristics of the fecal outputs, time for the onset of diarrhea, intestinal transit ratio, and intestinal fluid accumulation are the commonly measured parameters in assessing the antidiarrheal effects of medicinal plants [36–38]. The models employed and the parameters considered in this investigation are consistent with those used in previously conducted similar studies.

Due to their expanded polarity index, hydroalcoholic solvent combinations are often thought to provide good extraction yields. In general, hydroalcoholic co-solvents like 80% methanol appear to have the best solubility properties for initial extraction [39]. Therefore, 80% methanol was preferred to extract the plant material. In addition, the 80% methanolic crude extract was fractionated with solvents of different polarities to get insight about the polarity of the phytochemical components of the plant.

As shown in Tables 1, at least at the highest dose, the plant extract significantly delayed the onset of diarrhea and reduced the frequency, weight, and water contents of diarrheal drops in castor oil-treated mice in the 4-h observation period compared with the negative controls. Additionally, it was noted that an increase in the dose of the plant extract was accompanied by an increase in the percent reduction in the diarrheal outputs. Besides, the effect of the maximum dose of the crude extract was comparable to the effects of the standard treatment in all parameters examined. The significant delay in the onset of diarrhea caused by the highest dose of the extract, combined with an increasing pattern of percent inhibition in diarrheal episodes, suggests that the plant extract inhibits diarrhea more effectively at relatively higher doses. The percentage inhibition of diarrhea produced by the crude extract is comparable with the effects of the hydromethanolic root extract of *Idigofera spicata* [40] and greater than that of *Tetrastigma leucostaphylum* leaves

Table 7
In vivo antidiarrheal index of the solvent fractions of the root of *V. sinaiticum* in mice.

Group (treatment)	Dose (mg)	Delay in onset of diarrhea (D freq)	Reduction in length traveled by charcoal meal (G meq)	Reduction in diarrheal stools (P freq)	Antidiarrheal Index (ADI)
Group 1 (negative control)	–	–	–	–	–
Group 2 (positive control)	–	194.58	69.59	64.27	95.47
Group 3 (AQF)	100	19.46	40.05	8.57	18.83
Group 4 (AQF)	200	81.89	49.65	35.73	52.57
Group 5 (AQF)	400	179.44	58.32	45.78	78.25
Group 6 (EAF)	100	6.21	30.76	8.57	11.79
Group 7 (EAF)	200	73.78	49.69	28.62	47.17
Group 8 (EAF)	400	173.23	59.11	32.90	69.58

Negative control: a group of mice that received 10 ml/kg of 2% Tween 80. Positive control: group of mice received loperamide (3 mg/kg) in castor oil-induced diarrhea model and atropine (1 mg/kg) in gastrointestinal motility test model.

[41]. However, the inhibitory effects of the extract is less than the extracts of other medicinal plants including *Myrtus communis* [37], *Justicia schimperiana* [38], and *Ophiorrhiza rugosa* [42].

The results in castor oil-induced enteropooling model revealed that the 80% methanolic extract *V. sinaiticum* significantly reduced the weight and volume of intestinal contents at all the three serial doses in comparison to the vehicle. The percentage reductions in the weight and volume of intestinal contents were remarkably increased with the dose of the extract. The results demonstrated that the effect of the plant extract on percentage inhibition of castor oil-induced enteropooling is increased the the doses. Moreover, the results in this model revealed that the effect of the highest dose of the extract on intestinal fluid accumulation was found to be closer to and comparable with the inhibitory effect of loperamide. The findings in this model may indicate that the extract has a significant anti-secretory effect and this contributes to its antidiarrheal effect noted in castor oil-induced diarrhea model.

The reduction of gastrointestinal motility is one of the mechanisms by which antidiarrheal agents can act [43]. The crude extract was found to decrease intestinal motility as shown by significant reduction ($p < 0.001$) in the intestinal transit of charcoal meal and peristaltic index compared to the negative control. Furthermore, the results in this model showed that the antimotility effect of the highest dose of the extract is comparable to that of the standard drug, atropine. This can be viewed, for example, in terms of the percentage reductions in gastrointestinal transit by 400 mg/kg of the extract and atropine, which were 68.10 and 70.03%, respectively. A decrease in the intestinal motility increases the stay of intestinal contents in the intestine and this might significantly increase the time for the absorption of water and electrolytes from the small intestine. This may in turn be attributed to the observed effects of the extract in castor oil-induced diarrhea and enteropooling models.

Regarding the effect of the solvent fractions against castor oil-induced diarrhea, the AQF and EAF significantly delayed ($p < 0.001$) the onset of diarrhea at 400 mg/kg. Additionally, the middle ($p < 0.01$) and the highest doses ($p < 0.001$) of the fractions significantly reduced the frequency of diarrheal feces compared to the negative control. The antidiarrheal effects of AQF and EAF were further shown by the progressive percentage inhibitions of diarrhea with increasing doses. Furthermore, the fractions were shown to produce significant reductions in the weights of diarrheal stools as compared to the negative control. Besides, they also significantly decreased the fluid contents of diarrheal stools at all the serial doses compared to the negative control. Overall, the results in this model indicated that the AQF and EAF had significant activities against castor oil-induced diarrhea.

The effects of the solvent fractions against castor oil-induced enteropooling were also assessed, and the results (Table 5) revealed that the fractions produced remarkable effects against intestinal fluid accumulation. Both fractions produced increasing reductions in the weight and volume of intestinal contents with increasing doses. Their effects were further elaborated by the progressive increments in the percentage reductions of the weight and volume of intestinal contents in the treated groups. The effects of AQF and EAF determined in this model could be attributed to their antidiarrheal effects demonstrated in the castor oil-induced diarrhea model.

In the testing of the effects on intestinal transit of charcoal, all the serial doses of the fractions significantly decreased ($p < 0.001$) the intestinal transit of charcoal and peristaltic index compared to the negative control. The fractions were found to produce remarkable and consistent effects with increasing doses, as shown by a corresponding reduction in the mean intestinal length traveled by the charcoal meal and peristaltic index and an increasing percentage inhibition in intestinal transit. Therefore, the results in this model are in support of the effects of the fractions against castor oil-induced diarrhea.

The ADI value often provides a more reliable measurement of the effectiveness of extracts in treating diarrhea [44]. The ADI values increased with the doses of the crude extract and the fractions, suggesting that the crude extract and the solvent fractions caused dose-dependent antidiarrheal effects. Additionally, the crude extract exhibited the highest ADI value across all the test treatments at the corresponding doses, showing that it might have higher antidiarrheal activity than the solvent fractions. Regarding viewing the antidiarrheal effect in consideration of the ADI value, the result of this study is not consistent with a result reported by Ayalew et al. [45] which shows that the highest antidiarrheal activity might be produced by the chloroform fraction, whereas the highest activity was produced by the methanolic crude extract followed by the aqueous fraction in this study. The results are also differed from those of a study on the antidiarrheal effects of various solvent extracts of *Tetrastigma leucostaphylum* which reported that the percentage inhibition of diarrhea and antidiarrheal index values produced by the various organic solvents are generally greater than resulted by the methanolic extract [41].

The pathophysiologic mechanisms that cause diarrhea include altered intestinal motility that results in a shorter intestinal transit time, increased luminal osmolality and electrolyte release, and decreased electrolyte absorption [46,47]. Castor oil has been commonly employed to induce diarrhea in antidiarrheal activity studies because it releases ricinoleic acid, a metabolite that causes diarrhea, upon metabolism in the gut [48]. Ricinoleic acid causes diarrhea by irritating the GI mucosa and promoting the release of prostaglandin, which in turn accelerates gut motility and electrolyte secretion and lowers electrolyte absorption from the small intestine and colon [49]. In light of this, the effects of the crude extract and the solvent fractions against diarrhea may be the result of actions that counteract the activities of this metabolite to cause the pathophysiologic changes leading to diarrhea. It has been demonstrated that both the crude extract and the fractions significantly reduced the accumulation of fluid in the intestine. This suggests that they may promote water and electrolyte absorption and/or decrease the secretion. This in turn may lessen the overload and distension of the intestine. As a result, the intestinal motility may be decreased and this gives more time for the absorption of its contents. Hence, relatively lower water contents and frequency of diarrheal stools and longer onset of time for diarrhea episodes in groups received treatments may be due to underlying activities of the treatments to decrease secretion and/or promote the absorption of intestinal contents. This notion is compatible with the literature-presented mechanism of action of loperamide for its antidiarrheal effect [50]. Reduced motility and secretion of the intestine may be also through atropine activities [51].

The phytochemicals identified in the screening test may be principally responsible for the antidiarrheal effects of the extract and its solvent fractions. Anthraquinones, alkaloids, flavonoids, terpenoids, tannins, saponins (not in EAF), and phenols were identified in the extract and the fractions. The contents are similar to those of other plants having scientifically verified antidiarrheal activities, with

some variations, including *Zehneria scabra* [36], *Justicia schimperiana* [38], *Indigofera spicata* [40], and *Ophiorrhiza rugosa* [42]. Literature reports revealed that alkaloids and terpenoids (especially the monoterpenoid group) have antispasmodic activity [52], saponins suppress ilium contraction [53], flavonoids inhibit intestinal contractions and possess antispasmodic activity [52,54], tannins have an antispasmodic and muscle relaxant effect, and phenols reduce intestinal secretion and transit and have an astringent action [55]. All of these activities could be the undelaying mechanisms for the antidiarrheal effects of the test treatments of the study. These chemical components may therefore be responsible for the antidiarrheal properties of the plant extract and the solvent fractions. Regarding the phytochemical test results, there is some discrepancy with a previous report on the phytochemical contents of the methanolic root extract of the plant [56]. This difference may be attributed to seasonal variations in the collection of the plant material and/or differences in geographical area from which the plant material was collected.

Furthermore, the CE of the plant was determined to be safe because no considerable signs of toxicity were noted from the acute toxicity test. This shows that the plant may not have observable toxicity in short-term use, even at doses higher than those utilized in the three antidiarrheal models of this investigation. This supports that the plant is most probably safe in short-course usage in traditional settings as well.

This study did not include quantitative phytochemical determination and chemical characterization works. In addition, the study did not determine the possible chronic toxicities of the medicinal plant in the long run. These issues are considered the limitations of this study, and we recommend additional investigation on the plant to address these issues.

5. Conclusion

Overall, the results of this study showed that the crude extract and the solvent fractions of the root parts of *V. sinaiticum* had considerable *in vivo* antidiarrheal activities. The crude extract, especially at 400 mg/kg, produced the highest effect followed by the aqueous fraction at the same dose. This might indicate that the bioactive compounds responsible for the effects are more of hydrophilic in nature. Moreover, the antidiarrheal index values were increased with the doses of the extract and the fractions, suggesting that the treatments might have dose-dependent antidiarrheal effects. Additionally, the extract was shown to be free of observable acute toxic effects. Thus, this study corroborates the use of the root parts *V. sinaiticum* to treat diarrhea in the traditional settings. Furthermore, the findings of this study are encouraging and may be used as the basis to conduct further studies in the area including chemical characterization and molecular-based mechanism of actions of the plant for its confirmed antidiarrheal effects.

Author contributions

All authors took part in the title suggestion, proposal development, and report writing processes. SAW carried out the lab works, data organization, and analysis. ABA prepared the manuscript. Finally the manuscript was reviewed by SAT, and some changes were made in response to his feedback. Furthermore, all authors are responsible for the accuracy and originality of this work.

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Data availability

The data of this article can be obtained upon request.

Additional information

No additional information is available pertaining to this article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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