

Evaluation of Anti-Diarrheal Activity of 80% Methanol Extracts of *Vernonia amygdalina* Delile (Asteraceae) Leaves in Mice

This article was published in the following Dove Press journal:
Journal of Experimental Pharmacology

Biruk Mosisa Gudeta¹
Getu Melesie Taye¹
Teferra Abula²
Diriba Alemayehu Gadisa¹

¹Department of Pharmacy, College of Medicine and Health Sciences, Ambo University, Ambo, Ethiopia; ²Department of Pharmacology, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia

Introduction: Diarrhea is a cause of morbidity and mortality, especially in children. Patients with diarrhea have been suffering from limited treatment options due to poor drug tolerance, side effect, and multi-drug resistance to almost all the current drug treatments. Therefore, it is important to search for a new therapeutic medicine that can effectively prevent the disease and safe. *Vernonia amygdalina* is an indigenous medicinal plant used traditionally for the treatment of diarrhea in Ethiopia. The aim of this study was therefore to confirm the antidiarrheal activities of 80% methanol leaves extract using mice models.

Materials and Methods: The antidiarrheal activities of the 80% methanol leaves extract were investigated using castor oil-induced diarrhea, intestinal motility, and enteropooling models in mice. The negative control received distilled water orally, the test groups received three dose levels (100, 200, and 400 mg/kg) of the plant materials, and the positive control is given 3 mg/kg Loperamide orally.

Results: In the castor oil-induced diarrheal model, the extract delayed onset of diarrhea and reduced fecal parameters at all tested doses significantly as compared with the negative control. Results from the charcoal meal test revealed that the extract produced a significant anti-motility effect at all tested doses as compared with the negative control. In the enteropooling test, the extract produced a significant decline in the volume and weight of intestinal contents. The observed antidiarrheal activity could be associated with the phytochemicals present in this plant extract. It was also observed that the extracts have shown no acute toxicity at a dose of 2 g/kg.

Conclusion: This study provides the antidiarrheal activity of the crude extract in all three models. Hence, the findings provide scientific support for the traditional use of *Vernonia amygdalina* leaves as treatment of diarrhea.

Keywords: antidiarrheal activity, castor oil-induced diarrhea, mice, *Vernonia amygdalina*

Introduction

Diarrhea is a common gastrointestinal disorder characterized by an increase in the frequency, volume, and fluidity of bowel movement or a decrease in the form of stool (greater looseness of stool) for at least three times a day.¹ Diarrhea is the second cause of death next to respiratory infections among under-five years of age children throughout the world.² Diarrhea alone as a gastrointestinal disease causes 5–8 million deaths annually³ and kills 2195 children every 24 hours more than malaria, measles, and AIDS combined.⁴ Africa and South East Asia regions account for 78% of all diarrheal deaths occurring among under-five children in the developing world.⁵ Furthermore, in

Correspondence: Biruk Mosisa Gudeta;
Getu Melesie Taye
Department of Pharmacy, College of
Medicine and Health Sciences, Ambo
University, P.O. Box: 19, Ambo, Ethiopia
Email mail2bruke@gmail.com;
getumelese17@gmail.com

Ethiopia, diarrhea kills half a million under-five children annually next to pneumonia.⁶ Even though the availability of many drugs for treating diarrhea, the majority of them suffer from adverse effects like intestinal obstruction, the induction of bronchospasm, vomiting, constipation, and unwanted central effects by long-term use of opioids.⁷ Additionally, there is a growing warning of drug resistance and superinfection causing a global warning to the currently available anti-infective agents including antidiarrheal.⁸ Although globally herbal medicines account for 80%⁹ of the healthcare needs of the majority of developing countries including Ethiopia due to accessibility, affordability, and strong cultural belief,¹⁰ traditional medications are passed orally through generations, and the effective doses suggested by the traditional practitioner are not fully known nor is the effectiveness and safety profile also not described.¹¹ Due to the above-mentioned features, WHO inspire studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices.¹² A range of medicinal plants such as the bark extract of *Albizia gummifera*, leaf extract of *Calpurnia aurea*, seed extract of *Coffea Arabica*, root extract of *Ensete ventricosum*, leaves of *V. amygdalina* and *Caylusea abyssinica* have been widely used for the management of diarrhea by traditional healers in Ethiopia.¹³ *V. amygdalina* is native, to Congo, Eritrea, Ethiopia, Kenya, Nigeria, Sudan, Tanzania, Uganda, Yemen is used for the treatment of several human health problems. Leaf decoctions and bark infusions are used to treat fever, cough, malaria, diarrhea, hepatitis, sexually transmitted diseases and as a fertility inducer. Furthermore, leaves are pounded and mixed with warm water for bathing to treat spots on the skin and nausea.^{14,15} In Nigeria, alcoholic extracts of *V. amygdalina* were evaluated stem bark was effective against diarrhea in rat model.¹⁴ In Ethiopia, traditionally, the study revealed that orally administered *V. amygdalina* leaves powder mixed with water has been used for diarrhea treatment in Wonago Woreda and Halaba people, Southern Nations, Nationalities and people of Ethiopia and in Nekemte town, in East Wellega orally.^{16–18} Hence, the aim of this study was therefore to further confirm the antidiarrheal activities of 80% methanol extract of the leaves of *Vernonia amygdalina* using mice models of diarrhea.

Materials and Methods

Drugs and chemicals used in this study include Loperamide hydrochloride (Remedica, Cyprus), castor oil (Amman Pharmaceutical, Jordan), distilled water, activated charcoal (SD Fine Chem Ltd, India), methanol (Carlo Erba reagents,

France), chloroform, Tween-80 (Blulux, India), acetic acid (Sigma Aldrich, Germany), sulfuric acid and hydrochloric acid (Nice laboratory Reagent, India), Dragendorff's reagents (Fisher Scientific, UK), Mayer's reagent and ethyl acetate (May and Baker LTD Dagenham, England), ammonia (Lobe chemicals, India), and iron chloride (Supertek chemicals, India).

Plant Materials Collections and Preparation

Fresh leaves of *V. amygdalina* were collected in March, 2018 from Ambo Woreda, Oromia region, located about 125 km west of the capital Addis Ababa, Ethiopia. Then, the plant was identified by a botanist and a voucher specimen (BM-001) was given and deposited at the National Herbarium of College of Natural and Computational Sciences, Addis Ababa University for the future reference. Dust materials were removed from the plant materials by washing with distilled water dried at room temperature under shade for 2 weeks and then pulverized to a coarse powder using mortar and pestle to facilitate the extraction process. Finally, the coarse powder is stored in an airtight container until extraction begins.

Experimental Animals and Protocol

Healthy Swiss albino mice of both sexes weighing 20–30 g and aged 6–8 weeks were used for the experiment. The animals were obtained from the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University, and Ethiopian Public Health Institute. They were kept in an appropriate environment and on a 12-hour light-dark cycle with free access to pellet food and water up to the time of experimentation. The animals were acclimatized to laboratory conditions for 5 days before the actual experiments. The study was carried out according to the National Research Council Guide for the Care. All experimental animal protocols were in agreement with the standards set for the care and use of experimental animals by the National Research Council Guide for the Care of experiments on Animals¹⁹ and approved by the Department of Pharmacology Research and Ethics Review Committee.

Preparation of Crude Extract Plant Material

Eighty percent (80%) methanol leaves extract of the plant materials was prepared by cold method (maceration). Six hundred gram powders of *V. amygdalina* leaves were

macerated with 80% methanol (1:5 (w/v)) for 72 hours in a conical flask with occasional agitation using a mini orbital shaker at room temperature. The first extract was filtered by folded gauze and nylon clothing. Then, the extract was filtered over Whatman filter paper No.1 using a pressurized suction filtration system and the marc was remacerated twice using extra fresh methanol to fully extract the plant material in such a way to increase the yield. Then, filtrates from each extraction were combined and methanol was removed from the extract by evaporation under vacuum using Rotavapor. Then, the filtrates were frozen using a deep freezer and set water-free by using a lyophilizer. The crude extract obtained was stored in bottles at the refrigerator and fresh stock solution was prepared just before the experiment with 2% tween –80 for oral administration and distilled water.

Acute Oral Toxicity Test

The acute oral toxicity test was carried out according to the Organization for Economic Cooperation and Development (OECD) guidelines.²⁰ Five female mice have fasted overnight, the weight of each mouse was recorded just before use and it was then orally ingested with a single dose of 2000 mg/kg of 80% methanol extract. Then, the mice were kept separately in cages and strictly observed for the first 4 hours for any signs of change in behavioral characters such as restlessness, hyper-excitability, intermittent clonic convulsion, head tilt, ataxia, prostrations, and then once daily during the following 14 days.

Animal Grouping and Dosing

The experimental animals were randomly assigned to five groups, each consisting of six mice. All groups were administered orally their respective treatments by oral gavage. The animals of group I were considered as the negative control and orally administered distilled water 10 mL/kg under all experimental conditions. Group II to IV (test groups) was given extracts of *V. amygdalina* leaves orally at doses of 100, 200, and 400 mg/kg doses, respectively, during all the three antidiarrheal model tests. Group V was assigned as positive control and treated with the standard drug, Loperamide (3 mg/kg) orally. Loperamide served as a standard drug for all models. Dose selection was made based on the acute oral toxicity test as well as a pilot study and then the OECD15 guideline was followed and under all conditions, the solutions were prepared just before the experiments on the day of the experiments.

Determination of Antidiarrheal Activity Castor Oil-Induced Diarrhea Model

The method and formula described in another study was followed for this study.²¹ Thirty Swiss albino mice of either sex were denied food for 18 hours with free access to water and treated as described previously. One hour after administration, all mice received 0.5 mL of castor oil by oral gavage and then they were individually placed on the floor, which was covered with dry toner plain paper copier film (non-wetting transparent paper) cage. The floor lining was changed each time the mouse defecated. During the observation period of 4 hours, the time onset of diarrhea, the number and weight of wet stools, the total number and weight of fecal output (both diarrheal and non-diarrheal) excreted by the mice were recorded and compared with the control group. Finally, the percentages of diarrheal inhibition, as well as the weight of wet and total fecal output, were determined according to the formulae below.

% of inhibition of diarrhea =

$$\frac{\text{Mean number of wet defecation (negative control - test)}}{\text{Mean number of weight defecatin negative control}} \times 100$$

% of wet fecal output =

$$\frac{\text{Mean weight of wet feces of each treatment group}}{\text{Mean weight of wet feces of control}} \times 100$$

% of total fecal output =

$$\frac{\text{Mean fecal weight of each treatment group}}{\text{Mean fecal weight of control}} \times 100$$

Castor Oil-Induced Gastrointestinal Motility

A gastrointestinal motility test was evaluated according to the method and formula described in other studies.²² All mice have fasted for 18 hours with free access to water were randomly assigned to 5 groups and treated as described earlier. An hour later, 0.5 mL of castor oil was administered. Sixty minutes after the administration of castor oil, each mouse received 1 mL of 5% charcoal suspension in distilled water. Thirty minutes later each mouse was then sacrificed by cervical dislocation, the abdomen was opened and the small intestine was immediately dissected out from pylorus to caecum and placed lengthwise on a white paper. Thereafter, the distance traveled by the marker from the pylorus and the total length of the intestine were measured with a calibrated ruler. The

peristaltic index (PI) expressed as a percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine as well as the percentage inhibition of movement as a function of the control was calculated.²²

$$PI = \frac{\text{Mean distance travelled by charcoal meal}}{\text{Mean length of small intestine}} \times 100$$

Percentage inhibition of motility

$$= \frac{\text{Mean percent of distance travelled by the charcoal meal (negative control - test)}}{\text{Mean percent of distance travelled by the charcoal meal negative control}} \times 100$$

Castor Oil-Induced Enteropooling

Intestinal fluid accumulation was evaluated according to the method described.²⁰ Thirty mice were fasted for 18 hours and treated as described in the grouping and dosing section just one hour before oral administration of 0.5 mL CO. After 1 hour, the mice were sacrificed, the pyloric and cecum ends of the small intestine were tied and the intestine was removed. The dissected small intestine was weighed and intestinal contents were expelled into a measuring cylinder and then the volume was measured. Weight of the intestine after milking was taken and the difference between full and empty intestines was calculated. Finally, the percentage inhibitions of intestinal secretion were calculated.^{17,21}

% inhibition by using MVIC =

$$\frac{\text{Mean volume of intestinal fluid (negative control - test)}}{\text{Mean volume of intestinal fluid negative control}} \times 100$$

where MVIC is mean volume of the intestinal content

% inhibition by using MWIC =

$$\frac{\text{Mean weight of the intestinal fluid (negative control - test)}}{\text{Mean weight of the intestinal fluid negative control}} \times 100$$

where MWIC is mean weight of the intestinal content

In vivo Antidiarrheal Index

The in vivo antidiarrheal index (ADI) for the plant material extract and the standard drug was determined by combining three parameters taken from the aforementioned models. It was then expressed according to the formula developed.²¹

$$\text{In vivo antidiarrheal index (ADI)} = \sqrt[3]{D \text{freq} \times G \text{meq} \times P \text{freq}}$$

where D freq is the delay in defecation time or diarrheal onset obtained from castor oil-induced test (as % of control),

G meq is the gut meal travel reduction (as % of control) and Pfreq is the purging frequency or reduction in the number of wet stools (as % of control) from castor oil diarrhea model.

Statistical Analysis

SPSS Version 20 Software was used for the analysis of the results of the study. The results were then communicated as mean \pm standard error of the mean (SEM). Data analysis was performed with one-way ANOVA followed by Tukey post hoc test, test to find out the significant difference between control groups against each test group separately. The value of $P < 0.05$ was considered statistically significant.

Results

Acute Oral Toxicity Test

The present study was conducted as per the OECD guidelines 425 shown methanol extract of *V. amygdalina* did not yield any morbidity during the study period of 14 days at a dose of 2g/kg of body weight. No any neurological, behavioral and autonomic changes and physical changes. Moreover, the *V. amygdalina* leaves extract did not lead to death in the mice at a dose of 2g/kg at study period time. The finding indicated that a single dose of *V. amygdalina* extracts had no adverse effect, demonstrating that the LD₅₀ could be greater than 2g/kg/body weight in mice.

Effect on Castor Oil-Induced Diarrheal Model

The 80% methanolic extract of *V. amygdalina* leaves was found to be effective against castor oil-induced diarrhea in terms of delaying the onset of diarrhea at 100 mg/kg, 200 mg/kg, 400 mg/kg tested doses as well as reduced the fecal parameters (number and weight of wet and total stools) compared to the negative control. Besides, the data revealed that the percentage of diarrheal inhibitions were 49.02% ($p < 0.001$), 61.64% ($p < 0.001$) and 71.45% ($p < 0.001$) at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively. The number of wet feces was markedly reduced with 80% methanolic extract of *V. amygdalina* at a dose of 400 mg/kg when compared with a negative control. Loperamide 3 mg/kg (positive control) has also shown highest reduction of wet defecations with percentage inhibition of 75.49% ($P < 0.001$).

As depicted in Table 1, there was a dose-dependent reduction in the percentage of the weight of wet and total fecal outputs in 80% methanol extract ($R^2 = 0.996$; $R^2 = 0.964$, $p < 0.05$) with a maximum dose of 80% methanol

Table 1 Antidiarrheal Effects of 80% Methanol Extract of the Leaves of *V. amygdalina* on Castor Oil-Induced Diarrheal Model in Mice

Dose Administered	Onset of Diarrhea (Minutes)	Number of Wet Feces	Total Number of Feces	Average Weight of Wet Feces (gm)	Average Weight of Total Feces (gm)	% Reduction	% Wet Fecal Outputs	% Total Fecal Outputs
Control	68.67±1.87	8.16±0.74	9.00±0.85	0.41±0.03	0.43±0.04	—	—	—
100mg/kg	102.50±4.15 ^{a1}	4.15±0.54 ^{a3}	4.83±0.54 ^{a2}	0.21±0.02 ^{a2}	0.23±0.03 ^{a1}	49.02	51.21	53.49
200mg/kg	137.33±21.23 ^{a2}	3.13±0.79 ^{a3}	3.66±1.20 ^{a2}	0.16±0.04 ^{a3}	0.17±0.05 ^{a3}	61.64	39.02	39.53
400mg/kg	154.50±17.57 ^{a3}	2.33±0.84 ^{a3}	2.83±0.94 ^{a3}	0.12±0.04 ^{a3}	0.14±0.04 ^{a3}	71.45	29.26	32.55
3mg/kg Loperamide	158.00±16.80 ^{a3}	2.00±0.51 ^{a3}	2.50±0.56 ^{a3}	0.09±0.03 ^{a3}	0.12±0.03 ^{a3}	75.49	21.95	27.90

Notes: Data are expressed as mean ± SEM (n = 6); ^acompared with negative control; ¹p < 0.05, ²p < 0.01, ³p < 0.001.

extract revealed that greater effect to lessen percentage of fecal output but the effect was lower than standard drug Loperamide (21.95% and 27.90%).

respectively, and a more significant effect was produced by Loperamide (60.09%, P < 0.001), relative to negative control.

Effect on Castor Oil-Induced Gastrointestinal Motility

As presented in Table 2, all tested doses of the 80% methanol extract produce a significant reduction in the percent of intestinal motility in mice compared with the negative control group. The data revealed that the percentage reduction in gastrointestinal transit of charcoal meal was 52.65% (p < 0.001), 53.82% (p < 0.001), and 57.74% (p < 0.001) at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg,

Effect on Castor Oil-Induced Enter-Pooling

Compared to the negative controls, the three serial doses of 80% methanol extract of the leaves of *V. amygdalina* significantly inhibited castor oil-induced enteropooling in mice at doses of 100, 200, and 400 mg/kg as revealed by the reduction in volume and weight of intestinal contents. As the data revealed in Table 3, the 80% methanol extract decreased the mean volume of the intestinal content by

Table 2 Effects of 80% Methanol Leaves Extract of *V. amygdalina* on Castor Oil-Induced Gastrointestinal Motility in Mice

Dose Administered	Length of Small Intestine (cm)	Distance Moved by the Charcoal Meal (cm)	% Charcoal Meal Transit (Peristaltic Index)	% Inhibition
Control	56.00±1.37	39.50±2.52	70.54±4.35	—
100 mg/kg	59.26±2.37	19.38±2.39 ^{a3}	33.40±5.05 ^{a3}	52.65
200 mg/kg	56.33±1.99	18.35±1.26 ^{a3}	32.57±3.32 ^{a3}	53.82
400 mg/kg	59.65±1.99	17.78±2.16 ^{a3}	29.81±1.82 ^{a3}	57.74
3 mg/kg loperamide	59.83±1.19	16.83±1.10 ^{a3}	28.15±1.88 ^{a3}	60.09

Notes: Data are expressed as mean ± SEM (n = 6); ^acompared with negative control; ³p < 0.001.

Table 3 Effects of 80% Methanol Leaves Extract of *V. amygdalina* on Castor Oil-Induced Enteropooling in Mice

Dose Administered	Mean Volume of Small Intestinal Content (mL)	% Inhibition	Mean Weight of Small Intestinal Content (gm)	% Inhibition
Control	1.03±0.06	—	0.88±0.07	—
100 mg/kg	0.73±0.04 ^{a3b3c2}	29.13	0.68±0.04 ^{a1}	22.73
200 mg/kg	0.55±0.04 ^{a3}	46.60	0.51±0.07 ^{a2}	42.05
400 mg/kg	0.52±0.03 ^{a3}	49.51	0.48±0.04 ^{a3}	45.45
3 mg/kg loperamide	0.48±0.03 ^{a3}	53.39	0.43±0.04 ^{a3}	51.14

Notes: Data are expressed as mean ± SEM (n = 6); ^acompared with negative control, ^bcompared with Loperamide, ^ccompared with 400 mg/kg; ¹p < 0.05, ²p < 0.01, ³p < 0.001.

29.13% ($p < 0.001$), 46.60% ($p < 0.001$), and 49.51% ($p < 0.001$), and the weight of the intestinal content by 22.73% ($p < 0.05$), 42.05% ($p < 0.01$), and 45.45% ($p < 0.001$), at 100, 200, and 400 mg/kg doses, respectively, compared to the negative controls. However, the highest percentage of inhibition scored by Loperamide was 53.39% ($p < 0.001$) and 51.14% ($p < 0.001$), for percent reduction of volume and weight of intestinal contents, respectively.

In vivo Antidiarrheal Index

The in vivo antidiarrheal index (ADI) was measured by considering three parameters as shown in Table 4. These are delays in defecation (Dfreq), gut meal travel distance (Gmeq), and purging frequency in the number of wet stools (Pfreq). The 80% methanolic extract showed an antidiarrheal index of 50.28, 69.10, and 80.19 at doses of 100, 200, and 400 mg/kg, respectively, while standard drug produced a maximum index of 83.86. Moreover, 80% of methanol extract showed a dose-dependent increment in ADI value.

Preliminary Phytochemical Screening

Phytochemical analysis of the 80% methanol extract of the leaves of *V. amygdalina* revealed the presence of tannins, flavonoids, alkaloids, saponins, and terpenoids but steroids were absent in 80% methanol extract of the plant.

Discussion

Diarrhea is a main public health concern that causes high mortality, especially among children. Apart from orthodox medicine, the use of herbal drugs in the treatment of diarrhea disorder is a common practice in many developing countries including Ethiopia.²³ The medicinal value of the plant depends on the amount and purity of chemical constituents, which can be affected by different factors, such as climate condition, soil nutrient, method of preparation, and parts of the plant used.²⁴

An acute toxicity study was carried out to determine possible adverse reactions to a single dose or an overdose of the extract.²⁵ Generally, the absence of mortality and signs of overt toxicity up to five times the maximum effective dose of the extract suggested that 80% methanol leaves extract has a wider safety margin and LD50 value greater than 2g/kg in mice.¹⁵

The need for phytochemical screening has become vital importance since many plants accumulate biologically active (secondary plant metabolites) in their tissues that have antidiarrheal activity.^{26,27} Moreover, tannins, flavonoids, and alkaloids are the main chemical constituents that are responsible for the antidiarrheal activity of the plants and this may be due to antisecretory effects.²⁸ In the current study, the preliminary phytochemical screening of crude extracts revealed that the presence of tannins, flavonoids, alkaloids, and saponins and these secondary metabolites may be responsible for the antidiarrheal activity of the crude extract.

In the current study, diarrhea was induced experimentally by using castor oil, as this model is the general model to test the antidiarrheal activities of different substances.²⁹ Castor oil causes diarrhea due to poor absorption of its active metabolite, ricinoleic acid, which causes changes in the mucosal permeability, electrolyte transport, and intestinal peristalsis, leading to hypersecretory response and diarrhea.³⁰

In the castor oil-induced diarrhea model, the extract of *V. amygdalina* leaves at all tested doses significantly delayed the onset of defecation, reduced the number and weight of both wet and total fecal output. The highest doses, 400 mg/kg, significantly delay the onset of diarrhea caused by castor oil when compared with the negative controls and the percentage of inhibition of defecation produced was closer to inhibition produced by the standard drug. Furthermore, the extract displayed a dose-dependent reduction in percentage of weight of wet fecal output ($R^2 = 0.996$, $p < 0.05$) and

Table 4 In vivo Antidiarrheal Indices of 80% Methanol Extract of the Leaves of *V. amygdalina*

Dose Administered	Delay in Defecation (Time of Onset in Min. Dfreq) (%)	Gut Meal Travel Distance (Gmeq) (%)	Purging Frequency in Number of Wet Stools (Pfreq) (%)	Antidiarrheal Index (ADI)
Control	—	—	—	—
100mg/kg	49.26	52.65	49.02	50.28
200mg/kg	99.98	53.54	61.64	69.10
400mg/kg	124.98	57.74	71.45	80.19
3mg/kg loperamide	130.08	60.09	75.49	83.86

weight of total fecal output ($R^2 = 0.964$, $p < 0.05$). The reduction in the frequency of defecation, the weight of wet stools, and total stools indicate the efficacy of 80% methanol extract of *V. amygdalina* as an antidiarrheal agent. The results obtained support the report done in Nigeria.^{14,31}

These effects observed are believed to occur as a result of reduced water and electrolyte secretion into the small intestine, suggesting that the crude extracts that contain antidiarrheal secondary metabolites (flavonoid, tannins, and alkaloids) may increase electrolyte absorption from the intestinal lumen due to inhibition of hypersecretion produced by nitric oxides.¹⁴

To settle the antidiarrheal activity of leaves of *V. amygdalin* the possible mechanism of action was tested on intestinal motility and enteropooling models. In the castor oil-induced gastrointestinal motility model, it was observed that the extract significantly suppresses the movement of the charcoal marker at 100 mg/kg, 200 mg/kg, and 400 mg/kg doses as compared to the negative control. The higher percentage of inhibition (57.74%, $p < 0.001$), of the marker perceived at maximum dose was almost comparable to the standard drug (60.09%, $p < 0.001$ at the dose of 3 mg/kg). This finding showed that the extract can influence the peristaltic movement of the intestine thereby indicating the presence of intestinal antimotility activity. Concerning this, several plants have shown antidiarrheal activities by reducing the gastrointestinal motility and its secretions.^{32,33} Several studies suggested that the anti-motility properties of herbal are mostly due to flavonoids³⁴ by inhibiting the release of autacoids and prostaglandins results in inhibiting motility and hydro-electrolytic secretions induced by ricinoleic acid.^{35,36} Tannins may also show an anti-motility effect by reducing intracellular Ca^{2+} through decreasing Ca^{2+} inward current or increasing calcium outflow and finally resulting in reducing peristaltic movement and intestinal secretions due to induction of the muscle relaxation.³⁷ Pretreatment with 80% methanol extract significantly reduced peristaltic movements as evidenced by the decrease in the distance traveled by a charcoal meal in the GIT, showing that these crude extracts could have antimotility activity due to flavonoid and tannin constituents.

The last model used in the present study was the castor oil-induced enteropooling model that aimed to assess the secretory components of diarrhea. The anti-enteropooling properties of some medicinal plants have been attributed to their phytochemical constituents.³⁸ Flavonoids have been displayed secretion inhibition response by reducing synthesis inflammatory mediator prostaglandin E2.³⁶ Tannates

protein produced during tannin interaction with small intestinal mucosa may reduce the secretion.²⁹

Conclusion

The crude extract of *V. amygdalina* leaves showed antidiarrheal activity in an animal model by decreasing the number of wet feces, gastrointestinal motility, and intraluminal fluid accumulation in the intestine. The antidiarrheal activities may be ascribed to the presence of bioactive secondary metabolites including tannins, flavonoids, alkaloids, and saponins that bring antidiarrheal effect. These findings provide scientific support for the traditional claim of *V. amygdalina* as a treatment of diarrheal diseases.

Acknowledgments

We would like to thank all who gave us guidance and support through the work of this study. Additionally, we would like to thank Ambo University for sponsorship and the staff of Ethiopian public health institutions who assisted and permitted us to accomplish the detailed research at the institute.

Disclosure

The authors stated that there is no conflict of interest.

References

1. Yakubu MT, Salimon SS. Antidiarrhoeal activity of aqueous extract of *Mangifera indica* L. leaves in female albino rats. *J Ethnopharmacol.* 2015;163:135–141. doi:10.1016/j.jep.2014.12.060
2. Mihrete TS, Alemie GA, Teferra AS. Determinants of childhood diarrhea among under five children in Benishangul Gumuz Regional State, North West Ethiopia. *BMC Pediatr.* 2014;14(1):102. doi:10.1186/1471-2431-14-102
3. Rao GHJ, Lakshmi P. Anti-diarrhoeal activity of *Ziziphus Jujuba* leaf extract in rats. *Int J Pharma and Bio Sci.* 2012;3(1):533–538.
4. CDC. Global diarrhea burden; 2012. Available from <http://www.cdc.gov/healthywater/global/diarrhea-burden.html>. Accessed February 12, 2019.
5. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ.* 2008;86(9):710–717. doi:10.2471/BLT.07.050054
6. Gebru T, Taha M, Kassahun W. Risk factors of diarrhoeal disease in under-five children among health extension model and non-model families in Sheko district rural community, Southwest Ethiopia: comparative cross-sectional study. *BMC Public Health.* 2014;14:395.
7. Khansari MR, Sohrabi MR, Zamani F. The usage of opioids and their adverse effects in gastrointestinal practice: a review. *Middle East J Dig Dis.* 2013;5:5–16.
8. Adeyemi OO, Akindele AJ, Ogunleye EA. Evaluation of the anti-diarrheal effect of *Sansevieria Liberica* Gerome & Labroy (Agavaceae) root extract. *J Ethnopharmacol.* 2009;123(3):459–463. doi:10.1016/j.jep.2009.03.023
9. Kim HS. Do not put too much value on conventional medicines. *J Ethnopharmacol.* 2005;100(1–2):37–39. doi:10.1016/j.jep.2005.05.030
10. Kassaye KD, Amberbir A, Getachew B, Mussema Y. A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiop J Health Dev.* 2006;20(2):127–134.

11. Stark TD, Mtui DJ, Balembo OB. Ethnopharmacological survey of plants used in the traditional treatment of gastrointestinal pain, inflammation and diarrhea in Africa. Future perspectives for integration into modern medicine. *Animals*. 2013;3:158–227.
12. WHO. *World Health Report*. Geneva:WHO;2004: 120–125. Available from: <http://www.who.int/whr/2004/en/>. Accessed October 27, 2020.
13. Teklehaymanot T, Giday M. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *J Ethnobiol Ethnomed*. 2007;3(1):1–11. doi:10.1186/1746-4269-3-12
14. Olayemi AA, Temitope AO, David KB. Evaluation of antidiarrheal activity of the ethanolic stem bark extract of *Vernonia amygdalina* in experimental animals. *J Nat Sci Res*. 2016;6(10):61–66.
15. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestry database: a tree reference and selection guide version. 2009.
16. Mesfin F, Demissew S, Teklehaymanot T. An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J Ethnobiol Ethnomed*. 2009;5(1):1–18. doi:10.1186/1746-4269-5-28
17. Suleman S, Alemu T. A survey on utilization of ethnomedicinal plants in Nekemte town. *J Herbs Spices Med Plants*. 2012;18(1):34–57. doi:10.1080/10496475.2011.645188
18. Regassa R, Bekele T, Megersa M. Ethnobotanical study of traditional medicinal plants used to treat human ailments by Halaba people, southern Ethiopia. *J Med Plan Stud*. 2017;5(4):36–47.
19. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th ed. Washington, DC, USA: The National Academies Press; 2011.
20. OECD/OCDE. Acute oral toxicity. OECD Guidelines for the Testing of Chemicals. In: *Thirteenth Addendum to The, OECD, Guidelines for the Testing of Chemicals*. Paris: organization for economic cooperation, development; 2008:425.
21. Nwodo OFC, Alumanah EO. Studies on *Abrus precatorius* seeds II: antidiarrheal activity. *J Ethnopharmacol*. 1991;31(3):395–398. doi:10.1016/0378-8741(91)90024-8
22. Tan A, Kulkarni HJ, Hmone W, Ta SJ. Antidiarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. *Int J Crude Drug Res*. 1989;27(4):195–200. doi:10.3109/13880208909116903
23. Kebebew M. Diversity, knowledge and use of medicinal plants in Abay Chomen District, Horo Guduru Wollega Zone, Oromia Region of Ethiopia. *J Med Plants Res*. 2017;11(31):480–500. doi:10.5897/JMPR2016.6274
24. Mohammed A, Liman ML, Atiku MK. Chemical composition of the methanolic leaf and stem bark extracts of *Senna siamea* Lam. *J Pharmacognosy Phytother*. 2013;5(5):98–100.
25. Kelechi MG, Favour OO. Studies on antidiarrheal activity of *Cassia sieberiana* in Mice. *JABB*. 2015;3(4):139–144. doi:10.9734/JABB/2015/13810
26. Islam AMT, Uddin ME, Chowdhury MAU, Rahman MM, Habib MR, Rahman MA. In vivo antidiarrheal and cytotoxic potential of different fractions of *Pandanus foetidus* leaves. *Am J Biomed Sci*. 2013;5(3):208–216. doi:10.5099/aj130300208
27. Umer S, Tekewe A, Kebede N. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complement Altern Med*. 2013;13(1):21. doi:10.1186/1472-6882-13-21
28. Komal Kumar S, Rana A. Herbal approaches for diarrhea: a review. *Int Res J Pharm*. 2013;4:31–38.
29. Chowdhury A, Chowdhury A, Rahman A, Rahman A, Rahman A. Antidiarrheal and thrombolytic effects of methanol extract of *Wikstroemia indica* (L.) C. A. Mey leaves. *Int J Green Pharm*. 2015;9(1):8–13. doi:10.4103/0973-8258.150914
30. Saralaya MG, Patell P, Patel M, Roy SP, Patel AN. Antidiarrheal activity of methanolic extract of *Moringa oleifera* Lam roots in experimental animal models. *Int J Pharm Res*. 2010;2(2):35–39.
31. Okere OS, Sangodele JO, Tade OG, Obafemi OT, Falode JA. Antidiarrhea potential and acute toxicity studies of methanolic extract of *Vernonia amygdalina* and *Cymbopogon citratus* against castor oil induced diarrhea model in rats. *IJBcRR*. 2015;6(2):46–52. doi:10.9734/IJBcRR/2015/12293
32. Wansi SL, Nguetack-Mbuyo EP, Nchouwet ML, et al. Antidiarrheal activity of aqueous extract of the stem bark of *Sapium Ellipticum* (Euphorbiaceae). *Trop J Pharm Res*. 2014;13(6):929–935. doi:10.4314/tjpr.v13i6.15
33. Mekonnen B, Asrie AB, Wubneh ZB. Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. *Evid Based Complement Alternat Med*. 2018;2018:1–10. doi:10.1155/2018/3037120
34. Labu ZK, Laboni FR, Mamun A, Howlader SI. Antidiarrhoeal activity and total tannin content of ethanolic leaf extract of *Codiaeum variegatum*. *Pharm Sci*. 2015;14(1):87–90.
35. Ahmad MF. Antidiarrheal activity of aqueous leaf extract of *Swertia chirata* in rat. *EJPMR*. 2017;4(3):53–58.
36. Meite S, N' Guessan JD, Bahi C, Yapi HF, Djaman AJ, Guina FG. Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats. *J Pharm Res*. 2009;8(3):201–207.
37. Bamsaye FA, Odutuga AA, Minari JB, Dairo JO, Fagbohunka BS, Oluba OM. Phytochemical constituents and antidiarrhoeal effects of the aqueous extract of *Terminalia Superba* leaves on Wistar rats. *Afr J Pharm Pharmacol*. 2013;7(16):848–851.
38. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother Res*. 2006;20(9):717–724. doi:10.1002/ptr.1907

Journal of Experimental Pharmacology

Publish your work in this journal

The Journal of Experimental Pharmacology is an international, peer-reviewed, open access journal publishing original research, reports, reviews and commentaries on all areas of laboratory and experimental pharmacology. The manuscript management system is completely

online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-experimental-pharmacology-journal>

Dovepress