Journal of Evidence-Based Integrative Medicine
Volume 24: 1-9

DOI: 10.1177/2515690X19853264
journals.sagepub.com/home/cam
SAGE

Evaluation of the Antispasmodic and Antisecretory Activities of the 80% Methanol Extracts of *Verbena officinalis* L: Evidence From In Vivo Antidiarrheal Study

Mekonnen Sisay, MSc[1], Negussie Bussa, PhD[2], and Tigist Gashaw, MSc[1]

Abstract

Verbena officinalis L. has a folkloric repute for the management of digestive disorders, including diarrhea. However, the safety and efficacy of the plant material has not been scientifically validated yet. This study was, therefore, aimed to evaluate the overall antidiarrheal activity of the 80% methanol extracts of *V officinalis* in mice. The antidiarrheal activity of the 80% methanol extracts of the roots (R-80ME) and the leaves (L-80ME) of *V officinalis* was tested in castor oil–induced diarrhea in mice. R-80ME was further evaluated using charcoal meal and entero-pooling. In each test, group I and group II (controls) received 10 mL/kg distilled water and standard drug (5 mg/kg loperamide), respectively, whereas groups III, IV, and V (test groups) received 100, 200, and 400 mg/kg of the 80ME, respectively. The R-80ME at 200 mg/kg ($P < .01$) and 400 mg/kg ($P < .001$) significantly delayed the onset of diarrhea compared with negative control. Both R-80ME and L-80ME at 200 and 400 mg/kg significantly decreased the frequency of wet fecal outputs ($P < .01$). Generally, 70.24% inhibition of the number of wet fecal output was recorded at R-80ME 400 mg/kg. Results from the charcoal meal test revealed that the R-80ME at 200 ($P < .01$) and 400 mg/kg ($P < .001$) produced a significant antimotility effect. In entero-pooling test, the R-80ME, at 200 and 400 mg/kg doses ($P < .01$), showed a significant decline in both the volume and weight of intestinal contents. The maximum in vivo antidiarrheal index was determined to be 95.25 at dose of 400 mg/kg R-80ME. This study demonstrated that the 80ME, mainly the root extract, produced promising antidiarrheal activity and hence provides a scientific support for acclaimed traditional use of the plant material for treatment of diarrheal diseases.

Keywords

Verbena officinalis, castor oil, antidiarrheal, 80ME, roots, leaves

Received February 28, 2019. Received revised April 25, 2019. Accepted for publication May 4, 2019.

Since time immemorial, medicinal plants have played an important role in the development of potent therapeutic agents. Today, it is estimated that about 80% of population in developing countries still rely on traditional medicine for their primary health care. Herbal medicines are currently in great demand and their popularity is also increasing over time.[1-4] Among the medicinal plants, *Verbena officinalis* L has a folkloric repute for the management of various ailments including diarrhea. It is a perennial herb belongs to the genus *Verbena* and the family Verbenaceae (Figure 1). The plant is known with different vernacular names in different areas of Ethiopia: Atuch (Amharic), Atush (Tigiregna), Qoricha albaatii (Afan Oromo), and Guni tesha (Aari, Omotic). In Ethiopia, the plant is distributed in several regions, including Gondar, Gojam, Wollo, Shewa, Bale, Harerghe, Gamo Gofa, and Tigray. It is also wildly distributed in African countries, including West Eritrea, Democratic Republic of Congo, Sudan, Zambia, Zimbabwe, and South Africa.[5]

The anti-inflammatory and analgesic effects of *V officinalis* extract in different formulations have been studied and confirmed.[6] In addition, the extract of aerial parts of *V officinalis* has antipyretic, sedative,[7] and anticancer effects in traditional

[1] School of Pharmacy, Haramaya University, Harar, Ethiopia
[2] Food Science and Post-harvest Technology, Haramaya University, Dire Dawa, Ethiopia

Corresponding Author:
Mekonnen Sisay, Department of Pharmacology and Toxicology, School of Pharmacy, College of Health and Medical Sciences, Haramaya University, Harar, 235, Ethiopia..
Email: mekonnensisay2748@yahoo.com

Figure 1. Photograph of *Verbena officinalis*.

medicine.[8] Moreover, the plasters of aerial parts of *V officinalis* (sometimes with petrol) are used as hemostatic and antirheumatic in knees and elbows.[9] Apart from this, the extract or the juice of roots of *V officinalis* can be used for the treatment of tonsillitis and ascariasis. The leaf extract is also used in the treatment of ear disease by applying some drops of the extract through the ear[10] and control of snake bite poisoning.[11] With regard to treatment of diarrheal disease, the different parts of this plant are traditionally used for several purposes, especially for the management of stomachache, diarrhea, or dysentery. For instance, the extract of whole plant, after boiled with tea, is given orally for the treatment of dysentery.[12,13] In addition, the root extract, after maceration in water is drunk for the control of diarrhea.[14] On top of these, fresh leaves,[15] fresh roots,[16] or whole plant[10] are chewed and its juice is swallowed to treat stomachache or abdominal pain. Woldeab et al[17] has also reported the way that the traditional healers are using this plant material by grinding the root part into coarse powder; mixing it with water for some time and decanting/filtering it and finally the extract is drunk to treat diarrheal diseases. In some areas of Spain, *V officinalis* has been used for the management of varies digestive problems, including stomachache and abdominal cramp.[18] Based on this claim, this study was aimed to investigate the efficacy and safety profiles of crude extracts of *V officinalis* as antidiarrheal agent with possible antispasmodic and antisecretory potential.

Materials and Methods

Drugs, Chemicals, and Reagents

Distilled water, castor oil (Amman Pharmaceutical Industries, Jordan), activated charcoal (Acuro Organics Ltd, New Delhi, India), loperamide (Daehwa Pharmaceuticals, Republic of Korea), methanol (Carlo Erba reagents, SAS, France), Tween80 (Atlas Chemical Industries Inc, India) were used for the experiment. For phytochemical screening, reagents and chemicals including chloroform (Hi-Media Laboratory Reagents, India), glacial acetic acid, ammonia, hydrochloric acid, ferric chloride, acetic anhydride, Mayer's reagent, Dragendroff's reagent, sulfuric acid (all of them from BDH Chemicals Ltd, England) were used.

Plant Material Collection and Identification

Both the leaves and roots of *V officinalis* were collected from Debre Markos town and its surrounding, which is 300 km away to northwest of the capital of Ethiopia, Addis Ababa, in February 2017. The roots of the plant were carefully collected and gently washed with distilled water to remove dirt, soil, rootlets, and diseased portions. Care was maintained when digging out underground parts so as not to distract the medicinal components and to avoid microbial contamination and spoilage. The identification of the plant specimen was undertaken by a taxonomist at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, where a voucher specimen was deposited for future reference (ID: MS002/2017).

Experimental Animals

A total of 108 healthy Swiss albino mice of either sex, weighing 20 to 30 g and aged 6 to 8 weeks were used for the actual experiment (48, 30, and 30 for the castor oil–induced diarrhea, charcoal meal, and enteropooling models, respectively). The animals were obtained from animal center of Ethiopian Public Health Institute, Addis Ababa, and interbred in the animal house of central laboratory of Haramaya University. Five to 8 mice were housed in polyethylene cages having metallic cover with woodchip bedding at ambient room temperature, exposed to 12-hour light/dark cycles and with free access to standard pellet food and water. The animals were acclimatized to laboratory conditions for 1 week prior to the actual experiment.[19] The use and handling of mice was in accordance with the guidelines for the care and use of experimental animals.[20]

Preparation and Extraction of Plant Materials

The leaves were washed gently and dried at room temperature under shade for 2 weeks. The dried leaves were then reduced to appropriate size using mortar and pestle. The roots of *V officinalis* were manually cut into pieces and dried under shade for two weeks and then, ground in a coarse size powder using mortar and pestle. Then, 125 g of powdered root material and 150 g of coarse leaf powder were macerated separately in 2 L Erlenmeyer flasks with 400 mL 80% methanol for a period of 3 days with frequent agitation using mini-orbital shaker at room temperature. The extracts were filtered through double layered muslin cloth followed by Whatman No. 1 filter paper (Schleicher and Schuell Micro Science GmbH, Germany). The marcs of both extracts were remacerated twice with the same volume of fresh solvent to exhaustively extract the soluble components. Similar filtration procedure was taken place for the marc to get the final filtrates. The combined filtrates were concentrated to dryness with rotary evaporator (Buchi Labortechnik AG, Switzerland). The remaining aqueous residue was deep frozen to form ice and lyophilized (freeze dried) to get the final extract.[21-24] Then, the percentage yields of 80% methanol root (R-80ME) and leave extracts (L-80ME) were found to be 15.5% (w/w) and 9.25% (w/w), respectively. Finally, the dried extracts were kept in deep freeze with airtight container and reconstituted in distilled water at appropriate concentration during the day of experiment.

Acute Oral Toxicity Test

Acute oral toxicity test was performed according to the OECD guideline 425. Five female albino mice aged 6 to 8 weeks were used for

each test. For doing this, a single female mouse was given 2000 mg/kg of the either extracts as a single loading dose by oral gavage. As no death was observed within 24 hours, additional 4 female mice were recruited and dosed similarly in each extract. The animals were observed continuously for 4 hours with 30-minute interval and then for 14 consecutive days with an interval of 24 hours for the general signs and symptoms of toxicity.[19]

Animal Grouping and Dosing

In all models, animals were randomly divided into 5 groups (2 controls and 3 test groups) comprising 6 animals each. Negative controls received vehicle (10 mL/kg distilled water) and positive controls were treated with standard drug (5 mg/kg loperamide) in all models. The test groups (groups III, IV, and V) received 100, 200, and 400 mg/kg of each extract, respectively with oral gavage based on the acute oral toxicity result.

Determination of Antidiarrheal Activity

Castor Oil–Induced Diarrhea. The method described by previously published studies was considered with slight modification.[21-23,25] Swiss albino mice of either sex were fasted for 18 hours with free access to water. One hour after dosing of the respective treatments, each mouse was given 0.5 mL of castor oil orally for induction of diarrhea and was placed individually in plastic cage where the floor had been lined with white paper. The paper was changed every hour for a total duration of 4 hours. During the observation period, the onset of diarrhea, the frequency and weight of wet and total fecal outputs were recorded. Finally, the percentages of diarrheal inhibition and fecal output were calculated by using the formulae described below.[22,25]

$$\%\ \text{of inhibition} = \frac{\text{mean number of WFC} - \text{mean number of WFT}}{\text{mean number of WFC}} * 100$$

where, WFC = wet feces in control group and WFT = wet feces in test group

$$\%\ \text{of fecal output} = \frac{\text{mean fecal weight of each treatment group}}{\text{mean fecal weight of control}} * 100$$

Castor Oil–Induced Entero-Pooling. The activity of R-80ME on intraluminal fluid accumulation was determined using the method described by Robert et al.[26] Mice were fasted for 18 hours, grouped and treated accordingly. In this case, the mice were also deprived of water. After an hour of treatment, 0.5 mL of castor oil was administered, and the mice were sacrificed by cervical dislocation 1 hour following castor oil administration. The abdomen of each mouse was then opened; the small intestine was ligated at both ends (at pyloric sphincter and ileo-cecal valve) and dissected. The dissected small intestine was weighed and the intestinal content was then collected by milking into a graduated tube to measure the volume of the contents. The intestine, following milking, was reweighed and the difference was recorded as measure of weight of intestinal contents. Finally, percentage of reduction of intestinal contents (volume and weight) was calculated using the following formulae[22]:

$$\%\ \text{of } \textit{inhibition volume of intenstinal contents} = \frac{MVICC - MVICT}{MVICC} * 100$$

where, MVICC = mean volume of intestinal content of control group and MVICT = mean volume of intestinal content of test group

$$\%\ \textit{of inhibition of weight of intestinal contents} = \frac{MWICC - MWICT}{MWICC} * 100$$

where, MWICC = mean weight of intestinal content of control group and MWICT = mean weight of intestinal content of test group.

Gastrointestinal Motility Test. The mice were fasted for 18 hours with free access to water and divided and treated as described earlier. Then, 0.5 mL castor oil was administered for each mouse. One milliliter of the marker (5% activated charcoal suspension in distilled water) was administered orally 1 hour after castor oil induction. The mice were then sacrificed by cervical dislocation just 1 hour following charcoal meal. The small intestine was dissected out from pylorus to caecum and placed length wise on a white paper. The distance travelled by the charcoal marker and the total length of the small intestine was then measured. The peristaltic index and percentage inhibition were calculated by using the following formulae[22,25,27]:

$$\textit{Perstalstic index } (PI) = \frac{\textit{Distance traveled by charcoal meal}}{\textit{total length of small intestine}} * 100$$

$$\%\, \textit{inhibition} = \frac{PI\ control - PI\ test}{PI\ control} * 100$$

In Vivo Antidiarrheal Index. The in vivo antidiarrheal index (ADI) for the positive control and R-80ME was determined using the following formula[22,27]:

$$\textit{In vivo ADI} = \sqrt[3]{Dfreq \times Gmeq \times Pfreq}$$

where, *Dfreq* is the delay in the defecation time (minute) with reference to of negative control (%), *Gmeq* is reduction in distance moved by the charcoal meal with reference to negative control (%), and *Pfreq* is the reduction in the number of wet stools with reference to negative control (%).

Preliminary Phytochemical Screening

The presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, and steroids was evaluated in both crude extracts using standard testing methods.[22,28,29]

Data Analysis

Data were entered and analyzed using IBM Statistical Package for the Social Sciences (SPSS) Version 20.0 software (Armonk, NY, USA). Results were expressed as mean plus or minus standard error of the mean (M ± SEM). Normality of data distribution was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way analysis of variance (ANOVA) was performed followed by Dunnett's post hoc test for multiple comparisons. The analysis was performed at 95% confidence level and $P < .05$ was considered as statistically significant.

Results

Acute Oral Toxicity Test

Both R-80ME and L-80ME of *V officinalis* produced neither overt toxicity nor death during the 14 days observation period following oral administration of a single dose of 2000 mg/kg. The absence of mortality and any sign of overt toxicity up to 5 times the maximum effective dose of the extract suggested that

Table 1. The Antidiarrheal Activities of R-80ME and L-80ME of *Verbena officinalis* in Castor Oil–induced Diarrhea in Mice Model.[a]

Dose (mg/kg)	Onset of Diarrhea (min)	Number of Wet Feces	Number of Total Feces	Average Weight of Wet Feces (g)	Average Weight of Total Feces (g)	% Inhibition (Frequency of Wet Feces)
CON	40.33 ± 4.27	7.83 ± 0.60	10.67 ± 1.08	0.81 ± 0.08	0.89 ± 0.10	—
R-80ME100	71.50 ± 7.41	6.17 ± 0.48	7.17 ± 0.48*	0.67 ± 0.08	0.73 ± 0.04	21.20
R-80ME200	117.67 ± 20.77[b]**	3.50 ± 0.22[b]***	5.67 ± 0.33[b]**	0.52 ± 0.03	0.59 ± 0.03	55.30
R-80ME400	134.00 ± 15.22[b]***	2.33 ± 0.56[b]***	4.17 ± 1.14[b]***	0. 38 ± 0.09[b]*	0.45 ± 0.11[b]**	70.24
L-80ME100	46.17 ± 4.19	6.83 ± 0.30	9.33 ± 0.61	0.75 ± 0.07	0.82 ± 0.09	12.77
L-80ME200	63.33 ± 8.28	4.33 ± 1.05[b]**	7.50 ± 1.09	0.59 ± 0.09	0.71 ± 0.07	44.69
L-80ME400	86.83 ± 19.53	3.33 ± 0.80[b]***	5.83 ± 1.05[b]**	0.50 ± 0.12	0.61 ± 0.13	57.47
LOP 5	119.50 ± 13.99[b]***	2.67 ± 0.21[b]***	4.83 ± 0.70[b]***	0.47 ± 0.07[b]*	0.56 ± 0.07	65.90

Abbreviations: CON, negative control (10 mL/kg distilled water); LOP, loperamide; R-80ME, 80% methanol extract of roots; L-80ME, 80% methanol extracts of leaves.

[a]Values are presented as mean ± standard error of the mean (SEM) (n = 6); analysis was performed using one-way analysis of variance(ANOVA) followed by Dunnett's post hoc test.

[b]Compared with negative control value; *$P < .05$, **$P < .01$, ***$P < .001$.

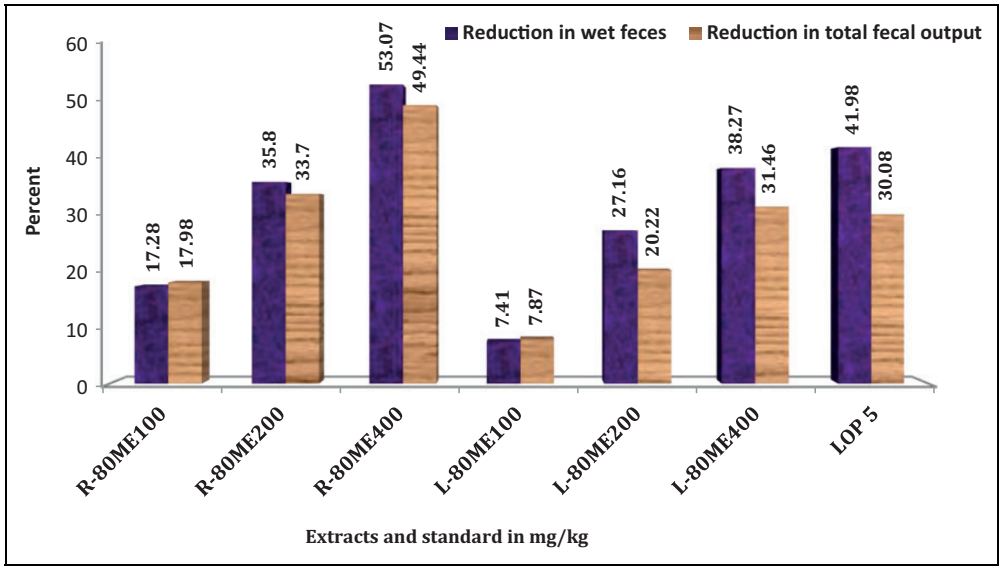

Figure 2. Percentage reduction of fecal outputs in R-80ME and L-80ME of *Verbena officinalis*. R-80ME, 80% methanol extract of roots; L-80ME; 80% methanol extracts of leaves.

the crude extracts have a wider safety margin and median lethal dose (LD_{50}) greater than 2000 mg/kg in mice model.

Effect on Castor Oil–Induced Diarrheal Model

In the general model, the R-80ME of *V officinalis* significantly prolonged the onset of diarrhea and reduced the frequency of wet and total fecal outputs at doses of 200 and 400 mg/kg compared with the negative control ($P < .01$). Besides, only 400 mg/kg of R-80ME significantly reduced the average weight of both wet and total fecal outputs ($P < .05$). Despite its modest effect on affecting urgency of defecation, the L-80ME at 200 and 400 mg/kg doses significantly inhibited the frequency of wet feces but not the weight of fecal outputs. The maximum percent inhibition of wet fecal output was observed at 400 mg/kg of R-80ME (70.24%) (Table 1).

There was a dose-dependent reduction in the percentage weight of wet and total fecal outputs in both R-80ME and L-80ME with 400 mg/kg of the R-80ME showing the highest effect. There was a linear increment in the percentage reduction of fecal outputs in both extracts. The highest reduction was observed at 400 mg/kg of R-80ME of *V officinalis* (53.07% and 49.44%) (Figure 2).

Castor Oil–Induced Intestinal Transit in Mice (Charcoal Meal Test)

The R-80ME significantly inhibited the distance moved by the charcoal meal and reduced peristaltic index at doses of 200 and 400 mg/kg. The data revealed that the percentage reduction of gastrointestinal motility was 33.52% ($P < .01$) and 52.97% ($P < .001$) at doses of 200 and 400 mg/kg, respectively. The

Table 2. The Antispasmodic Effect of R-80ME of *Verbena officinalis* in Castor Oil–Induced Gastrointestinal Transit (Charcoal Meal) in Mice.[a]

Dose (mg/kg)	Length of Small Intestine (cm)	Distance Moved by the Charcoal Meal (cm)	Peristaltic Index (%)	Inhibition of Motility (%)
CON	52.83 ± 2.02	40.00 ± 3.27	75.17 ± 4.41	—
R-80ME100	53.00 ± 1.29	35.00 ± 2.26	65.81 ± 3.35	12.45
R-80ME200	53.83 ± 1.62	26.67 ± 1.98[b]**	49.97 ± 4.68[b]**	33.52
R-80ME400	53.17 ± 1.66	18.83 ± 2.92[b]***	35.35 ± 5.33[b]***	52.97
LOP 5	54.17 ± 0.70	22.17 ± 1.19[b]****	40.74 ± 2.12[b]****	45.80

Abbreviations: CON, negative control (10 mL/kg distilled water); LOP, loperamide; R-80ME; 80% methanol extract of roots.
[a]Values are presented with mean ± SEM (n = 6); analysis was performed using one way ANOVA followed by Dunnett's post hoc test.
[b]Compared with negative control; *$P < .05$, **$P < .01$, ***$P < .001$.

Table 3. The Antisecretory Effect of R-80ME of *Verbena officinalis* in Castor Oil–Induced Gastrointestinal Fluid Accumulation (Entero-Pooling) in Mice.[a]

Dose Administered (mg/kg)	Volume of Intestinal Contents (mL)	% Inhibition	Weight of Intestinal Contents (g)	% Inhibition
CON	0.81 ± 0.06	—	1.02 ± 0.07	—
R-80ME100	0.68 ± 0.04	16.05	0.89 ± 0.05	12.74
R-80ME200	0.53 ± 0.04[b]***	34.57	0.74 ± 0.04[b]**	27.45
R-80ME400	0.42 ± 0.01[b]***	48.15	0.63 ± 0.02[b]***	38.23
LOP 5	0.45 ± 0.02[b]***	44.44	0.64 ± 0.01[b]***	37.25

Abbreviations: CON, negative control (10 mL/kg distilled water); LOP, loperamide; R-80ME; 80% methanol extract of roots.
[a]Values are presented with mean ± SEM (n = 6); analysis was performed using one-way ANOVA followed by Dunnett's post hoc test.
[b]Compared with negative control value; *$P < .05$, **$P < .01$, ***$P < .001$.

maximum dose of R-80ME showed slightly higher antimotility effect than that of the standard (45.80%, $P < .001$) (Table 2).

Castor Oil–Induced Entero-Pooling (Intraluminal Fluid Accumulation)

In this test, the R-80ME revealed significant reduction in the average volume and weight of intestinal contents at 200 and 400 mg/kg doses as compared with control ($P < .01$). The percentage inhibition of volume and weight, respectively, was 34.57% and 27.45% at 200 mg/kg and 48.15% and 38.23% at 400 mg/kg dose (Table 3).

In Vivo Antidiarrheal Index

The in vivo antidiarrheal index (ADI) was measured by considering 3 important parameters such as delay in defecation (time of onset, *Dfreq*), gut meal travel distance (*Gmeq*), and purging frequency in number of wet stools. The highest ADI value was recorded at 400 mg/kg R-80ME (95.25), which is slightly higher than that of standard drug dosed at 5 mg/kg (Table 4).

Preliminary Phytochemical Screening

The presence of phytochemical constituents was tested on L-80ME and R-80ME. Flavonoids and terpenoids are rich secondary metabolites in both crude extracts of *V officinalis*. Tannins were detected in the roots but not in the leaves. Glycosides, steroids, and alkaloids were not detected in both crude extracts (Table 5).

Discussion

There is a great demand for newer, more efficacious, less costly, and safer herbal medicines to overcome the current challenges, including drug resistance, toxicity, and treatment failure.[4,30] Plants have been traditionally used for treatment of various diseases, including diarrhea without assessing the safety and efficacy. It is of paramount importance to screen and validate the safety and efficacy profile of herbal medicines that are under use in complementary and alternative medicines. This study was aimed to validate the safety and efficacy of the antidiarrheal agent *V officinalis* based on folkloric medicine.

Hydro-alcoholic cosolvents are generally considered to give high extraction yields, owing to their expanded polarity index.[31] Generally, cosolvents such as 80% methanol seem to possess the optimum solubility characteristics for crude extraction of plant materials and therefore, 80% methanol has been used as a universal solvent for extended extraction of bioactive metabolites. Hence, the roots and leaves of *V officinalis* were extracted with this solvent mixture.

The acute oral toxicity of R-80ME and L-80ME of *V officinalis* was evaluated based on OECD guideline 2008:425.[19] To this end, the LD_{50} was found to be >2000 mg/kg for both extracts. Generally, if the LD_{50} value of the test substance is greater than 3 times the minimum effective dose, the substance is considered as a good candidate for further studies.[32] Since both extracts had an LD_{50} value of more than 3 times the minimum effective dose, it was considered as a good candidate for further studies. In toxicology, LD_{50} has also been used for classification of chemicals. Based on the World Health Organization hazard classification schemes, both extracts of *V*

Table 4. The *In Vivo* Antidiarrheal Index of the R-80ME of *Verbena officinalis.*

Dose Administered (mg/kg)	Delay in Defecation (*Dfreq*) (%)	Gut Meal Travel Distance (*Gmeq*) (%)	Purging Frequency (*Pfreq*) (%)	In Vivo Antidiarrheal Index (ADI)
CON	—	—	—	—
R-80ME100	77.29	12.45	21.20	27.32
R-80ME200	191.77	33.52	55.30	70.84
R-80ME400	232.26	52.97	70.24	95.25
LOP 5	196.31	45.80	65.90	83.99

Abbreviations: CON, negative control (10 mL/kg distilled water); LOP, loperamide; R-80ME, 80% methanol extract of roots.

Table 5. Preliminary Phytochemical Screening of R-80ME and L-80ME of *Verbena officinalis.*

	Morphological Parts of *V officinalis*	
Constituents	Root	Leaf
Cardiac glycosides	−	−
Flavonoids	+	+
Alkaloids	−	−
Saponins	−	−
Steroids	−	−
Tannins	+	−
Terpenoids	+	+

Abbreviations: R-80ME, 80% methanol extract of roots; L-80ME, R-80ME, 80% methanol extract of leaves; "+", present, "−", absent.

officinalis with LD_{50} > 2000 mg/kg are treated as "unlikely to be hazardous."[33]

Diarrhea is a symptom of many diseases/disorders whereby an imbalance between the absorptive and secretary processes of gastrointestinal tract and/or an alteration of motility of intestinal smooth muscles play a pivotal role.[34] The use of castor oil as diarrhea-inducing agent is very well documented.[35,36] On oral administration, it induces irritant laxative effect mediated by its active metabolite, ricinoleic acid—a hydroxylated fatty acid released by intestinal lipases. Ricinoleic acid induces local irritation of the intestinal mucosa, causing the release of prostaglandins that eventually increase net secretion of water and electrolytes and gastrointestinal motility.[26,37]

Castor oil–induced diarrheal model was aimed to evaluate the potential of a test substance in reducing the urgency of defecation, frequency, and weight of fecal output as well as in modifying the consistency of stools. The R-80ME (at middle and higher doses) significantly delayed the onset of diarrhea and reduced the frequency of both wet and total fecal outputs. The maximum dose of R-80ME revealed a significant effect in reducing the weight of fecal outputs. Despite its modest effect in hindering diarrheal urgency, the L-80ME at middle and high doses significantly reduced the number of wet fecal outputs. However, the overall percentage inhibition is less than the effect of R-80ME. Generally, diarrhea is characterized by fecal urgency and incontinence.[38,39] Substances exhibiting potential antidiarrheal activity may have a tendency to retard the onset of diarrhea significantly as observed in the root extract of *V officinalis* Even though diarrhea has been defined over time by various scientific groups and organizations in different ways, greater emphasis is given on the consistency of stools than the number.[39] Therefore, determination of percentage inhibition has mainly focused on the reduction of frequency of wet fecal outputs as a good marker of antidiarrheal activity. Diarrhea is also presented with an increase in weight of defecation.[39,40] Accordingly, both extracts displayed a dose-dependent reduction in weight of fecal outputs, with greater intensity seen in root extract, indicating the antidiarrheal potential of the extract. With regard to other medicinal plants having confirmed antidiarrheal activity, the antidiarrheal activity of the root extract of this plant revealed comparable effect (74.9%) with the 80ME of leaves of *Myrtus communis* at the same maximum doses (400 mg/kg).[22] 80ME of the leaves of *Lantama camara* (87.6%),[21] and *Mimusops kummel* (85.7%)[41] also exhibited better antidiarrheal activity at 400 mg/kg. Other medicinal plants, including *Ajuga remota*,[23] *Idigofera spicata*,[42] and *Osyris quadripartia*,[43] showed relatively lower antidiarrheal activities compared with the same dose of the root extracts of *V officinalis* though they exhibited comparable effects when compared with the leave counterparts.

Observing the overall antidiarrheal activity of both morphological parts of the plant material (leaves and roots), the root had exhibited a better antidiarrheal potential and chosen for further *in vivo* antimotility and antisecretory studies elucidating the potential mechanisms of action at glimpse. The reduction of gastrointestinal motility is one of the mechanisms by which antidiarrheal agents act.[44] To this end, the R-80ME, particularly at middle and maximum doses, significantly suppressed the movement of charcoal marker along the small intestine. This finding suggests that the extract has the ability to influence the peristaltic movement of intestine thereby indicating the presence of an antimotility activity in-vivo.

The enter-opooling model was designed to evaluate the secretory components of diarrhea. In this model, the R-80ME, at middle and maximum doses, showed significant reduction in both the volume and weight of intestinal contents relative to negative control. Mascolo et al[45] reported that the active metabolite, ricinoleic acid can activate the nitric oxide pathway and induces nitric oxide (NO)–mediated intestinal secretion. A growing body of evidence has revealed that phytochemical constituents such as terpenoids[46] and flavonoids[47] are implicated in attenuation of NO synthesis. In line with the present preliminary phytochemical screening, previous

phytochemical studies indicated that flavonoids and terpenoids are major secondary metabolites found in this plant.[48-50] It is further supported by the study conducted by Carnat et al[51] who isolated luteolin 7-diglucuronide as a major flavonoid in *V officinalis*. Moreover, as it is rich in tannin, the root of *V officinalis* has been treated as a potential astringent.[49] Although based on qualitative phytochemical analysis only, current data suggest that the difference in antidiarrheal activity of the 2 morphological parts of this medicinal plant can partly be ascribed to the presence of tannins in roots but not in leaves.

Generally, the *in vivo* ADI value indicates the potential of a given extract in treating diarrhea.[35] The ADI has shown a linear increment with dose, suggesting the dose dependency nature of this parameter. The R-80ME at 400 mg/kg showed highest in vivo ADI value, which is slightly higher than the standard drug, reinforcing the notion that this plant is endowed with promising antidiarrheal activity. Besides, having a broad-spectrum antimicrobial activity,[52,53] the plant is a good candidate for management of diarrheal diseases with diverse pathophysiology, including those with infectious etiology.

Conclusion

This study revealed that the extracts of *V officinalis* are endowed with secondary metabolites having promising antidiarrheal activity. The root extract has better antidiarrheal potential than that of the leaf counterparts. Considering the high safety margin (median lethal dose >2000 mg/kg) and dose dependent nature of the extracts, the recommended effective dose should be between 200 and 400 mg/kg. Generally, this finding provides a scientific evidence for acclaimed traditional use of *V officinalis* for treatment of diarrheal diseases, including those with infectious component.

Acknowledgments

We would like to acknowledge Haramaya University for granting finance and materials to conduct this laboratory-based research work.

Author Contributions

MS conceived and designed the study; MS, NB, and TG conducted the experiment and acquired the data. MS also analyzed, and interpreted the data, drafted the manuscript and prepared the final version for publication. All authors have read and approved the final version.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Haramaya University covered financial costs for this research work.

ORCID iD

Mekonnen Sisay https://orcid.org/0000-0001-6611-1174

Ethical Approval

Ethical approval of this research project was obtained from Institutional Health Research Ethics Review Committee of College of Health and Medical Sciences, Haramaya University. The ARRIVE guidelines were also strictly followed while conducting this research work.

References

1. Olajuyigbe O, Afolayan A.Ethnobotanical survey of medicinal plants used in the treatment of gastrointestinal disorders in the Eastern Cape Province, South Africa. *J Med Plants Res*. 2012;6:3415-3424. doi:10.5897/JMPR11.1707
2. Pathak K, Das RJ. Herbal medicine—a rational approach in health care system. *Int J Herbal Med*. 2013;1:86-89.
3. Tiwari S. Plants: a rich source of herbal medicine. *J Nat Prod*. 2008;1:27-35.
4. Kumar R, Sharma RJ, Bairwa K, Roy RK, Kumar A. Pharmacological review on natural antidiarrhoel agents. *Der Pharma Chemica*. 2010;2:66-93.
5. Hedberg I, Kelbessa E, Edwards S, Demissew S, Person E. *Flora of Ethiopia and Eritrea: gentianaceae to cyclocheilaceae*. Vol 5. Addis Ababa, Ethiopia: Addis Ababa University; 2006.
6. Calvo MI. Anti-inflammatory and analgesic activity of the topical preparation of *Verbena officinalis* L. *J Ethnopharmacol*. 2006;107:380-382.
7. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J Ethnopharmacol*. 2005;96:145-150.
8. Rigat M, Bonet MÀ, Garcia S, Garnatje T, Valles J.Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula). *J Ethnopharmacol*. 2007;113:267-277.
9. Guarrera PM, Forti G, Marignoli S.Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). *J Ethnopharmacol*. 2005;96:429-444.
10. Teklay A, Abera B, Giday M.An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *J Ethnobiol Ethnomed*. 2013;9:65.
11. Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk JGEthno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. *J Ethnobiol Ethnomed*. 2013;9:32.
12. Molla AE, Asfaw Z, Kelbessa E, Nagappan R.Ethnobotanical study of traditional medicinal plants in and around Fiche District, Central Ethiopia. *Curr Res J Biol Sci*. 2014;6:154-167. doi:10.19026/crjbs.6.5515
13. Gebeyehu G, Asfaw Z, Enyew A, Raja N. Ethnobotanical study of traditional medicinal plants and their conservation status in Mecha Wereda West Gojjam Zone of Ethiopia. *Int J Pharm Health Care Res*. 2014;2:137-154.
14. Gedif T, Hahn HJ. The use of medicinal plants in self-care in rural central Ethiopia. *J Ethnopharmacol*. 2003;87:155-161.
15. Parvez N, Yadav S.Ethnopharmacology of single herbal preparations of medicinal plants in Asendabo district, Jimma, Ethiopia. *Indian J Traditional Knowledge*. 2010;9:724-729.

16. Teklehaymanot T, Giday M.Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *J Ethnobiol Ethnomed*. 2007;3:12.
17. Woldeab B, Regassa R, Alemu T, Megersa M. Medicinal plants used for treatment of diarrhoeal related diseases in Ethiopia. *Evid Based Complement Alternat Med*. 2018;2018:4630371.
18. Calvo MI, Akerreta S, Cavero RYThe pharmacological validation of medicinal plants used for digestive problems in Navarra, Spain. *Eur J Integr Med*. 2013;5:537-546.
19. Organization of Economic Cooperation and Development. *Guidelines for Testing of Chemicals: Guideline 425: Acute Oral Toxicity*. Paris, France. The Organization of Economic Co-operation and Development. http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-ofchemicals-section-4-health-effe. Accessed May 14, 2019.
20. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th ed. Washington, DC: National Academies Press; 2001.
21. Tadesse E, Engidawork E, Nedi T, Mengistu G. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (*Verbenaceae*) in mice. *BMC Complement Altern Med*. 2017;17:190.
22. Sisay M, Engidawork E, Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of *Myrtus communis* Linn (*Myrtaceae*) in mice model. *BMC Complement Altern Med*. 2017;17:103.
23. Yacob T, Shibeshi W, Nedi T. Antidiarrheal activity of 80% methanol extract of the aerial part of *Ajuga remota* Benth (*Lamiaceae*) in mice. *BMC Complement Altern Med*. 2016;16:303.
24. Degu A, Engidawork E, Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extract of *Croton macrostachyus* Hocsht. ex Del. (*Euphorbiaceae*) in mice model. *BMC Complement Altern Med*. 2016;16:379.
25. Igboeli N, Onyeto CA, Okorie AN, Mbaoji FN, Nwabunike IA, Alagboso DI. Antidiarrheal activity of methanol leaf extract of *Lophira lanceolata* Tiegh (*Ochnaeceae*). *Merit Res J Environ Sci Toxicol*. 2015;3:2350-2266.
26. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay: a test for diarrhea produced by prostaglandins. *Prostaglandins*. 1976;11:809-828.
27. Than A, Kulkarni HJ, Hmone W, Tha S. Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. *Int J Crude Drug Res*. 1989;27:195-200.
28. Zohra SF, Meriem B, Samira S, Muneer MA. Phytochemical screening and identification of some compounds from mallow. *J Nat Prod Plant Resource*. 2012;2:512-516.
29. Bhandary SK, Kumari NS, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. https://pdfs.semanticscholar.org/027e/9f8f5c856ba6c73fac15060c058ce32198a7.pdf. Accessed May 14, 2019.
30. Komal KS, Rana AC. Herbal approaches for diarrhoea: a review. *Int Res J Pharm*. 2013;4:31-38.
31. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. *Int J Appl Nat Sci*. 2012;1:8-26.
32. Carol SA, Michael JD. Acute, sub chronic and chronic toxicology. In: Derelanko MJ, Hollinger MA, eds. *Handbook of Toxicology*. Boca Raton, FL: CRC Press; 1995:51-104.
33. World Health Organization. The guidebook to the registration of public health pesticides and repellents against vectors: hazard classification-acute LD50 values of formulated products. https://www.nea.gov.sg/docs/default-source/resource/guidebook-for-the-registration-of-public-health-pesticides-and-repellents.pdf. Accessed May 14, 2019.
34. Gidudu J, Sack D, Pina M, et al; Brighton Collaboration Diarrhea Working Group. Diarrhea: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine*. 2011;29:1053-1071.
35. Okpo S, Ching F, Ekeleme I. Evaluation of the anti-diarrhoeal activity of the aqueous extract from leaves of *Pterocarpus santalinoides*. *Res J Pharm Biol Chem Sci*. 2011;2:590-597.
36. Shiferie F, Shibeshi W. In vivo antidiarrheal and ex-vivo spasmolytic activities of the aqueous extract of the roots of *Echinops kebericho* (*Asteraceae*) in rodents and isolated guinea-pig ileum. *Int J Pharm Pharmacol*. 2013;2:110-116.
37. Horton EW, Main IH, Thompson CJ, Wright PM. Effect of orally administered prostaglandin E1 on gastric secretion and gastrointestinal motility in man. *Gut*. 1968;9:655-658.
38. World Gastroenterology Organization Guidelines. Acute diarrhea in adults and children: a global perspective. http://www.worldgastroenterology.org/UserFiles/file/guidelines/acute-diarrhea-english-2012.pdf. Accessed September 5, 2018.
39. World Health Organization. Diarrheal disease. http://www.who.int/mediacentre/factsheets/fs330/en. Accessed October 8, 2018.
40. Thomas PD, Forbes A, Green J, et al. Guidelines for the investigation of chronic diarrhoea. *Gut*. 2003;52:v1-v15.
41. Molla M, Gemeda N, Abay SM. Investigating potential modes of actions of *Mimusops kummel* fruit extract and solvent fractions for their antidiarrheal activities in mice. *Evid Based Complement Alternat Med*. 2017;2017:4103410.
42. Birru EM, Asrie AB, Adinew GM, Tsegaw A. Antidiarrheal activity of crude methanolic root extract of *Idigofera spicata* Forssk (*Fabaceae*). *BMC Complement Altern Med*. 2016;16:272.
43. Teferi MY, Abdulwuhab M, Yesuf JS. Evaluation of in vivo antidiarrheal activity of 80% methanolic leaf extract of *Osyris quadripartita* Decne (*Santalaceae*) in Swiss Albino Mice. *J Evid Based Integr Med*. 2019;24:2515690X19833340.
44. Schiller LR, Ana CAS, Morawski SG, Fordtran JS. Mechanism of the antidiarrheal effect of loperamide. *Gastroenterology*. 1984;86:1475-1480.
45. Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and castor oil-induced diarrhea. *J Pharmacol Exp Ther*. 1994;268:291-295.
46. Jang DS, Min HY, Jeong YH, Lee SK, Seo EK. Di-and sesquiterpenoids isolated from the pods of *Sindora sumatrana* and their potential to inhibit lipopolysaccharide-induced nitric oxide production. *Arch Pharm Res*. 2004;27:291-294.
47. Messaoudene D, Belguendouz H, Ahmedi ML, et al. Ex vivo effects of flavonoïds extracted from *Artemisia herba alba* on

cytokines and nitric oxide production in Algerian patients with Adamantiades-Behçet's disease. *J Inflamm (London)*. 2011;8:35.
48. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. *Iran J Pharm Res*. 2003;2:77-82.
49. Rehecho S, Hidalgo O, de Cirano MGI, et al. Chemical composition, mineral content and antioxidant activity of *Verbena officinalis* L. *LWT-Food Sci Technol*. 2011;44:875-882.
50. Schönbichler S, Bittner L, Pallua J, et al. Simultaneous quantification of verbenalin and verbascoside in *Verbena officinalis* by ATR-IR and NIR spectroscopy. *J Pharm Biomed Anal*. 2013;84:97-102.
51. Carnat A, Carnat AP, Chavignon O, Heitz A, Wylde R, Lamaison JL. Luteolin 7-diglucuronide, the major flavonoid compound from *Aloysia triphylla* and *Verbena officinalis*. *Planta Med*. 1995;61:490.
52. Ahmed D, Qasim KA, Ashraf CM, Maab H. *Verbena officinalis*: a herb with promising broad spectrum antimicrobial potential. *Cogent Chemistry*. 2017;3:1363342. doi:10.1080/23312009.2017.1363342
53. Casanova E, García-Mina J, Calvo MI. Antioxidant and antifungal activity of *Verbena officinalis* L. leaves. *Plant Foods Hum Nutr*. 2008;63:93-97.