

Evaluation of In Vivo Antidiarrheal Activity of 80% Methanolic Leaf Extract of *Osyris quadripartita* Decne (Santalaceae) in Swiss Albino Mice

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Abstract

The leaf of *Osyris quadripartita* is traditionally used for the management of diarrhea in different parts of Ethiopia. However, its use has not been scientifically validated for its efficacy. The aim of this study was to investigate antidiarrheal activity of hydroalcoholic leaf extract of *O. quadripartita* in mice models. Different doses of the methanolic leaf extract of *O. quadripartita* (100, 200, and 400 mg/kg) were tested for antidiarrheal activity using castor oil-induced diarrhea, enteropooling, and gastrointestinal motility models in Swiss Albino mice. The activities of the extract at different doses were compared with standard drugs and negative control groups of mice. The extract at all tested doses resulted in significant reduction ($P < .01$) in number of wet feces, whereas significant reduction ($P < .01$) in frequency of defecation in castor oil-induced diarrhea was seen at a dose of 400 mg/kg. It also showed a dose-dependent and significant reduction of volume of intestinal content in the enteropooling model at all tested doses and the observed results in 200 and 400 mg/kg were better than the standard drug, loperamide. However, significant antimotility effect was not observed at any of the tested doses. From these results we can conclude that methanolic leaf extract of *O. quadripartita* showed antidiarrheal activity.

Keywords

Osyris quadripartita, antidiarrheal index, castor oil, medicinal plants

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Diarrhea is a gastrointestinal disorder, characterized by an alteration in a normal bowel movement, an increase in the water content, volume or frequency of stools.¹ A decrease in consistency (i.e, soft or liquid), an increase in frequency of bowel movements, and 3 or more stools per day have often been used as a definition for epidemiological investigations.² It is often accompanied by pain, urgency, perianal discomfort, and incontinence.³ It is a sign or symptom, not a disease by itself, and can be caused by numerous conditions.^{1,4}

The epidemiology of diarrhea varies in developed versus developing countries. The burden of diarrheal illness sits firmly in the developing world, both for morbidity (6-7 episodes per child per year compared with 1 or 2 in the developed world) and mortality.⁵ It is the second gravest cause of mortality of children younger than 5 years worldwide next to pneumonia especially in developing countries along with its long-term impact on growth and cognitive development.⁶⁻⁸ In Ethiopia, diarrhea kills half million under-5 children annually secondary to pneumonia.⁹ The 2010 report of the Ministry of Finance and

Economic Development (MOFED) showed that 20% of childhood deaths in Ethiopia were due to diarrhea.¹⁰ Diarrhea accounts for approximately 14% of outpatient visits and 16% hospital admissions.¹¹ And despite the availability of vast spectrum of approaches for diarrheal management, majority of people in developing countries rely on herbal drugs for the management of diarrhea.¹²⁻¹⁴ Currently used drugs are important in the management of diarrhea, but they still are linked with adverse effects and contraindications.^{15,16} For instance, racecadotril and loperamide are used to treat secretory diarrhea

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but they produce bronchospasm, vomiting, and fever.¹⁵ Serious heart problems are also associated with high doses of loperamide (Imodium).¹⁶ Moreover, some are contraindicated in children less than 6 years of age (loperamide) and intestinal obstruction.¹⁵ Traditionally, *O. quadripartita* is used for treatment of cancer, diarrhea,¹⁷ tuberculosis,¹⁸ peptic ulcer disease,¹⁹ skin lesion and infection,^{20,21} jaundice,²² abdominal pain, and urine problem.²³ Experimental studies indicated that *O. quadripartita* has shown anti-inflammatory,²⁴ antioxidant,²⁵ antimicrobial, and antifungal,²⁶ and antimalarial²⁷ activities.

In developing countries, majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea.^{12,13} It would be interesting to search for plants with antidiarrheal activities that could be used against any type of diarrheal disease. A range of medicinal plants with antidiarrheal properties have been widely used by traditional healers. However, therapeutic potentials of some of these medicines have not been scientifically evaluated.¹³ Evaluating the therapeutic potential of these medicinal plants scientifically is too worthy to get new antidiarrheal drugs with novel mechanism of action and with minimal side effects, as a single medicinal plant will not be representative for all. Among these medicinal plants, *O. quadripartita*, widely possesses a number of ethnomedical uses in Ethiopia. But there is no pharmacological study done to confirm the antidiarrheal activity of *O. quadripartita* leaf. Therefore, it is necessary to establish the scientific basis for antidiarrheal activity of *O. quadripartita*.

Material and Methods

Drugs and Chemicals Used

The following drugs and chemicals were used in this study with their source written in parentheses. Methanol (Nice Chemicals Pvt Ltd, India), atropine sulfate (Aculife, Health Care Pvt Ltd, India), activated charcoal (SD Fine Chem Ltd, India), loperamide hydrochloride 2 mg (Remedica, Cyprus), castor oil (Amman Pharmaceutical, Jordan), chloroform (Lab Tech Chemicals), iron chloride (Supertek Chemicals, India), lead acetate trihydrate (Guangdong Chemical Reagent Engineering, China), sulfuric acid (Hi Media Laboratory Pvt Ltd, India), hydrochloric acid (Nice Laboratory Reagents, India), potassium iodide (Supertek Chemicals, India), benzene (Nice Laboratory Reagents, India), ammonia solution (Lab Tech Chemicals, India), acetic acid (Sigma Aldrich, Germany), and mercuric chloride (Supertek Chemicals, India).

Plant Material Collection and Identification

Enough amounts of the fresh leaves of *O. quadripartita* were collected in Gondar, Ethiopia during the month of January 2016. The plant was identified by botanist and a voucher specimen (001) was deposited at the Department of Biology, Addis Ababa University.

Experimental Animal

Swiss Albino mice of both sexes weighing (31.25 ± 0.74 g) were used for the experiment. The mice were obtained from animal house of Department of Pharmacology, School of Pharmacy, University of Gondar. They were kept in 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 condition for 5 days prior to the actual experiments. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals²⁸ and Organisation for Economic Cooperation and Development (OECD) guidelines.²⁹ A total of 90 mice were used in this study.

Extraction Procedure

Fresh leaves of *O. quadripartita* were collected, washed, dried under shade and extracted as described by Girma et al²⁷ with slight modification. The dried plant was coarsely powered by using a mortar and pestle. 600 g plant extract material was macerated in 2400 mL of 80% methanol for 72 hours. The beakers were sealed with aluminum foil and accompanying occasional shaking and stirring. After 72 hours, the mixture was first filtered using muslin cloth and then with filter paper No. 3 (Labsman, India). The residue was remacerated in 1800 mL of 80% methanol for the second time for another 72 hours and filtered similar to the first one. Finally, the residue was macerated in 1500 mL of 80% methanol for the third time for 72 hours, successively and filtered using muslin cloth and filter paper. Then the filtrate was dried in an oven at a temperature of 40°C. After drying, the dried extract was weighed and kept in desiccators in screw cap vials until use.

The percentage yield of the extract was calculated using the following formula³⁰:

$$\text{Yield} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100$$

Preliminary Phytochemical Screening of *Osyris quadripartita* Leaves

The crude methanol extract was assessed for secondary metabolites such as flavonoids, tannins, anthraquinones, glycoside, steroid, phenols, terpenoids, alkaloids, and saponins using standard screening tests.^{23,31}

Grouping and Dosing of Animals

The animals were randomly assigned to 5 groups, each consisting of 6 mice. The animals of group I were considered as the negative control and treated with distilled water 10 mL/kg, group II served as positive control and treated with standard drugs (loperamide for castor oil-induced diarrhea and enteropooling, atropine sulfate for gastrointestinal motility test), animals of group III, IV, and V were treated with doses of 100, 200, and 400 mg/kg of methanolic leaf extract of *O. quadripartita*, respectively, based on acute toxicity test result.²⁷ According to Girma et al,²⁷ with the acute toxicity test at the limit test dose of 2000 mg/kg the experimental mice did not show any indication of gross physical or behavioral changes such as hair erection, reduction in feeding and motor activities within the 24 hours monitoring period as well as within the observation period of 2 weeks.

As a result, 10% of the limit dose (200 mg/kg) was selected as middle dose, half of it (100 mg/kg) as lower dose and 2 times the middle dose (400 mg/kg) was taken as higher dose based on OECD guideline.²⁹

Models for Antidiarrheal Activity

Castor Oil–Induced Diarrhea. The method described by Shoba and Thomas³² was followed for this study with slight modification. Thirty mice fasted for 12 hours were randomly allocated to 5 groups of 6 animals each. Group I (received distilled water 10 mL/kg) served as control group, group II received the standard drug loperamide 3 mg/kg orally, and groups III, IV, and V received the methanolic leaf extract of *O. quadripartita* at doses of 100, 200, and 400 mg/kg, respectively. The extract and the standard drug, loperamide were dissolved with distilled water and volume administered was 10 mL/kg for all mice. One hour after administration, all mice received 0.5 mL of castor oil by an oral gavage and then they were individually placed on the floor, which was covered with dry toner plain paper copier film (nonwetting transparent paper). The floor lining was changed each time the mouse defecated. During an observation period of 4 hours, the time of onset of diarrhea, the total number of fecal output (frequency of defecation), consistency of feces (wet and dry diarrheal drops) excreted by the mice were recorded and compared with the control group. The results were expressed as a percentage of inhibition of diarrhea:

% Inhibition of diarrhea

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

Castor Oil–Induced Enteropooling. The effect of the extract on inhibition of intraluminal fluid accumulation was determined by measuring the volume of fluid accumulated in intestine of mice. Thirty mice were fasted for 18 hours prior to experiment and divided into 5 groups of 6 mice each. Group I (negative control) were treated with 10 mL/kg

distilled water. Group II were treated with standard drug (loperamide 3 mg/kg orally). Groups III, IV, and V were treated with the extract at doses of 100, 200, and 400 mg/kg, respectively. One hour later, all the mice were challenged with 0.5 mL of castor oil orally. After 1 hour, the mice were sacrificed, the pyloric and cecum ends of the small intestine were tied and the intestine was removed. The intestinal contents were expelled into a measuring cylinder and then the volume was measured. Then, the percentage inhibition was calculated.³⁰

% Inhibition of diarrhea

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

Gastrointestinal Motility Test by Charcoal Meal. Gastrointestinal motility test was evaluated according to the method described by Ezeja and Anaga.³³ Mice that were fasted for 18 hours were randomly assigned to 5 groups: group I received distilled water (10 mL/kg orally), group II received atropine sulfate (5 mg/kg intraperitoneally), and groups III, IV, and V received different dose of the extract at doses of 100, 200, and 400 mg/kg, respectively, 30 minutes before the administration of 0.5 mL of castor oil. Thirty minutes after the administration of castor oil, each mouse received 0.5 mL of 10% charcoal suspension in 5% of acacia gum by oral gavages. Thirty minutes later, mice were sacrificed, the abdomen opened, and small intestine was removed. Then the total length of small intestine was measured with a calibrated ruler. Thereafter, the distance covered by charcoal from the pylorus to the cecum was measured and expressed as a percentage of the overall length of the small intestine from where the percent inhibition of movement was calculated.

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

Peristalsis index

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

The in vivo antidiarrheal index (ADI in vivo) was then expressed according to the formula developed by Aye-Than et al³⁴:

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

where *D freq* is the delay in defecation time or diarrhea onset obtained from castor oil diarrhea test, *G meq* is the gut meal travel reduction (as % of control) obtained from charcoal meal test (% inhibition), and *P freq* is the purging frequency or reduction in the number of wet stools (as % of control) obtained from castor oil–induced diarrheal model (% inhibition of defecation).

Data Analysis

All results were expressed as mean \pm standard error of the mean (SEM). Statistical analysis was performed using SPSS statistical software version 20. Statistical significance differences within and between groups were assessed by one-way analysis of variance followed by post hoc Tukey's multiple comparison test. The results were considered for statistical significance at *P* value less than .05.

Ethical Clearance

The animals were handled according to the guidelines for Care and Use of Laboratory Animals²⁸ and OECD guidelines.²⁹ The proposal was submitted for approval to Department of Pharmacology and ethical clearance was obtained from Department of Pharmacology (SOP/51/2016) to continue the research.

Results

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the 80% methanolic extract of *O. quadripartita* leaves revealed the presence of tannins, saponins, flavonoids, terpenoids, alkaloids, and phenols. On the other hand, steroids, anthraquinones, and glycoside were absent (Table 1).

Effect of *Osyris quadripartita* on Castor Oil–Induced Diarrhea

The 80% methanolic extracts of *O. quadripartita* leaves were found to be effective against castor oil–induced diarrhea on

Table 1. Preliminary Phytochemical Screening Results of 80% Methanolic Extract of *Osyris quadripartita* Leaf.

Secondary Metabolites	Results
Tannins	Positive
Saponins	Positive
Flavonoids	Positive
Terpenoids	Positive
Steroids	Negative
Alkaloids	Positive
Phenols	Positive
Anthraquinones	Negative
Glycosides	Negative

mice at all tested doses in terms of reduction of frequency of defecation and number of wet feces (consistency of feces).

Number of wet feces was markedly reduced ($P < .01$) with methanolic extracts of *O. quadripartita* at a dose of 400 mg/kg with percentage inhibition of 57.42%. The extract has also shown significant reduction ($P < .01$) of the number of wet defecations at doses of 200 and 100 mg/kg with similar percentage inhibition of 55.75%. Loperamide 3 mg/kg (positive control) has also shown highly significant reduction ($P < .001$) of wet defecations with percentage inhibition of 70.5% (Table 2).

The standard drug, loperamide 3 mg/kg significantly ($P < .05$) delayed the onset of diarrhea (161.17 ± 41.12 minutes) or prolonged diarrhea-free period induced by castor oil when compared with the distilled water-treated group (44.67 ± 4.64 minutes) (Table 2).

In terms of decreasing total number of defecation (frequency of defecation) within 4 hours, the methanolic extract of *O. quadripartita* at dose of 100 mg/kg showed significant reduction ($P < .01$) of the frequency of defecation with percentage inhibition of 50.69%. The extract also showed significant reduction ($P < .05$) of the frequency of defecation at doses of 200 and 400 mg/kg ($P < .01$) with percentage inhibitions of 43.48% and 49.30%, respectively. The positive control, loperamide 3 mg/kg showed significant reduction of frequency of defecation ($P < .001$) with percentage inhibition of 62.35% (Table 3).

Effect of *Osyris quadripartita* on Castor Oil-Induced Enteropooling

The methanolic extract of *O. quadripartita* leaves at dose of 400 mg/kg significantly ($P < .001$), reduced the volume of fluid accumulation in the intestine with percentage inhibition of 53.16% as compare to negative control group. At dose of 200 and 100 mg/kg, the extract significantly ($P < .01$) reduced the volume fluid accumulation in the intestine with percentage inhibition 39.24% and 35.44%, respectively, as compared with negative control. The standard drug loperamide 3 mg/kg significantly ($P < .01$) reduced the volume of intestinal fluid produced by castor oil with percentage inhibition 35.44% as compare with the distilled water-treated group. The extract at

doses of 200 and 400 mg/kg had better effect than the standard drug (Table 4).

Effect of *Osyris quadripartita* on Gastrointestinal Motility by Charcoal Meal

The effect of the extract on the intestinal motility is shown in Table 5. All tested doses of the extract did not produce significant reduction in the percent of intestinal motility in mice compared with negative control group. The negative control group resulted in $84.05\% \pm 6.22\%$ intestinal motility by the charcoal meal marker. The extract at 100, 200, and 400 mg/kg oral doses exhibited $80.83\% \pm 9.51\%$, $77.25\% \pm 9.03\%$, and $76.76\% \pm 14.04\%$ inhibition of intestinal motility, respectively. However, the standard drug atropine sulfate produced significant inhibition ($44.59\% \pm 6.17\%$, $P < .05$) in intestinal motility as compared with the negative control.

In Vivo Antidiarrheal Index (ADI In Vivo)

Results for the in vivo ADI were 30.06, 35.85, and 29.19 at the dose of 100, 200, and 400 mg/kg oral doses of the plant extract, respectively, while standard drug produced a maximum index of 91.39 as shown in Table 6.

Discussion

The traditionally claimed antidiarrheal activity of *O. quadripartita* leaves on experimentally induced diarrhea in mice was evaluated by using 3 antidiarrheal activity testing models: castor oil-induced diarrhea, castor oil-induced enteropooling, and castor oil-induced gastrointestinal motility in Swiss Albino mice.

Castor oil induces diarrhea within 1 to 2 hour just after administration of 0.1 to 0.3 mL castor oil for mice as different researches have reported.^{15,35} In castor oil-induced diarrhea model of this study, diarrheal episode was seen within 1 hour in most of the experimental mice probably due to high dose of castor oil (0.5 mL/kg) as seen in a similar study.¹⁵ In castor oil-induced diarrhea model, the methanolic extract of *O. quadripartita* leaves at all doses significantly ($P < .01$) reduced number of wet feces. It also showed significant reduction of frequency of defecation with $P < .01$, $P < .05$, and $P < .01$, respectively, at doses of 100, 200, and 400 mg/kg. Loperamide 3 mg/kg significantly ($P < .001$) decreased the number of wet feces, decreased frequency of defecation, and also delayed the onset of diarrhea. The results obtained from this model are in line with reports elsewhere, where methanolic leaf extract of *Pterocarpus erinaceus*³⁰ and methanolic leaf extract of *Bombax buonopozense*,³⁶ showed significant reduction of number of wet feces. After 1 hour of administration of castor oil, diarrhea was observed for 4 hours in negative control whereas reduced diarrheal episode was observed in loperamide-treated group (positive control) and in the *O. quadripartita* leaf extracts-treated groups (Table 3).

Table 2. Effect of *Osyris quadripartita* Leaf Extract on Castor Oil–Induced Diarrhea in Mice (n = 6).

Groups	Time of Onset of Diarrhea (Minutes) or Diarrhea-Free Period, Mean ± SEM	Number of Dry Feces, Mean ± SEM	Number of Wet Feces, Mean ± SEM	% Inhibition of Diarrhea
CON	44.67 ± 4.64	1.33 ± 0.42	10.17 ± 0.60	—
LOP	161.17 ± 41.12 ^{a,1}	1.33 ± 0.42	3.00 ± 1.26 ^{a,3}	70.50
MOQ 100	107.17 ± 23.65	1.17 ± 0.54	4.50 ± 0.50 ^{a,2}	55.75
MOQ 200	103.17 ± 10.71	2.00 ± 0.57	4.50 ± 1.25 ^{a,2}	55.75
MOQ 400	70.67 ± 23.86	1.50 ± 0.67	4.33 ± 1.31 ^{a,2}	57.42

Abbreviations: SEM = standard error of the mean; CON, negative control (distilled water 10 mL/kg, orally); MOQ 100, methanolic extract of *O. quadripartita* 100 mg/kg; MOQ 200, methanolic extract of *O. quadripartita* 200 mg/kg; MOQ 400, methanolic extract of *O. quadripartita* 400 mg/kg; LOP, loperamide 3 mg/kg, orally.

^aCompared with negative control.

^bCompared with 100 mg/kg of methanolic extract.

^cCompared with 200 mg/kg of methanolic extract.

^dCompared with 400 mg/kg of methanolic extract.

^eCompared with loperamide 3 mg/kg, orally.

¹*P* < .05.

²*P* < .01.

³*P* < .001.

Table 3. Effect of *Osyris quadripartita* Extract on Frequency of Defecation on Castor Oil–Induced Diarrhea Within 4 Hours in Mice (n = 6).

Group	Number of Defecations in Specific Time, Mean ± SEM				Total Number of Defecations, Mean ± SEM	% Inhibition of Defecation
	On the 1st Hour	On the 2nd Hour	On the 3rd Hour	On the 4th Hour		
CON	2.83 ± 0.78	5.67 ± 0.61	1.83 ± 0.31	1.17 ± 0.31	11.50 ± 0.62	—
LOP	0.50 ± 0.50 ^{a,3}	1.33 ± 0.71 ^{a,3}	1.67 ± 0.61	0.67 ± 0.21	4.33 ± 1.36 ^{a,3}	62.35
MOQ 100	1.17 ± 0.48	2.17 ± 0.54 ^{a,2}	1.83 ± 0.31	0.50 ± 0.22	5.67 ± 0.61 ^{a,2}	50.69
MOQ 200	0.83 ± 0.40	2.83 ± 0.54 ^{a,1}	1.67 ± 0.61	1.17 ± 0.40	6.50 ± 1.18 ^{a,1}	43.48
MOQ 400	1.83 ± 0.60	2.17 ± 0.48 ^{a,2}	0.83 ± 0.40	1.00 ± 0.45	5.83 ± 1.30 ^{a,2}	49.30

Abbreviations: SEM = standard error of the mean; CON, negative control (distilled water 10 mL/kg, orally); MOQ 100, methanolic extract of *O. quadripartita* 100 mg/kg; MOQ 200, methanolic extract of *O. quadripartita* 200 mg/kg; MOQ 400, methanolic extract of *O. quadripartita* 400 mg/kg; LOP, loperamide 3 mg/kg, orally.

^aCompared with negative control.

^bCompared with 100 mg/kg of methanolic extract.

^cCompared with 200 mg/kg of methanolic extract.

^dCompared with 400 mg/kg of methanolic extract.

^eCompared with loperamide 3 mg/kg, orally.

¹*P* < .05.

²*P* < .01.

³*P* < .001.

In castor oil–induced enteropooling test, the methanolic extract of *O. quadripartita* leaves reduced diarrhea significantly by reducing volume of intraluminal fluid accumulation at doses of 100, 200, and 400 mg/kg body weight with *P* < .01, *P* < .01, and *P* < .001, respectively, in a dose-dependent manner.

The extract at doses of 200 and 400 mg/kg significantly decreased the volume of intestinal fluid accumulation, with percentage inhibition of volume of intestinal fluid 39.24% and 53.16% respectively, which is better than the loperamide-treated groups (35.44%). This may be because the standard drug loperamide has mild antisecretory activity and acts mainly by altering intestinal motility (antimotility activity).³⁷ So this result indicated that the plant is a promising candidate for new antidiarrheal drug development. The results are in line with reports elsewhere, where methanolic root extract *Moringa oleifera* Lam, which showed better reduction in intraluminal fluid accumulation than the standard drug atropine.¹² This activity of

the extract is probably because of the ability of the extract to inhibit intestinal secretion and/or increase water and electrolyte absorption and the extract probably inhibit prostaglandin biosynthesis and release. During milking process the fluid accumulation occurred in the small intestine within the pylorus and the ileocecal junction in loperamide- and extract-treated groups was thick where very watery fluid was accumulated in distilled water–treated groups (negative control group).

The anti-inflammatory property of a methanolic extract of *O. quadripartita* leaves was demonstrated in rats by using various chemical mediators involved in inflammatory process, like histamine, 5-hydroxy-triptamine, and bradykinin²⁴; some of these mediators are noninfectious causes of diarrhea.³⁸ So, these activities of the extract may be responsible for the reduced intraluminal fluid accumulation by preventing intestinal mucosa from inflammation and by decreasing intestinal secretion. That means the anti-inflammatory effect partly contributes for

Table 4. Effect of *Osyris quadripartita* Leaf Extract on Intestinal Fluid Accumulation in Mice (n = 6).

Groups	Volume of the Intestinal Content (mL), Mean \pm SEM	% Inhibition of Volume of Intestinal Content
CON	0.79 \pm 0.05	—
LOP	0.51 \pm 0.03 ^{a,2}	35.44
MOQ 100	0.51 \pm 0.05 ^{a,2}	35.44
MOQ 200	0.48 \pm 0.03 ^{a,2}	39.24
MOQ 400	0.37 \pm 0.08 ^{a,3}	53.16

Abbreviations: SEM = standard error of the mean; CON, negative control (distilled water 10 mL/kg, orally); MOQ 100, methanolic extract of *O. quadripartita* 100 mg/kg; MOQ 200, methanolic extract of *O. quadripartita* 200 mg/kg; MOQ 400, methanolic extract of *O. quadripartita* 400 mg/kg; LOP, loperamide 3 mg/kg, orally.

^aCompared with negative control.

^bCompared with 100 mg/kg of methanolic extract.

^cCompared with 200 mg/kg of methanolic extract.

^dCompared with 400 mg/kg of methanolic extract.

^eCompared with loperamide 3 mg/kg, orally.

¹P < .05.

²P < .01.

³P < .001.

antidiarrheal effect. The secretory diarrhea is also associated with an activation of chloride channels, causing chloride efflux from the cell, the efflux of chloride results in massive secretion of water into the intestinal lumen and cause watery diarrhea.¹² The methanolic extract of *O. quadripartita* leaves may have inhibited the secretion of water into the lumen by blocking this mechanism.

In the gastrointestinal motility test, all tested doses of the extract failed to produce significant reduction in percentage of intestinal motility, and this indicated that the extract has poor antimotility activity. The result obtained from this model is in line with reports elsewhere, where methanolic leaf extract of *Zehneria scabra* failed to produce significant reduction in percentage of intestinal motility similarly.¹⁵ Cholinergic stimulation often cause diarrhea by increasing gastrointestinal motility³⁹ and anticholinergics like atropine sulfate prevent diarrhea by blocking cholinergic stimulation. The insignificant inhibition of gastrointestinal motility by the extract suggested that the extract has poor anticholinergic activity on intestinal mucosa. The positive control, atropine sulfate, showed significant ($P < .05$) peristalsis index (% charcoal meal transit)

Table 5. Effect of *Osyris quadripartita* Leaf Extract on Intestinal Motility in Mice Using Charcoal Meal as a Marker (n = 6).

Groups	Total Length of Intestine (cm), Mean \pm SEM	Distance Traveled by Charcoal (cm), Mean \pm SEM	% Charcoal Meal Transit (Peristalsis Index)	% Inhibition of Motility Compared With Negative Control
CON	50.92 \pm 2.07	43.00 \pm 3.85	84.05 \pm 6.22	—
ATR	49.83 \pm 2.20	21.83 \pm 2.33 ^{a,1}	44.59 \pm 6.17 ^{a,1}	46.95
MOQ 100	52.58 \pm 2.15	42.25 \pm 5.17 ^{e,1}	80.83 \pm 9.51	3.83
MOQ 200	54.50 \pm 1.63	42.33 \pm 5.40 ^{e,1}	77.25 \pm 9.03	8.09
MOQ 400	50.17 \pm 2.26	44.50 \pm 3.27 ^{e,2}	76.76 \pm 14.04	8.67

Abbreviations: SEM = standard error of the mean; CON, negative control (distilled water 10 mL/kg, orally); MOQ 100, methanolic extract of *O. quadripartita* 100 mg/kg; MOQ 200, methanolic extract of *O. quadripartita* 200 mg/kg; MOQ 400, methanolic extract of *O. quadripartita* 400 mg/kg; ATR, atropine sulfate 5 mg/kg, intraperitoneally.

^aCompared with negative control.

^bCompared with 100 mg/kg of methanolic extract.

^cCompared with 200 mg/kg of methanolic extract.

^dCompared with 400 mg/kg of methanolic extract.

^eCompared with atropine 5 mg/kg, intraperitoneally.

¹P < .05.

²P < .01.

³P < .001.

Table 6. The In Vivo Antidiarrheal Index of Methanolic Extract of Leaf of *Osyris quadripartita*.

Groups	Dose, mg/kg	D freq (From Castor Oil-Induced Diarrhea)	G meq (From Charcoal Meal Test)	P freq (From Castor Oil-Induced Diarrhea)	ADI (In Vivo)
CON	—	—	—	—	—
POS	—	260.8	46.95	62.35	91.39
MOQ	100	139.91	3.83	50.69	30.06
MOQ	200	130.96	8.09	43.48	35.85
MOQ	400	58.2	8.67	49.30	29.19

Abbreviations: CON, negative control (distilled water 10 mL/kg, orally); POS, positive control (loperamide 3 mg/kg, orally, in case of castor oil-induced diarrhea and castor oil-induced enteropooling; atropine sulfate 5 mg/kg intraperitoneally, in case of gastrointestinal motility test). MOQ, methanolic extract of *O. quadripartita*. D freq, the delay in defecation time or diarrhea onset obtained from castor oil diarrhea test; G meq, the gut meal travel reduction (as % of control) obtained from charcoal meal test (% inhibition); P freq, the purging frequency or reduction in the number of wet stools (as % of control) obtained from castor oil-induced diarrheal model (% inhibition of defecation); ADI (in vivo), in vivo antidiarrheal index.

compared with the negative control. This indicated that atropine sulfate prevented the movement of charcoal meal in the intestine significantly, confirming its antiperistalsis effect as anticholinergic drug. On the other hand, extracts at all doses did not show significant peristalsis index.

Antidiarrheal activity was found in plants possessing tannins, alkaloids, saponins, flavonoids, reducing sugars, steroids, and/or terpenoids.⁴⁰ In general, tannins and flavonoids have been reported to have several pharmacological activities, including antidiarrheal activity, which has been attributed to antimicrobial action and antisecretory effects.⁴¹ Preliminary phytochemical screening of methanolic extract of *O. quadripartita* leaves performed in this study revealed possible presence of alkaloids, tannins, flavonoids, terpenoids, and saponins. These constituents may be responsible for the in vivo antidiarrheal activity of methanolic extract of *O. quadripartita* leaves. The highest phenolics and flavonoids contents were detected in leaves of *O. quadripartita*,²⁵ which may be responsible for the antidiarrheal activity of the extract. Flavonoids, which are presumed to be responsible for the inhibitory effects exerted on several enzymes, including those involved in the arachidonic acid metabolism,⁴² and which inhibits the synthesis of prostaglandins (PGE₂), acts directly on the intestinal mucosa and increases water, electrolyte, and mucus secretion. So, the ability of the extract to decrease the intestinal secretion may be due the presence of flavonoids. Tannins are also plant-derived compounds that react to and bond strongly with protein and form poorly soluble protein tannate salts, which reduce secretion and produce antidiarrheal effects,^{13,43} may be responsible for antisecretory activity of the extract.

The ADI is a measure of the combined effects of these different parameters of diarrhea such as defecation frequency, onset of diarrheal stools, as well as intestinal motility. The plant extract produced a high antidiarrheal index at dose of 200 mg/kg from other tested doses (see Table 6); although it was lower than that produced by the positive control and this suggested that the antidiarrheal activity of the extract revolves around this dose. In general, lower doses of methanolic extract of *O. quadripartita* showed more antidiarrheal effect than higher dose as also evidenced by significant reduction ($P < .01$) of frequency of defecation by 100 mg/kg with higher percentage of inhibition (50.69%) than 200 and 400 mg/kg (see Table 3).

Although the specific mechanisms of action of this extract need to be investigated, the possible mechanisms of antidiarrheal activity of the methanolic extract of *O. quadripartita* leaves could be related to inhibition of secretion, reducing intraluminal fluid accumulation induced by castor oil or enhancing water and electrolyte absorption but not by reducing gastrointestinal motility. In this study, the percentage yield of 80% methanolic extract of *O. quadripartita* leaves was found 34.5%, which has similarity with other study, where the percentage yield of *O. quadripartita* during in vitro antimicrobial activity study was found 40.11%.²⁶

Conclusion

The 80% methanolic extract of *O. quadripartita* leaves showed antidiarrheal activity in animal model by decreasing number of

wet feces, frequency of defecation and by reducing intraluminal fluid accumulation in the intestine. As the plant extract and standard drugs were administered ahead of induction of diarrhea, the percentage inhibition of diarrhea was calculated by using animal models of antidiarrheal activity. Although the experimental anti-diarrheal models are not complete predictors of the clinical effectiveness of the extract, the overall findings from this study indicated the antidiarrheal potential of the methanolic extract of *O. quadripartita* leaves, even better result than loperamide-treated group was found in reducing of volume of intestinal content in the castor oil-induced enteropooling model. These findings provided a scientific support for the traditional use of this plant in the treatment of diarrheal diseases. However, the extract did not decrease castor oil-induced gastrointestinal motility at all tested doses.

Authors' Note

Most of data used/analyzed in this study are contained in the article. Additional data can be obtained from the corresponding author on reasonable request.

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Author Contributions

MYT was responsible for conception of the idea of the study, designing the study, performing the laboratory work, analysis and interpretation of the data as well as writing study. MA participated in selecting the animal models for antidiarrheal activity, analysis, and interpretation of the results, and also in the preparation of the manuscript. JSY participated in modifying initial idea of the study, in selection of animal models for antidiarrheal activity, in the laboratory work, in interpretation of the result, and authored the manuscript. All authors read and approved the final manuscript.

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

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Ethical Approval

The animals were handled according to the guide lines for Care and Use of Laboratory Animals and OECD Guidelines. The proposal was submitted for approval to Department of Pharmacology and ethical

clearance was obtained from Department of Pharmacology (SOP4/51/2016) to continue the research.

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