

Phytochemical Investigation and Anti-Diarrheal Activity of Hydroalcoholic Extract of Fruits of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae)

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Abstract

Background: Cucurbitaceae family is one of the best genetically assorted accumulations of restorative plants in the plant kingdom. Previous studies have suggested that *Citrullus colocynthis* (L.) Schrad. plant parts (root, stem, leaf, fruits, and seeds) have been utilized in the traditional system of medicine. Pharmacological activities reported for this plant include antioxidant activity, antimicrobial activity, anti-diabetic activity, anti-hyperlipidemic activity. The anti-diarrheal activity of hydroalcoholic extract of fruits of this plant is reported for the first time in the present study.

Objective: To evaluate the anti-diarrheal activity of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae) in experimentally induced diarrhea in Wistar rats.

Materials and methods: Hydroalcoholic extract of fruits of *Citrullus colocynthis* was examined for its acute toxicity on rats, in order to establish the safe doses. Castor oil induced diarrhea model and gastrointestinal motility test using barium sulfate milk were done to assess the anti-diarrheal activity of plant extracts. Extract of *Citrullus colocynthis* at the dose of 50 mg/kg, 100 mg/kg and per se group (100 mg/kg) were used in Wistar rats of either sex. Loperamide (2 mg/kg) was taken as a standard drug in both the models.

Results: Phytochemical analysis showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. The acute toxicity studies revealed that extract is relatively safe when given orally; no death was recorded at a dose of 2000 mg/kg. The dose of 100 mg/kg ($P < 0.001$) and 50 mg/kg ($P < 0.01$) of plant fruit extract significantly reduced defecation frequency in 6 h and also increased the latency time which showed similar effects as produced in loperamide treated group. Both doses of fruit extract and loperamide reduced the gastrointestinal motility in Wistar rats significantly ($P < 0.001$).

Conclusion: The hydroalcoholic extract of fruits of *Citrullus colocynthis* showed significant anti-diarrheal activity and supports its use as a complementary and alternative medicine for treatment of diarrhea.

Keywords: *Citrullus colocynthis*; Hydroalcoholic extract; Anti-diarrheal activity; Acute toxicity; Cucurbitaceae; Phytochemical analysis

Introduction

Enough experimental and epidemiological researchers have indicated that diarrhea is one of the common gastrointestinal disorders that make a passage to mortality and morbidity in children especially in developing countries including India. Diarrhea is an intestinal disorder involving abnormal fluid content and defecation frequency resulting in increased motility in the colon [1,2]. Different multidrug-resistant pathogenic microbes' have driven treatment of this sickness more troublesome. The subclass of secretory looseness of the bowels is normally prompted by bacterial pathogens like *Vibrio cholerae*, enterotoxin *E. coli*. It is portrayed by the dynamic discharge of chloride or potentially bicarbonate into the digestive tract and consequently by lost liquid [3]. Clinically diarrhea shows increased liquidity of stool, along with increased stool frequency and weight. Despite the understanding causes, treatment and counteractive action of diarrheal illnesses, an expected 4.6 million individuals, with 2.5 million kids, bite the dust from looseness of the bowels consistently, especially in developing countries [4]. Regardless of the variety of etiologies of the runs, literary works express that there are four noteworthy pathophysiologies that lead to diarrhea. These incorporate increased electrolytes secretion (secretory diarrhea), increased luminal osmolarity (osmotic diarrhea), deranged intestinal motility and decreased electrolytes absorption causing a decreased transit time. Conversely, purgatives and diarrhea-causing agents improve gastrointestinal motility. For example, castor oil; which is utilized as an inducer of looseness of the bowels in this investigation, is known for its purgative effects due to active principle, retinoic acid [5].

Although WHO launched Diarrhea Disease Control Program in 1983 to eradicate diarrhea in developing countries included measures like use of traditional remedies, but more than 85% of plants still wait for scientific results in terms of their pharmacological activities [6]. Herbal medicines indicate potential uses in future in light of the fact that the greater part of the plants, their activities, and pharmacological actions have not been investigated totally [7]. The more noteworthy efficiency of plant-origin medicines is because of the antioxidative role which prevents oxidation and provides protection to living beings from harm caused by excessive generation of ROS, lipid peroxidation, DNA strand breaking and protein damage. Prevention of cell oxidative damage, therefore, limits the events of the vast majority of the illnesses. It is the world's third most elevated executioner illness to undernourished, children [8]. It is explicitly stated that diarrhea mars the intestinal antioxidant defense system which will make it confused and cause other oxidative stress disorders and in this way,

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Received November 10, 2017; **Accepted** November 20, 2017; **Published** November 23, 2017

Citation: Dhakad PK (2017) Phytochemical Investigation and Anti-Diarrheal Activity of Hydroalcoholic Extract of Fruits of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae). J Mol Genet Med 11: 305 doi:10.4172/1747-0862.1000305

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antioxidants may take up an essential application in the treatment of diarrhea [9]. The revelation of viable anti-microbial agents, vaccines have diminished the overwhelming effect of irresistible maladies and enhanced personal satisfaction. However, the efficacy of many antimicrobials is being undermined by the development of microbial resistance to existing chemotherapeutic agents [10]. Despite many efforts taken by the government and international organizations as well as advancement in medicines current rate of diarrheal patients is still high at large. It is necessary to identify and evaluate the indigenous plants for the treatment of diarrhea as allopathic medicines have both adverse effects and toxicities.

Citrullus colocynthis (L.) Schrad. *Citrullus colocynthis* Schrad (family Cucurbitaceae), also known as colocynth or bitter apple or Indian wild gourd (Hindi-Indrayan) is a desert plant widely distributed in hot arid areas of the world, including Pakistan, India and Saudi Arabia [11]. It is a perennial herbaceous crawling plant, possessing rough and angular stems. Leaves are rough, 5 to 10 cm long, 3 to 7-lobed and fruits are almost globular, 4 to 10 cm in breadth about the extent of a little orange. Customarily, the poultice of colocynth is utilized to battle rheumatic agony. The leaves have been utilized for the agonizing feminine cycle and in the treatment of asthma. The fruit pulp is laxative, utilized as diuretic, cathartic and furthermore utilized against gonorrhoea [12]. The watery pulp extract of *Citrullus colocynthis* fruits is used for the treatment of kidney, liver-related diseases. The fruits and leaves of this plant contain cucurbitacins A, B, C and D and a elaterin and probably other constituents. Phytochemical investigations of its bitter principles 'cucurbitacins' were numerous [13]. Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc Cucurbitacins are reported to be the main constituent of fruits of this plant [14]. The various antidiarrheal therapies and their mechanisms of action include proabsorptive action by ORS/RS-ORS (Oral rehydration solution/ Resistant starch-based oral rehydration solution) responsible for increase in glucose/SCFA (Short-chain fatty acids) absorption, antisecretory action through following ways – (a) CFTR (Cystic fibrosis transmembrane conductance regulator) and CaCC (Ca²⁺ activated chloride channels), (b) decrease in bile salt in lumen (c) decreasing PG (Prostaglandins) synthesis which results in reduction of intestinal secretions. Antimotility action has also been seen by various compounds like loperamide act by increasing μ opioid receptor hence decreasing the motility, hyoscyamine reduces the acetylcholine (ACh) action on muscles thereby decreasing muscle contractions, Alosetron works by inhibiting serotonergic (5 HT₂) receptor action and decreases the intestinal motility as well as secretion, Racecadotril increases enkephalin endogenously which also decreases gut secretions. Clonidine acts on adrenergic receptors and activates the α_2 adrenoceptor which results in increasing the absorption. Anti-inflammatory action is seen by corticosteroids through immunosuppression by decreasing prostaglandins and increasing IL-10 (Interleukins), while Anti-TNF α causes a decrease in blood and tissue TNF α (Tumor necrosis factor) [15].

Currently, there is no scientific evidence in the literature on the effect of *Citrullus colocynthis* on diarrhea. Hence present investigation was undertaken to evaluate the effect of dried fruit extract of *C. colocynthis* on the experimentally induced diarrhea in Wistar rats. Acute toxicity study and phytochemical investigations of dried fruit extract were also done.

Materials and Methods

Plant materials

Citrullus colocynthis plant widely grows in Jaipur region and

the fruits of this plant were collected fresh from Smriti Van, Jaipur, Rajasthan, India in the month of August 2016. They were taxonomically identified and authenticated by Dr. Manju Sharma, Convener Herbarium committee, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (R.No: RUBL 211645) has been deposited at the Herbarium of Department of Botany, University of Rajasthan, Jaipur, India.

Preparation of crude extract

Citrullus colocynthis fruits were washed with tap water followed by distilled water and then cut and dried under the shade. The dried fruits were comminuted into moderately coarse powder and passed through sieve no. 40, stored in a tightly closed container. The dried and powdered plant material was Soxhlet extracted with water and alcohol. The extraction was carried out for 24 hr at room temperature with mild shaking. The extract was filtered and concentrated at 48°C and weight of residue was recorded. The percentage yield of hydro-alcoholic extract was found to be 38.5%. The collected extract was stored in a sterile container for further use.

Experimental animals

Healthy Albino Wistar rats of both sexes weighing 150-250 g were obtained from Central Animal Facility AIIMS New Delhi. The experimental protocol was approved by Institutional Animal Ethics Committee CPCSEA No. - 1149/PO/ERe/07/CPCSEA. Animals were housed under standard conditions of temperature (24 \pm 2°C) and relative humidity (30% to 70%) with 12:12 light: dark cycle. The animals were given standard pellet diet and water ad libitum. All the experimental procedures involving animals were conducted in accordance with Institutional Animal Ethics Committee (IAEA) (OECD guideline no.420) and approved by IAEA.

Chemicals

Castor oil (Jayant Agro-Organics, Mumbai Maharashtra), Loperamide (Arene Life Sciences, Andhra Pradesh), Barium Sulfate (Oasis Fine Chem, Vadodara) and distilled water were used in this study.

Phytochemical analysis

The extract was analyzed for the presence of pharmacologically active constituents such as phenols, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and carbohydrates [16].

Test for phenolic compounds

50 mg of *C. colocynthis* extract was dissolved in 5 ml of distilled water and few drops of 5% ferric chloride were added. The appearance of bluish black color indicated the presence of phenolic compounds.

Test for flavonoids

Few drops of dilute sodium hydroxide solution were added into the *C. colocynthis* extract (0.5 ml) to give intense yellow color which disappears after addition of dilute hydrochloric acid showed the presence of flavonoids.

Test for terpenoids

The extract (0.5 mg) of *C. colocynthis* was added with few ml of chloroform followed by concentrated sulphuric acid to form a layer. Formation of the reddish-brown ring at the interface indicated the presence of terpenoids.

Test for saponins

C. colocynthis extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min using hands. The formation of two cm layer of foam layer indicated the presence of saponins.

Test for alkaloids

About 50 mg of *C. colocynthis* extract was shaken with few ml of dilute hydrochloric acid and filtered. Few drops of Wagner's reagent were added at the side of the test tube. The appearance of reddish-brown precipitate indicated the presence of alkaloids.

Test for cardiac glycosides

C. colocynthis extract (50 mg) was treated with 2 ml of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface is a feature of cardenolide deoxy sugar and appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

Test for steroids

C. colocynthis extract (1 gm) was dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The color of the upper layer changed to red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [17].

Test of carbohydrates

Molisch test: To 2–3 ml of the aqueous. *C. colocynthis* extract added two drops of alpha-naphthol solution in alcohol, shaken and added conc. H_2SO_4 from the sides of the test tube. Violet ring was formed [18].

Monosaccharide Barfoed's test: Equal volumes of Barfoed's reagent and the *C. colocynthis* extract were mixed to form a solution. Heated for 1–2 min in a boiling water bath and cooled. Red color indicated the presence of monosaccharides [18].

Test for tannins

C. colocynthis extract (1 gm) dissolved in water in a test tube and diluted with chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully to the side of the test tube to the solution. A green color was formed which showed the presence of tannins [19].

Acute toxicity studies

Lorke method [20] was used in this study. Twenty-five Wistar rats of both sexes were randomly grouped into five with five rats in each group and were fed orally with graded doses 100, 500, 1 000, 1 500 and 2 000 mg/kg of a hydroalcoholic extract of *Citrullus colocynthis* by gastric gavage. The animals were allowed free access to feed and water. They were observed over a 48 h period for acutely toxic signs and death.

Experimental Methods

Castor oil-induced diarrhea (COID) in rats

The antidiarrheal activity of *C. colocynthis* extract was estimated as per the method of Awouters et al. [21]. Thirty Wistar rats were allowed to fast for 18 h. Animals were divided randomly into five groups of six animals each (n=6) as a control group, standard group, and test groups. At first, *C. colocynthis* extract and the standard drug will be provided

orally and after 1 h castor oil (2 ml/rat) will be supplied for inducing diarrhea. Only distilled water (2 ml/rat) will be supplied for the control group and standard drug loperamide (2 mg/kg) will be provided for the positive control group. Treated Groups III, IV received *C. colocynthis* extract at the dose of 50 mg/kg and 100 mg/kg respectively. Group V i.e., per se group will receive only plant extract at the dose of 100 mg/kg. Separate cages will be used for each rat and sheets of paper will be placed below the cage for the collection of fecal matters. The presence of stool with fluid material that stained the paper will be placed beneath the cages indicated diarrhea. During the observation period of 6 h, parameters such as latency time, defecation frequency, no. of wet defecations, the weight of stool and water content of feces were recorded. The total score of disease control group was considered as 100%. The water content of feces was expressed in terms of percentages using the formula:

$$W_c(\%) = \frac{F_w - D_w \times 100}{F_w}$$

Where W_c = Water content of feces; F_w = Fresh weight (g); D_w = Dry weight (g).

Gastrointestinal motility test with barium sulfate milk (BSM) model for diarrhea

This experiment was carried out by the method developed by Chatterjee [22]. By random selection Wistar rats (overnight fasted for 18 h) was divided into five groups of six rats each. Group, I recognized as normal control was administered distilled water of 2 ml/rat orally. Commercially available reference antidiarrhoeal drug loperamide at the dose of 2 mg/kg was provided orally for Group II marked as a positive control group. The hydroalcoholic extract of *C. colocynthis* was orally treated at a dose of 50 mg/kg and 100 mg/kg for groups III, and IV respectively assigned as treated groups and group V as Per se group where an only hydroalcoholic extract of *C. colocynthis* with 100 mg/kg was administered. After 30 min, 2 ml of 10% barium sulfate solution was administered in all groups. Rats were sacrificed after 30 min of extract and drug administration. The distance traversed by barium sulfate milk was measured and expressed as a percentage of the total length of the small intestine (from the pylorus to the ileocecal junction). The percentage of inhibition compared with the control group was determined by using the following equation:

$$\text{Inhibition } (\%) = \frac{\text{extract} - \text{control} \times 100}{\text{control}}$$

Statistical Analysis

The data were represented as a mean \pm standard error of the mean (SEM). Statistical significance was carried out employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests where $P < 0.05$ was considered statistically significant using Graph Pad Prism version 5.03 software.

Results

Preliminary qualitative phytochemical screening and acute toxicity studies

Preliminary qualitative phytochemical analysis of hydroalcoholic fruit extract of *C. colocynthis* showed the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. Acute toxicity studies did not show any signs of death at administered graded doses of 100, 500, 1000, 1500 and 2000

mg/kg of a hydroalcoholic extract of *C. colocynthis* by gastric gavage. The LD₅₀ value for oral administration of the plant extract was found to be greater than 2000 mg/kg body weight. Based on the results of acute toxicity studies the doses of 50 and 100 mg/kg of plant extract were selected for administration.

Effect of *C. colocynthis* extract on Castor oil induced diarrhea (COID) model

The results about the antidiarrheal effect of loperamide and hydroalcoholic extract of *C. colocynthis* in COID on Wistar rats are shown in Table 1. The results indicated that both the doses of *C. colocynthis* extract (100 mg/kg and 50 mg/kg) showed protection against COID model. The hydroalcoholic extract of *C. colocynthis* at 100 mg/kg showed highly significant results such as prolonged the latency time, reduced the defecation frequency, number of wet defecations, the weight of stool and water content of feces when compared with the negative control group ($P < 0.001$). In addition, this dose of plant extract had shown results comparable to loperamide while at a dose of 50 mg/kg the results were same as the significant value of $P < 0.01$. Per se group showed a highly significant result ($P < 0.001$) on all the parameters discussed above as compared to negative control group and it contributes to the beneficial effects of *C. colocynthis* extract in the treatment of diarrhea.

Effect of *C. colocynthis* extract on Barium sulfate milk (BSM) model

The results of the gastrointestinal motility test with BSM of hydroalcoholic extract of *C. colocynthis* and loperamide on Wistar rats have been shown in Table 2. The treatment with standard drug loperamide and with all the doses of hydroalcoholic extract of *C. colocynthis* significantly inhibited the gastrointestinal motility of rats. The percentages of inhibition of 100 mg/kg, 50 mg/kg, and per se groups compared to control group was 26.57%, 17.30%, 23.74% respectively. While standard group exhibited 38.89% inhibition.

Discussion

In our experiment, the anti-diarrheal activities were evaluated in

two *in-vivo* study models (COID and BSM). The hydroalcoholic extract of *C. colocynthis* (fruits) inhibited the Castor oil induced diarrhea at doses- 100 mg/kg and 50 mg/kg. While per se group (only 100 mg/kg of *C. colocynthis* extract administered without induction of diarrhea) also showed highly significant results which were used to evaluate the beneficial effects of *Citrullus colocynthis* extract. In COID model the hydroalcoholic extracts showed decrease in number of wet defecations in 6 h [100 mg/kg ($P < 0.001$), 50 mg/kg ($P < 0.01$), per se ($P < 0.001$)], weight of stool [100 mg/kg ($P < 0.001$), 50 mg/kg ($P < 0.05$), per se ($P < 0.001$)] and water content of feces [100 mg/kg ($P < 0.001$), 50 mg/kg ($P < 0.01$), per se ($P < 0.001$)] (Table 1). The experimental groups of *C. colocynthis* extract (CCE 100 mg/kg, CCE 50 mg/kg and per se 100 mg/kg) significantly diminished the severity of diarrhea with respect to decreasing in the rate of defecation and watery content of feces in Wistar rats. All the extracts showed significant anti-diarrheal activity demonstrating 52.48%, 21.29% and 71.32% reductions in diarrhea respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg as compared to that of loperamide that demonstrated 72.16% reductions in diarrhea. While the percent inhibition of water content was found to be significant displaying 34.34%, 07.63% and 83.19% decrease in water content respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg as compared to that of the standard drug loperamide that showed 100% reductions in water content in diarrhea. In the gastrointestinal motility test, all the doses of extract produced a significant decrease in intestinal motility. In gastrointestinal motility test with BSM, all the doses of hydro alcoholic extract of *C. colocynthis* decreased intestinal transmit significantly ($P < 0.001$). The normal control group showed 56.93% intestinal motility by the Barium Sulfate milk in gastrointestinal motility test. *C. colocynthis* extracts showed intestinal motility as 41.8%, 47.08% and 43.41% respectively in CCE 100 mg/kg, CCE 50 mg/kg and Per se 100 mg/kg groups. CCE extracts also gave a significant inhibition of intestinal motility with values as 26.57% (CCE 100 mg/kg), 17.3% (CCE 50 mg/kg), and 23.74% (Per se 100 mg/kg) while loperamide (2 mg/kg) had a value of 38.89% ($P < 0.001$) of decrease in intestinal motility (Table 2).

Similar results were also ascertained in prior anti-diarrhoeal studies conducted which substantiates the medicinal use of *Citrullus colocynthis* in the treatment of diarrhea.

Groups	Dose (mg/kg)	Latency Time (min)	Defecation Frequency in 6 hrs.	% Inhibition of defecation	No. of wet defecation within 6 hrs.	% Inhibition of defecation	Wt. of stool (gm)	Wt. of wet stool (gm)	Water content of feces (%)	% Inhibition of water content
Control	2 ml/rat	107.33 ± 2.24	20.33 ± 0.88		12.66 ± 0.88		0.63 ± 0.04	0.57 ± 0.03	87.60 ± 1.14	
Loperamide	2 mg/kg	304.16 ± 1.74 ^c	5.66 ± 0.66 ^c	72.16	2.66 ± 0.33 ^c	78.98	0.13 ± 0.02 ^c	0.10 ± 0.02	00 ± 000	100 ^c
CCE	100 mg/kg	233.66 ± 1.94 ^c	9.66 ± 0.88 ^c	52.48	5 ± .73 ^c	60.5	0.32 ± 0.01 ^c	0.23 ± 0.03	57.51 ± 1.25	34.34 ^c
	50 mg/kg	117.83 ± 0.98 ^b	16 ± 0.44 ^b	21.29	9.5 ± 0.42 ^b	24.96	0.51 ± 0.02 ^a	0.41 ± 0.01	80.91 ± 0.63	07.63 ^b
Per se	100 mg/kg of CCE	200.66 ± 2.51 ^c	5.83 ± 0.30 ^c	71.32	2 ± .36 ^c	84.2	0.27 ± 0.009 ^c	0.06 ± 0.006	14.72 ± 1.50	83.19 ^c

Note: Values are expressed as mean ± SEM (n=6); ^{a,b,c}p<0.001 refers to significant difference compared to control with positive control and extract.

Table 1: Effects of a hydroalcoholic extract of *C. colocynthis* on castor oil induced diarrhea model in wistar rats.

Group	Dose (mg/kg)	Length of GIT (cm)	Distance passed by BaSO ₄ (cm)	BaSO ₄ Transverse (%)	Inhibition (%)
Control	2 ml/rat	119.24 ± 0.94	67.89 ± 0.31	56.93	--
Loperamide	2 mg/kg	112.16 ± .60	39.03 ± 0.37	34.79 ^c	38.89
CCE	100 mg/kg	99.95 ± 0.42	41.78 ± 0.27	41.8 ^c	26.57
	50 mg/kg	106.74 ± 0.63	50.26 ± 0.35	47.08 ^c	17.3
Per se	100 mg/kg of CCE	103.10 ± 0.44	44.76 ± 0.50	43.41 ^c	23.74

Note: Values are expressed as mean ± SEM (n=6); ^cP<0.001 refers to significant difference compared to control with positive control and extract.

Table 2: Effects of a hydroalcoholic extract of *C. colocynthis* on gastrointestinal motility with barium sulphate milk model in rats.

In prior anti-diarrheal studies, ethanolic extracts of *Cynodon dactylon* Pers. aerial parts (EECA) in Wistar rats demonstrates that EECA viably restrains the recurrence of wetting feces and defecation as well as inhibit the water content of total feces. In gastrointestinal motility test with BSM, the most astounding decrease of gastrointestinal motility is for loperamide at a dose of 2 mg/kg and inhibition of the distance traveled by BaSO₄ milk is 39.6%. While the plant extracts decrease the distance of gastrointestinal motility of rats ranging from 58.57% (control group) to 47.12% and inhibition of distance traveled by barium sulfate milk is 19.55% at the dose of 1 g/kg of extract dose as compared to control [23].

In another anti-diarrheal investigation of methanol (MEHO), ethanol (EEHO) and water (AEHO) extracts of *H. odorata* leaves demonstrate critical ($p < 0.001$) inhibition against castor oil-induced diarrhea. At the 400 mg/kg dose, the extracts show significant anti-diarrheal activity ($P < 0.001$) demonstrating $47.76 \pm 2.36\%$, $58.21 \pm 6.92\%$ and $56.72 \pm 5.48\%$, reductions in diarrhea respectively in AEHO, EEHO and MEHO comparable to that of the standard drug loperamide with $59.70 \pm 2.99\%$ reduction in diarrhea. The normal control group demonstrates intestinal motility as $84.85 \pm 2.88\%$. The 200 and 400 mg/kg (p.o) of the extracts displays intestinal motility as $51.51 \pm 0.97\%$ to $62.05 \pm 1.41\%$. Also, the extracts significantly inhibit intestinal motility as $22.82 \pm 1.76\%$ to $35.93 \pm 1.21\%$ at all the doses. Be that as it may, Loperamide (5 mg/kg) shows a significant inhibition ($43.6 \pm 2.14\%$) in intestinal motility [8].

Castor oil is obtained from the seeds of *Ricinus communis* (Family-Euphorbiaceae). Castor oil acts as a stimulant laxative which hydrolyzes to form ricinoleic acid, a local irritant and instigate changes in gastrointestinal mucosal fluid and electrolyte transport bringing about hypersecretory reaction and diarrhea. Experimental studies demonstrated that inflammatory response occurring due to ricinoleic acid causes generation of prostaglandins PGE₂. Ricinoleic acid diminishes the active Na⁺ and K⁺ retention and declines Na⁺-K⁺ ATPase pump in small intestine and colon and henceforth hindering the mucosal c-AMP intervened dynamic secretion. Loperamide is an opioid derivative that functions through mu receptors on neurons in submucosal neural plexus of intestinal wall and moderates the intestinal motility, aside from it, likewise, indicates antimuscarinic action in the gastrointestinal tract. So clearly loperamide protected the Wistar rats through above mechanism [24].

Standard chemical test carried out during the phytochemical screening of *C. colocynthis* showed the presence of a number of bioactive constituents such as phenols, alkaloids, terpenoids, flavonoids, saponins, cardiac glycosides, steroids, tannins, and carbohydrates. The counter diarrheal action could be ascribed to these compounds. Previous literature survey and experimental studies also showed the presence of these compounds in the fruit of *C. colocynthis* [25-27]. In earlier studies, anti-diarrhoeal activity is showed by the plants having alkaloids, tannins, saponins, steroids, terpenoids, and flavonoids. Hostile to the looseness of the bowels exercises of flavonoids have been credited to their capacity to hinder intestinal motility and hydroelectrolytic discharges which are known to be modified in diarrhoeic conditions. Tannins denature proteins in the intestinal mucosa by shaping protein tannates which may lessen secretion [28]. Studies on the on the useful part of tannins additionally uncovered that they can likewise lessen the peristaltic developments and intestinal discharges by decreasing the intracellular Ca²⁺ inward current or by the enactment of the calcium pumping system (which actuates the muscle unwinding) ascribed by calcium channel blocking and spasmodic

activities of tannins present in the plant extract. Sesquiterpenes, terpenes, diterpenes and various terpenoid derivatives are known for repressing release of prostaglandins and autocooids, in this way restrain the motility and secretion instigated by castor oil [29].

Conclusion

The present results validate the use of a hydroalcoholic extract of *Citrullus colocynthis* as an anti-diarrheal agent as it showed significant inhibition of defecation-related parameters in castor oil induced diarrhea model and gastrointestinal motility and secretion in barium sulfate milk model. Phytochemical screening of *Citrullus colocynthis* (L.) fruits rationalizes the presence of active phytoconstituents in this plant. We suppose that the anti-diarrheal effect of *Citrullus colocynthis* is related to its bioactive components such as polyphenols, flavonoids, alkaloids, saponins, terpenoids. Therefore, further investigations are ongoing to isolate and identify specific constituents which will explicate the mechanism of action for the anti-diarrheal potential of *Citrullus colocynthis*. Hence the evaluation of the phytoconstituents and anti-diarrheal activity of hydroalcoholic extract of fruits of *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae) in experimentally induced diarrhea in Wistar rats affirm fruits of this plant for its effective anti-diarrheal use.

Conflicts of Interest

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Dr. Kailash Agrawal and Dr. Manju Sharma, Department of Botany, University of Rajasthan, Jaipur for their kind cooperation during authentication of plants. The author wishes to thank Mr. Ramji Gupta, Assistant Professor, R.V. Northland Institute of Pharmacy, Gautam Budh Nagar, U.P. for his valuable advice and support through the project work.

References

1. Pal BB, Khuntia HK, Samal SK, Kar SK, Patnaik B (2010) Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the *ctxB* gene of the classical biotype in Orissa, India. Int J Infect Dis 14: e384-e389.
2. Krishna BVS, Patil AB, Chandrasekhar MR (2006) Fluoroquinolone-resistant vibrio cholerae isolated during a cholera outbreak in India. Trans R Soc Trop Med Hyg 100: 224-226.
3. Schulzke JD, Andres S, Amasheh M, Fromm A, Günzel D (2011) Anti-diarrheal mechanism of the traditional remedy uzara via reduction of active chloride secretion. PLoS One 6: 1-9.
4. Hossain SM, Alam BM, Asadujjaman M (2011) Antidiarrheal, antioxidant and antimicrobial activities of the musa sapientum seed. Avicenna J Med Biotechnol 3: 95-105.
5. Tadesse WT, Hailu AE, Gurmu AE, Mechesso AF (2014) Experimental assessment of the antidiarrheal and anti-secretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. BMC Complement Altern Med 14: 460.
6. Wardlaw T, Salama P, Brocklehurst C, Chopra M, Mason E (2010) Diarrhoea: Why children are still dying and what can be done. Lancet 375: 870-872.
7. Ali N, Alam H, Khan A (2014) Antispasmodic and antidiarrhoeal activity of the fruit of *Rosa moschata* (J). BMC Complement Altern Med 14: 485.
8. Kabir MSH, Hossain MM, Kabir MI (2016) Antioxidant, antidiarrheal, hypoglycemic and thrombolytic activities of organic and aqueous extracts of *Hopea odorata* leaves and in silico PASS prediction of its isolated compounds. BMC Complement Altern Med 16: 1-13.
9. Birru EM, Asrie AB, Adinew GM, Tsegaw A (2016) Antidiarrheal activity of crude methanolic root extract of *Idigofera spicata* Forssk (Fabaceae). BMC Complement Altern Med 16: 272.
10. Billah MM, Islam R, Khatun H (2013) Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonducella* (L.) Roxb leaves. BMC Complement Altern Med 13: 101.

11. Hussain AI, Rathore HA, Sattar MZA (2013) Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* (L.) from the Pakistani flora. Ind Crops Prod 45: 416-422.
12. Nessa F, Khan S (2014) Evaluation of antioxidant and xanthine oxidase inhibitory activity of different solvent extracts of leaves of *Citrullus colocynthis*. Pharmacognosy Res 6: 218.
13. Salama HMH (2012) Alkaloids and flavonoids from the air dried aerial parts of *Citrullus colocynthis* 6: 5150-5155.
14. Mehrzadi S, Shojaii A, Pur SA, Motevalian M (2016) Anticonvulsant activity of hydroalcoholic extract of *Citrullus colocynthis* fruit: Involvement of benzodiazepine and opioid receptors 16: 21.
15. Cheng SX (2016) Calcium-sensing receptor: A new target for therapy of diarrhea. World J Gastroenterol 229: 2711-2724.
16. Khanam Z, Wen CS, Bhat IUH (2015) Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). J King Saud Univ - Sci 27: 23-30.
17. Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis. (2nd edn). Chapman and Hall, London.
18. Boxi M, Rajesh Y, Kumar VR, Praveen B, Mangamma K (2000) Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of *Commicarpus chinensis* (aqueous leaf extract). Int J Pharma BioSci 1: 537-547.
19. Hossain MA, Al-Raqmi KAS, Al-Mijizy ZH, Weli AM, Al-Riyami Q (2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac J Trop Biomed 3: 705-710.
20. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54: 275-287.
21. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ (1978) Delay of castor oil diarrhea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharm Pharmacol 30: 41-45.
22. Chatterjee TK (1993) Handbook on laboratory mice and rats. (1st edn). Kolkata, India.
23. Rahman MS, Akter R, Mazumdar S (2015) Antidiabetic and antidiarrhoeal potentials of ethanolic extracts of aerial parts of *Cynodon dactylon* Pers. Asian Pac J Trop Biomed 5: 658-662.
24. De Sales IRP, Machado FDF, Marinho AF, Lúcio ASSC, Filho JMB, et al. (2015) *Cissampelos sympodialis* Eichl. (Menispermaceae), a medicinal plant, presents antimotility and antidiarrheal activity *in vivo*. BMC Complement Altern Med 15: 253.
25. Hussain AI, Rathore HA, Sattar MZA, Chatha SAS, Sarker SD, et al. (2014) *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses, and nutritional potential. J Ethnopharmacol 155: 54-66.
26. Kumar V, Rathee P, Kohli K, Chaudhary H, Rathee S (2009) Phytochemical and Biological Potential of Indrayan: An Overview. Pharmacogn Rev 3: 193-205.
27. Najafi S, Sanadgol N, Nejad BS, Beiragi MA, Sanadgol E (2010) Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) schrad against *Staphylococcus aureus*. J Med Plants Res 4: 2321-2325.
28. Umer S, Tekewe A, Kebede N (2013) Anti-diarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. BMC Complement Altern Med 13: 21.
29. Yacob T, Shibeshi W, Nedi T (2016) Antidiarrheal activity of 80% methanol extract of the aerial part of *Ajuga remota* Benth (Lamiaceae) in mice. BMC Complement Altern Med 16: 303.