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LAXATIVE EFFECT OF 70% ETHANOL EXTRACT OF CONVOLVULUS SPINOSUS BURM. F. IN MICE

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Abstract
Convolvulus spinosus Burm.f. (*C. spinosus*) is a woody shrub and is traditionally used in Pakistan for its laxative virtue in indigenous culture. Despite the usage of *C. spinosus* by traditional healers and local communities for the management of constipation, it lacks scientific evidence for its claimed traditional use. The aim of the study is to evaluate the laxative effect of 70% ethanolic extract of *C. spinosus* in mice. The laxative potential of 70% ethanolic extract of *C. spinosus* (100/200 and 400 mg/kg P.O.) was evaluated and analyzed for the mean number of fecal pellets, percentage of fecal pellet water content (laxative test), and gastrointestinal transit ratio by charcoal meal movement in the small intestine in the Loperamide-induced constipated mice model, and intestinal fluid accumulation in normal mice. The phytochemical screening and ATR-FTIR analysis of *C. spinosus* 70% ethanolic extract were performed. The laxative test demonstrated that *C. spinosus* 70% ethanolic extract indicated a significant increase in both the number of fecal pellets at 200 mg/kg ($p<.05$) and 400 mg/kg ($p=.0001$), the percent fecal water content at 100 mg/kg ($p<.0001$), 200 mg/kg ($p<.0001$) and 400 mg/kg ($p<.0001$) in loperamide-induced constipated mice model. The gastrointestinal motility test of *C. spinosus* 70% ethanolic extract showed significant accelerated propulsion of charcoal meal in loperamide-induced constipated mice and produced a significant increase in Gastrointestinal (GI) transit ratio at 100 mg/kg ($p<.05$), 200 mg/kg ($p<.0001$) and 400 mg/kg ($p<.0001$). The findings of the Gastrointestinal secretion test presented that *C. spinosus* extract produced significantly higher fluid accumulation at 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.0001$). The phytochemical screening of *C. spinosus* extract showed the presence of tannins, alkaloids, terpenoids, and flavonoids. This study provides scientific evidence for the traditional use of *C. spinosus* for the treatment of constipation and highlights the need for the identification of biologically active compound(s).

Keywords: Convolvulaceae, Constipation, Laxative, Animal Model, Medicinal plant, *Convolvulus spinosus*

List of Abbreviations:
CFTR = Cystic fibrosis transmembrane conductance regulator
FTIR – ATR = Fourier transform infrared spectroscopy-attenuated total reflectance
GMP = Guanosine monophosphate
Na+/K + -ATPase = Sodium Potassium Adenosine triphosphatase
OECD = Organization for Economic Co-operation and Development
V/V = Volume/Volume

1. Introduction

Constipation is a global troublesome gastrointestinal disorder that adversely affects the quality of life [1]. Approximately 20 % of the global population is a victim of this disease [2]. Several other complications are associated with constipation, and these vary with the patient's age. The main characteristics of constipation are infrequent or hard stools and an incomplete sense of defecation [3]. Constipation can range from mild and temporary medical conditions to complex and permanent health issues. Constipation can be due to primary or secondary causes. The primary cause of constipation includes a physiological defect in colon/anorectal function and a secondary cause includes organic or systemic disease and medication [4]. Non-pharmacological interventions are the first choice for the management of constipation. Non-pharmacological interventions include lifestyle modifications like the use of a high-fiber diet, exercise, and stress-free life [5]. Laxatives are the mainstay component of pharmacological intervention for the management of constipation when non-pharmacological interventions cannot relieve symptoms [6]. Several natural, semisynthetic, and synthetic laxatives are available, but every available laxative has limitations for its use. These limitations include availability, high cost, low efficacy, and safety. Therefore, searching for a novel moiety with laxative potential is needed to overcome these limitations.

Plants exhibit diverse biological and pharmacological activities as they are a valuable source of natural therapeutically active compounds [7]. The family Convolvulaceae, also known as morning glory, contains important medicinal plants that are used to treat several disorders, and its oldest use is as a purgative [8]. Convolvulus is a medicinally and economically important Genus of the family Convolvulaceae. Convolvulus plants have been used to treat many serious ailment [9]. *Convolvulus scammonia* Linn and *Convolvulus arvensis* L. are two important members of the genus Convolvulus that have the potential to treat constipation [10]. *Convolvulus spinosus* Burm. f. from the genus Convolvulus too has the potential to treat constipation and have been used traditionally as a laxative in Pakistan [11]. It has been reported that 25 g bark of *C. spinosus* is ground to a fine powder and then taken with water once a day in South Balochistan [12].

The folklore use of *C. spinosus* as a laxative has not been validated experimentally, and there is a lack of scientific validation of its claimed laxative potential. Hence, the current pre-clinical study was conducted to evaluate the potential of a 70% ethanolic extract of this plant to treat constipation using animal models.

2. Materials and Methods

2.1. Drugs and Chemicals

The drugs/chemicals utilized for this study included absolute ethanol (Merck & Co, Darmstadt, Germany), Loperamide HCl (Janssen cilag A) Bisacodyl (Merck private ltd), Castor oil (Marhaba laboratories), Distilled water (Lab), and Charcoal (Sigma Aldrich). Moreover, the chemicals/reagents utilized in the phytochemical testing included concentrated Sulphuric acid, Hydrochloric acid, Sodium hydroxide, Chloroform and Ammonia were purchased of Merck & Co, Darmstadt, Germany. Bromine water, Mayer’s reagent and Fehling solution were purchased from Sigma Aldrich.

2.2. Plant Material and its collection

The plant name *Convolvulus spinosus* Burm. f. (Convolvulaceae) locally known as Ritachak was checked on April 2020 in “World Flora Online” and it also corresponds to the latest revision in

"World Flora Online" [13]. The entire plant of *C. spinosus* was collected along the Tubat-Makran road, which links Turbat with Makran in June 2020. Turbat is in the south region of the province of Balochistan, Pakistan, and the administrative center of district Kech. The plant was identified by a botanist Mohammad Anwar working as an Assistant professor at Government Attashad Degree College Turbat. A voucher specimen of the plant was deposited in the herbarium of the above-mentioned institute (voucher|# 06) for any future reference.

Figure 2.1. *Convolvulus spinosus* Burm. f.

2.3. Preparation of Extract

The entire collected plant was made free of adulterants and rinsed with tap water. It was shade dried and coarsely ground into powder. Approximately 750 g of powdered plant material was soaked in aqueous ethanol in a 30:70 v/v ratio for 7 days using the technique of cold maceration. The temperature was maintained at 25 degrees centigrade. The filtrate pass passed through muslin cloth to remove vegetative debris before its filtration through Whatman filter paper (grade 3). The filtrate was evaporated in a rotovap under reduced pressure at 25 °C [14]. The obtained concentrated crude ethanolic extract was freeze-dried for the removal of water molecules. The color of the extract was light brownish green, and its calculated percentage yield was 18%. The extract was put into vials and stored in the refrigerator for use in experimental procedures in the future.

2.4. Experimental Animals

♂ / ♀ Swiss albino mice, weighing 30-35 g, were obtained from the animal house of Faculty of Pharmacy, University of Karachi , Karachi, Pakistan. The mice were kept in plastic cages and a standard environment (controlled temperature of 23 ± 2°C and 12 hr. light-dark cycles) was maintained for them. Internationally accepted guidelines for Animal handling were used to handle animals throughout the study [15]. Animals were provided with standard food pellets and water ad libitum. Animals were allowed to get adapted to their environment for one week before beginning the experimental study.

2.5. Acute toxicity

According to OECD guidelines for testing, Test No. 425 Acute oral toxicity was conducted to observe any visible sign of toxicity. The limit dose of *C. spinosus* extract was 2000 mg/kg body weight in mice [16].

2.6. Induction of constipation

Loperamide 5mg/kg dissolved in 10ml/kg distilled water was given orally for 6 days to induce constipation in mice. The normal control group was administered distilled water (10ml/kg) for a similar duration [17,18].

2.7. Laxative potential test in loperamide-constipated mice

To determine the laxative potential, animals were divided into 6 groups. Every group contained 5 experimental animals. Animals from Group 1 were treated with 10ml/kg distilled water and Group 1

was marked as normal control (NOC). Animals from groups 2-6 were loperamide-induced constipated animals. Group 2 animals were treated with 10ml/kg distilled water and Group 2 was marked as negative control (NEC). Animals from Groups 3, 4, and 5 were administered (100/200/400) mg/kg *C. spinosus* extract respectively, and these groups were marked as the test groups. Animals from Group 6 were administered 0.25 mg/kg of bisacodyl, and this group was marked positive control (POC). All groups were provided with the above-mentioned treatment for five days.

Before administration of the dose on the 5th day, mice were kept on fasting for 12 hrs. and then every mouse was housed in a separate cage sheeted with unabsorbent paper. Then the number of fresh fecal pellets from every cage was counted every 2 hrs. for 16 hrs., weighed, and kept on drying for a day at room temperature. The percentage of fecal water content was evaluated by the following formula [19].

$$\text{Fecal Water Conten (\%)} = \frac{(weight\ of\ wet\ fecal\ matter - weight\ of\ dry\ fecal\ matter)}{(\text{weight of wet fecal matter})} \times 100$$

2.8. Gastrointestinal (GI) motility test in loperamide constipated mice

The mice were divided into groups and treated with distilled water, *C.spinosus* extract, and bisacodyl as described in 2.7 activity. They were made to fast for 12 hours before the fifth dose. However, mice were allowed to access water.

After 20 minutes of the fifth dose, each animal of every group received orally 0.3 ml (freshly prepared) 10% aqueous suspension of the charcoal meal. Then after 20 minutes of charcoal meal administration, each animal was euthanized and dissected. The charcoal's leading small intestine edge of each animal was located and to avoid peristalsis after excision (pylorus-caecum) it was immersed in formalin solution. The gastrointestinal transit ratio was calculated by the following standard formula [19,20].

$$\text{GI Transit Ratio (\%)} = \frac{(\text{Distance travel by charcoal meal})}{(\text{Total length of small intestine})} \times 100$$

2.9. Gastrointestinal secretion test in normal mice

To determine the gastrointestinal secretion capacity, mice were sectioned into 5 separate groups. There were five mice in each group. Group 1 was treated with 10ml/kg distilled water and was marked normal control (NOC). Groups 2-4 were administered (100,200, and 400) mg/kg of *C. spinosus* extract, respectively, and marked test groups. Every mouse from Group 5 was administered with castor oil 0.5 ml and this group was marked positive control (POC). Every animal was sacrificed after approximately 1 hour of administration. Then, the small intestine of each mouse was excised from the pylorus to the cecum. Then it was weighed immediately. The technique of milking was employed to remove its contents. Then the small intestine was reweighed and the calculation for each animal regarding the difference in weight of the small intestine before and following milking was done [19,21]

$$G\text{I secretion(g)} = \text{Weightof small intestine before milking(g)} - \text{Weight of small intestine after milking(g)}$$

2.10. Preliminary phytochemical analysis

The Standard methods of phytochemical screening were used to screen secondary metabolites (tannins/alkaloids/flavonoids/steroids/glycosides/terpenoids/resins/saponins/carbohydrates) in *Convolvulus spinosus* extract [22,23].

2.11. FTIR-ATR characterization

The FTIR - ATR analysis was conducted on the dry 70% ethanolic extract of *C. spinosus*. The equipment used was a Bruker Alpha-E spectrometer (serial number 10046639, Germany) in the

region of 4000-400 cm^{-1}. The characteristic peaks were recorded to detect specific functional groups.

2.12. Data analysis

Graph pad Prism 9 was used for all statistical analyses. Statistical differences among groups were analyzed by one-way analysis of variance (ANOVA) and then Tucky's test was performed. All results are mentioned as mean ± standard error mean (SEM) and *p*-values less than or equal to 0.05 were accepted to have a certain significance.

3. Results

3.1. Acute toxicity

The acute toxicity testing showed that 2000 mg/kg dose was safe for each animal and there was no visible sign of toxicity. Breathing and motor activity were normal. There was no convulsion and restlessness in any animal. There was no fatality in mice for 2 weeks. So, it is assumed that the lethal dose is larger than 2000 mg/kg.

3.2. Laxative Potential of the *C. spinosus* extract in loperamide induced constipated mice

Oral administration of loperamide in mice produced a significant decrease in the number of fecal output and percentage of fecal water content. Table 1 depicted that plant extract counteracted these effects of loperamide and enhanced the number of pellets and percentage of fecal water content. Figure 3.1 a depicted that plant extract expressed a significant increase in the number of fecal output at the dose of 200mg/kg (13.80±1.28, $p<.05$) and 400mg/kg (18±1.05, $p=.0001$) compared to the NEC group. The plant extract significantly increased the percentage of fecal water content at doses 100mg/kg (60.05%±2.05%, $p<.0001$), 200mg/kg (63.02%±1.52%, $p<.0001$), and 400mg/kg (68.42%±1.01, $p<.0001$) as shown in figure 3.1 b. Data demonstrated that bisacodyl produced the most significant increase in both the number of pellets (18.40±1.43, $p<.0001$) and the percentage of fecal water content (70.08%±1.33%, p<.0001) compared to the NEC group. Moreover, plant extract enhanced the percentage of fecal water content with certain significance as compared to the NOC group.

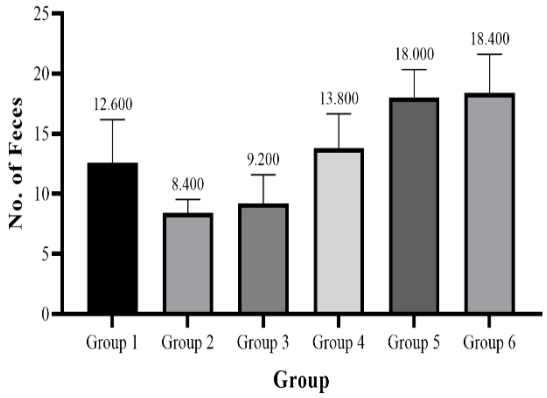

Figure 3.1. a. Effect of *C. spinosus* extract on the number of feces

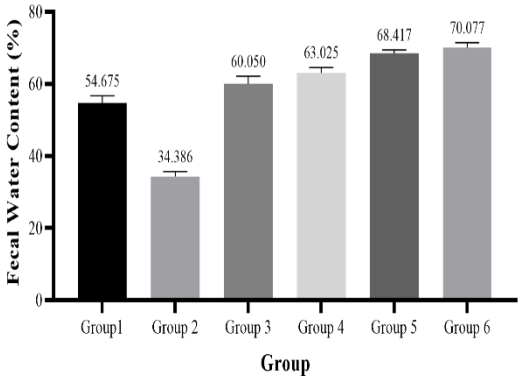

Figure 3.1. b. Effect of *C. spinosus* extract on the percentage of water content in feces

3.3. Effects of *C. spinosus* extract on Charcoal GI transit in loperamide induced constipated mice

Figure 3.2 shows that *C. spinosus* extract produced a significant increase in gastrointestinal motility at all doses as compared to the NEC and NOR groups. The gastrointestinal transit ratio as compared to the NEC group was 46.78% ($p<.05$), 67.29% ($p<.0001$), and 79.30% ($p<.0001$) at the doses of 100mg/kg, 200mg/kg, and 400mg/kg respectively as shown in table 2. The positive control

bisacodyl also expressed a rise in gastrointestinal motility with certain significance as compared to both NOC and NEC groups (p<.0001).

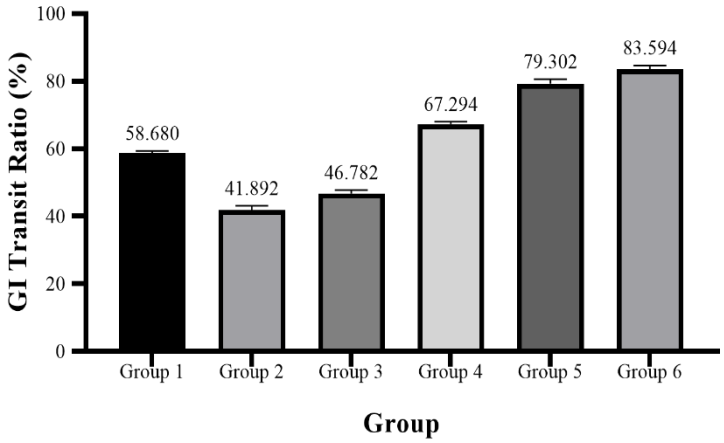

Figure 3.2. Effects of *C. spinosus* extract on GI transit ratio

3.6. Effects of *C. spinosus* extract on GI secretion in normal mice

The results of the GI secretion test expressed in Figure 3.3 shows that as compared to the NOC group at the dose of 200mg/kg (1.05 ±.07, $p<0.05$) and 400mg/kg (1.42±.03, $p<0.0001$) produced an increased fluid accumulation that was statistically significant. Castor oil (1.48±.04, $p<0.0001$) produced the highest fluid accumulation as compared to the NOC group.

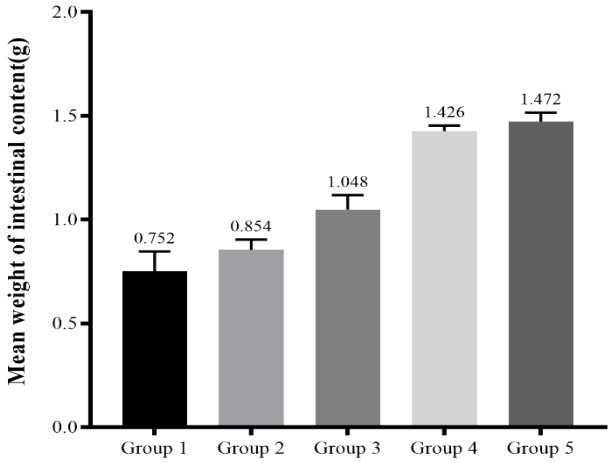

Figure 3.3. Effects of *C. spinosus* extract on GI secretion.

3.4. Phytochemical screening

The results of the preliminary phytochemical analysis of *C. spinosus* extract showed the presence of tannins, alkaloids, terpenoids, and flavonoids.

3.5. ATR-FTIR spectroscopy characteristics

The ATR-FTIR spectra of *C. spinosus* extract presented in Figure 3.4. The spectra had significant vibrational bands at wavelengths 3312, 2931, 1611, 1569, 1363, 1272, 1127, and 1045. The broader peak at 3312 cm^{-1} is assigned to O-H absorption. The peak near 2931 cm^{-1} corresponds to the stretch vibration of $–CH_3$ and $–CH_2$. The absorption near 1611 cm^{-1} corresponds to aromatic C=C Bend (α, β-unsaturated ketone or aromatic compound). The peak at 1569 cm^{-1} is related to the stretch vibration of C=C (cyclic alkenes). The peak at 1363 cm^{-1} would be related to O-H bending (phenol). The peak near 1,272 and 1,127 cm^{-1} is attributed to C–O (carboxylic acid, esters, ethers,

and alcohol) stretch vibrations and C–N (amine) stretch vibrations. The peak at 1045 cm^{-1} would be related to C-N stretch vibrations (amine) shown in Table 3.

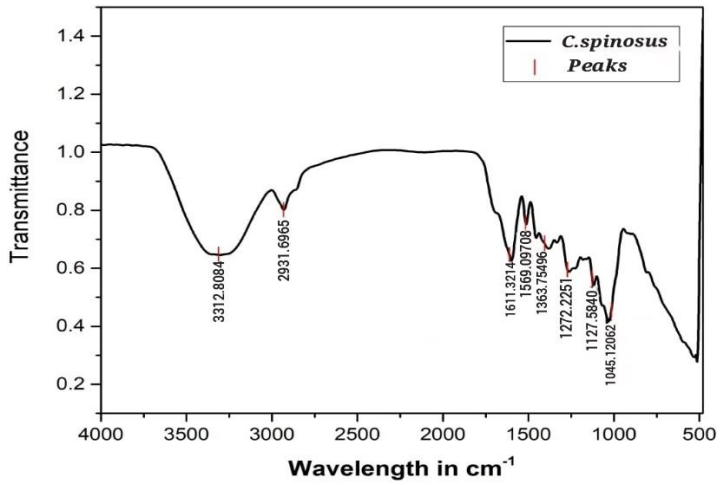

Figure 3.4. ATR-FTIR spectra of dry *C. spinosus* extract

Table 1. Laxative potential of the *C. spinosus* extract in loperamide induced constipated mice.

Group (treated with)	Dose	No. of Faeces in 16 hrs	*p*- value		Weight of wet faeces	*p*- value		Weight of dry faeces	*p*- value		% Faecal Water Content	*p*- value	
			NOC	NEC		NOC	NEC		NOC	NEC		NOC	NEC
Group 1 Distilled water	10 ml/kg	12.6±1.6	…	0.18	1.07±.0.04	…	<0.0001 ****	0.48±0.01	…	0.73	54.67±2.1	…	<0.0001 ****
Group 2 Distilled water	10 ml/kg	8.4±0.5	0.18	…	0.69±0.03	<0.0001 ****	…	0.45±0.02	0.73	…	34.4±1.3	<0.0001 ****	…
Group 3 Extract	100 mg/kg	9.2±1.1	0.38	0.97	1.09±0.04	0.99	<0.0001 ****	0.44±0.02	0.45	0.99	60.1±2.1	0.20	<0.0001 ****
Group 4 Extract	200 mg/kg	13.8±1.3	0.98	0.04*	1.25±0.03	0.02*	<0.0001 ****	0.46±0.01	0.93	0.99	63.2±1.5	0.01**	<0.0001 ****
Group 5 Extract	400 mg/kg	18±1.1	0.04*	0.0001****	1.47±0.02	<0.0001 ****	<0.0001 ****	0.46±0.01	0.97	0.99	68.4±1.1	<0.0001 ****	<0.0001 ****
Group 6 Bisacodyl	0.25 mg/kg	18.4±1.4	0.03*	<0.0001***	1.57±0.04	<0.0001 ****	<0.0001 ****	0.47±0.01	0.99	0.97	70.1±1.3	<0.0001 ****	<0.0001 ****

Results expressed as Mean ± SEM (n=5) P≤.05*, P≤.01**, P≤.001***, P≤.0001****
Group 1 and Group 2 are Normal Control (NOC) and Negative Control (NEC) respectively.
Group 6 is Positive Control (POC), all animals from Group 2 to Group 6 are constipated.

Table 2. Effect of *C. spinosus* 70% Ethanolic Extract on Gastrointestinal Transit in Loperamide- Induced Constipated Mice

Group (treated with)	Dose	Distance travelled by charcoal (mm)	*p* value		Gastrointestinal ratio (%)	*p* value	
			NOR	NEC		NOR	NEC
Group 1 distilled water	10ml/kg	187.4 ±1.7	…	<0.0001	58.68 ±0.7	…	<0.0001
Group 2 distilled water	10ml/kg	133.6 ±4.3	<0.0001****	…	41.89 ±1.3	<0.0001	…
Group 3 Extract	100mg/kg	149.4± 3	<0.0001****	0.0296*	46.78 ±1	<0.0001****	0.0369****
Group 4 Extract	200mg/kg	214.2 ±1.7	0.0001****	<0.0001****	67.29 ±0.7	<0.0001****	<0.0001****
Group 5 Extract	400mg/kg	252.8 ±4.2	<0.0001****	<0.0001****	79.30 ±1.3	<0.0001****	<0.0001****
Group 3 Bisacodyl	0.25mg/kg	266.2±3.2	<0.0001****	<0.0001****	83.59 ±1.1	<0.0001****	<0.0001****

Results expressed as Mean±SEM(n=5) P≤.05*, P≤.01**, P≤.001***, P≤.0001****
Group 1 and Group 2 are Normal Control (NOC) and Negative Control (NEC) respectively.
Group 6 is Positive Control (POC), all animals from Group 2 to Group 6 are constipated.

Table 3. ATR-FTIR spectrum representing potential bands in the dry 70% Ethanolic Extract of *C. spinosus*

Band range (cm^{-1}) (Literature)	Band range (cm^{-1}) (Experimental)	Band Interaction	Band Assignment	Possible compounds
4000-3000	3312	Stretch	0 ─ H	Alcohol
3000-2500	2931	Stretch	C ─ H	Alkane, Alkene, Aldehyde
1670-1600	1611	Bend	C = C	α-ß unsaturated ketone, Aromatic compound
1600-1300	1569	Stretch	C = C	Cyclic Alkenes
	1363	Bend	O ─ H	Phenol
1400-1000	1272	Stretch	C ─ O C ─ N	Aromatic Amine, Alkyl Aryl Ether, Esters, Carboxylic acids
	1127	Stretch	C ─ O C ─ N	Aliphatic ether, Aliphatic amine, Alcohol
	1045	Stretch	C ─ N	Amine

4. Discussion

Convolvulus spinosus Burm. f. is generally known as Ritchak, Dolako, Titok, and Sahsa in different local communities of Balochistan. The bark of the plant is mostly used orally with water by tribal and some other communities of Balochistan to manage constipation in both young and old people. Despite its traditional use in the management of constipation, there is a lack of scientific support for its laxative potential. Hence, this scientific investigation was undertaken to evaluate the laxative potential of *C. spinosus* using standard preclinical experimental models in Swiss albino mice [24].

The *C. spinosus* extract at different doses/concentrations was investigated for its pharmacological effect on the number of pellets, percentage of the water content of feces, GI transit ratio, and GI fluid accumulation. The methods adopted in this study are standard methods that have been used in previous studies to evaluate the laxative potential of plants used traditionally to manage constipation [25]. The present study used In vivo models in Swiss albino mice to determine the potential of 70% ethanolic *C. spinosus* extract to counteract constipation. One of the models used in this study is loperamide-induced constipation model. Loperamide, a potent μ-opioid receptor agonist, was used to induce constipation in this study [24]. Loperamide use can be justified based on its pharmacological actions as it decreases both gastrointestinal motility and intestinal fluid. This results in a decrease in the number and weight of feces along with a reduction in fecal water content. These all parameters are considered the key markers of constipation. Furthermore, Previous literature documented that loperamide effectively induces constipation in rodents when it is administered orally or subcutaneously for 3-7 days [26]. So, the plant is considered to possess laxative potential if it counteracts the decrease in above mentioned parameters produced by loperamide. Furthermore, a gastrointestinal secretion test in a normal Swiss albino mice model was used to evaluate the enteropooling effect of *C. spinosus* using castor oil as a standard drug. This explains the effect of *C. spinosus* on the amount of intestinal fluid accumulated in the small intestine. Castor oil possesses an eneteropooling effect and due to this reason, it is used as a standard drug.

In this study, oral administration of 70% ethanolic *C. spinosus* extract expressed a significant increase in the number of defecations and percentage of fecal water content as compared to the NEC group in the loperamide-induced constipation mice model. Both parameters are evidence of the laxative potential of plant extract. The 200 mg/kg (p<.01) and 400mg/kg concentrations significantly increased the pellets frequency (p<.01). The number of pellets from these doses is approximately 14 and 18 respectively. The increase in the no. of pellets refers to the increase in the number of bowel movements or frequency of defecation by the action of *C. spinosus* extract. The highest such effect (no. of feces 21) was observed in the case of the positive control bisacodyl (p<.0001). A certain significant increase in the percentage of fecal water content was observed at 100mg/kg (60.04%), 200mg/kg (63.02%), and 400mg/kg (68.41%) concentrations (p<.0001). The highest percentage of fecal water content produced by the positive control bisacodyl is 70.08 (p<.0001). In addition, compared to the NOC group an increase in the number of pellets at the

concentration of 400mg/kg was observed and an increase in the percentage of fecal water content was observed at the concentrations of 200mg/kg (p<.05) and 400mg/kg (p=.0001). The plant exerted a significant increment in wet fecal weight and percentage of fecal water content with increasing concentration. This may be due to an increased number of bioactive compounds with the increase in dose. *C. spinosus* extract possibly increased bowel movements due to stimulation of colon nerves that increased the rhythmic muscle contractions of the small intestine and facilitated pushing stools through the small intestine more quickly and it increased the defecation frequency. The plant extract increased the percentage of the fecal water content of pellets possibly due to less time available to the colon to absorb moisture and/ or water and electrolyte secretion into the lumen. The luminal water retention leads to an increase in fecal water content [27,28]. So, the plant extract reversed the anti-absorptive and anti-secretory action of loperamide.

The measurement of gastrointestinal transit time demonstrates propulsion of intestinal contents from proximal to distant segments of the colon as it affects luminal content mixing to promote water absorption. Transit assessment of the small intestine is adopted to evaluate multiple gastrointestinal disorders including constipation. It is also helpful to identify any fluctuation in intestinal tone and capacitance. In this study, Charcoal was used as a marker to assess the intestinal transit ratio. The loperamide administration decreased intestinal motility. The results of this preclinical study showed that *C. spinosus* extract increased intestinal motility that led to accelerated colonic peristalsis. A significant increase in intestinal transit ratio was observed at all doses of *C. spinosus* extract compared to NEC. The value of intestinal transit ratio at the concentration of (100/200/400) mg/kg are 46.78 (p<.05), 67.24 (p <.0001), and 79.38 (p<.0001) respectively. Furthermore, an apparent increase in intestinal transit ratio was observed at all concentrations of *C. spinosus* extract compared to NOC group (p<.0001). Enhancing intestinal motility means a decrease in stool transit time and an increase in the frequency of defecation. Furthermore, enhanced motility does not provide enough time for the absorption of water and electrolytes which also play a role in accelerated colonic transport. The possible mechanism behind increased intestinal motility is the stimulation of intestinal mucosa and/or colonic nerve plexus to secrete more water and electrolytes that induce peristaltic contractions and result in accelerated colonic transport [29].

The effect of *C. spinosus* extract on the small intestinal fluid accumulation was evaluated through a GI secretion model in normal mice using castor oil as a positive control. Castor oil releases ricinoleic acid after its metabolism by intestinal lipases. Ricinoleic acid is responsible for the laxative action of castor oil by several mechanisms. It blocks intestinal Na+/K + -ATPase activity, releases prostaglandins as it irritates the intestinal wall and activates EP3 prostanoid receptors on the intestine and produces contractions of smooth muscles of the intestine. These all-mediate propulsive effects on the gut promote laxation. The *C. spinosus* extract expressed a significant rise in the mean weight of the GI content at the concentration of 200mg/kg (p<0.05) and 400mg/kg (p<0.0001) compared to the NOC group. The possible mechanism might be mediated by the mechanism of action of castor oil as explained earlier. Moreover, laxative agents activate GC-C receptors to increase cyclic GMP that indirectly activates CFTR and it induces secretion of chloride and bicarbonate ions and inhibition of sodium ion absorption [30,31]. This facilitates intestinal water secretion and causes intestinal dilatation and hence promotes intestinal movement.

The secondary metabolites like saponins, phenolic compounds, flavonoids, tannins, and alkaloids have been documented to possess a plethora of pharmacological activities in previous literature including the laxative potential of plant species [32,33,34]. There is no literature available on the presence of secondary metabolites in *C. spinosus*. The phytochemical analysis of the *C. spinosus* extract was carried out in order to deal with this lacuna and results expressed the presence of carbohydrates, tannins, flavonoids, alkaloids, and resins and these may be attributed to the laxative potential of *C. spinosus*.

The ATR-FTIR provides real time analysis, and its characteristic peaks are used to distinguish several functional groups of bioactive substances [35]. There is no literature available for functional groups of compounds present in *C. spinosus*. So, the ATR-FTIR spectral analysis of the dry extract of *C. spinosus* was done and it showed characteristic peaks at different wavelengths. Each peak is

due to infrared absorption from metabolites in dry *C. spinosus* extract and corresponds to a functional group [36]. The ATR-FTIR spectra of dry extract of *C. spinosus* showed the vibrational bands at 3312 ,2931, 1611, 1569, 1363, 1272, 1127 and 1045 wavelengths. The band range of (4000 -2500) cm^{-1} confirmed the presence of O-H and C-H bonds that correspond to the presence of alcohols, alkanes, alkenes, and aldehydes [37]. The band range of (167-1300) cm^{-1} showed the stretch and bend vibrations of C=C and 0-H and showed the possibility of the presence of α-ß unsaturated ketone, cyclic Alkenes, phenol, and aromatic compounds [37,38]. The band range of (1400-1000) cm^{-1} showed vibrations of C-O and C-N and provide evidence for the possible presence of aromatic and aliphatic amines, alkyl aryl ethers, aliphatic ethers, esters, carboxylic acid and alcohols [39,40]. The ATR-FTIR spectrum profile of the dry extract of *C. spinosus* provided the evidence that *C. spinosus* is enriched with bio-active phytochemical compounds and the medicinal properties of these phytochemicals could be a novel approach in developing a safe and cost-effective herbal formulation.

The present study has evaluated and confirmed experimentally the traditional laxative use of *C. spinosus* in the management of constipation, and this is the important public health implication of this preclinical study. This study provides scientific validation of the laxative potential of *C. spinosus*. Furthermore, *C. spinosus* can provide cost-effective and easy access to drug treatment to patients who cannot afford or access modern-day medicines.

5. Conclusions

This research concludes that the crude 70% ethanolic extract of *C. spinosus* has active phytochemicals and possesses laxative potential. The crude extract showed an increase in the number of defecations, percentage of fecal water content, GI motility, and intestinal fluid accumulation. Therefore, current research outcomes provide pharmacological authentication for the use of *C. spinosus* in the management of constipation in ethnomedicine.

Ethics approval: All in-vivo experiments were conducted after acquiring ethical approval for laboratory animal usage from the Advanced Studies and research board (ASRB/No./06101/pharm.) of the University of Karachi, Karachi. Furthermore, all experiments were carried out in accordance with the Internationally accepted guidelines for Animal handling.

Data availability: The data for this study is available at a reasonable request from the corresponding author.

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Credit authorship contribution statement: Javeria Arif has planned the project, Methodology, Performed the experiments, Formal analysis, Results interpretation, Writing – original draft, corresponding author. **Nuzhat Sultana** planned the project, Formal analysis, Supervision, review. **Rabail Urooj** contributed in **w**riting, review and editing**.** All authors read carefully and approved the final manuscript. **Rida Arif** performed phytochemical analysis, and reviewed.

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