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In vivo evaluation of antidiarrhoeal and insecticidal potential of *Loranthus falcatus* Linn. stem extracts

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Abstract
Loranthus falcatus Linn. is a parasitic plant which is importantly used in folk medicine. Aim of the study was to assess antidiarrhoeal and insecticidal potential of *L. falcatus* stem extracts. Castor oil–induced diarrheal model and activated charcoal induced intestinal transit were evaluated in mice for antidiarrhoeal study while Film residue method on *Sitophilus oryzae* L. was followed for insecticidal study. Among the extracts, chloroform fraction showed the highest antidiarrhoeal effect having protection at 79.67% faecal output, 69.75% fluid accumulation and 69.75% peristaltic index at 200 mg/kg dose. On the other hand, petroleum ether fraction showed the maximum insecticidal effect at 93.33%, 96.66% and 100% mortality at 50, 100 and 200 µg/ml concentration, respectively. These findings may potentiate to unveil specific compounds for development of antidiarrhoeal and insecticidal drugs.

Keywords: *Loranthus falcatus*, antidiarrheal effect, insecticidal activity, *Sitophilus oryzae*

Introduction
Diarrhoea is one of the prime causes of child mortality under the age of five which is characterized by increased frequency of bowel movement, wet stool and abdominal pain [1]. The main factors associated with the pathogenesis of the disease are trans epithelial hyper secretion in gastrointestinal (GI) tract and defective water absorption in colon [2]. Diarrhea can either be anindicator of any chronic disease or due to contagious etiology. Treatment is necessaryin chronic diarrheal diseases if the symptoms last which could be for weeks, months or even lifetime. Management of chronic diarrhea includes anti-motility and/or bulk-forming agents which are associated with distention, bloating, nausea and constipation, particularly in long term use [3]. Considering the adverse reaction and untoward effects to the patient triggered bycurrent therapy with antidiarrheal medicine, the search for new antidiarrheal agents are still going on and the medicinal plants are the major sources of them [4]. On the other hand, alternative medicine constituting mainly of herbs offers relatively safe and cost-effective treatment. Still now, almost 25% of drugs isolated from plant sources are using in the treatment of disease such as in malaria, diarrhea, dysentery, skin diseases etc [5].
Sitophilus oryzae L. is one of the main insect pests which is found in stored food grains especially of cereals and their products, responsible for the physical, nutritional and quality deterioration of food grains [6]. They provoke serious damage to grains which are stored at 25-30 °C and at low relative humidity as these conditions assist the pest development. It is the most damaging and pervasive cereal pest in the world which are responsible for around18.30% losses to stored grains [7]. Hence, heavy invasion may take place if control measures are not taken. In many countries, several chemical insecticides have either been banned or prohibited considering environmental concerns and human health hazard issues [8]. Therefore, there is a must to exploration of environmentally friendly, safe, degradable and target specific insecticides. Plants are the soundest source of natural products which are found in the most effective way and with selectivity [9].
L. falcatus is an evergreen perennial climbing woody plant which belongs to the Loranthaceae family. About 7 species out of 20are found in Indian subcontinent [10], which are mainly originated in tropical and sub-tropical regions especially in India, China, Bangladesh, Malayasia, Myanmar and Thailand. In folk medicine it is widely used as aphrodisiac, astringent and narcotic. Moreover, it is used to control asthma, menstrual disorders, swellings, wounds, ulcers etc. [11]. Preliminary phytochemical screening revealed alkaloids, phytosterols, fixed oils and phenolic compounds in it. Besides, it has been reported to have biologically active substances such as quercetin, tannins, β-sitosterol, β-amyrin,

oleanolic acid [12]. Furthermore, stigmasterol, kaempferol, rutin, gallic acid, etc.have been isolated from the plant [13]. The main objective of our present research was to screen antidiarrhoeal and insecticidal potential of aqueous (ALFS), ethanol (ELFS), chloroform (CLFS) and petroleum ether (PLFS) extracts of *L. falcatus* stem in Swiss albino mice and on *Sitophilus oryzae* respectively. This effort may help us to find out safe and effective natural agent for the management of diarrheal disorder and environmentally friendly insecticides.

Materials and Methods

Plant materials

L. falcatus stems were collected from Comilla, Bangladesh in October 2018 and identified by an expert of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. DACB39432. The collected stem was separated from undesirable materials or plant parts, cleaned, dried for one week and subjected to grinding to create coarse powder employing a suitable grinder. The powder was then kept in a dark, cool and dry place and stored in an airtight container.

Extract preparation

Firstly, 700g of stem powder was placed in a clean, flat-bottomed glass container and soaked in ethanol and then sealed. The container with its content was kept for a period of 7 days accompanying occasional shaking and stirring by sonication (40 minutes) using ultrasonic sound bath. After that, the whole mixture undertook a coarse filtration by a piece of clean, white cotton material. Again, the filtrate was filtered through Whatman filter paper. Then, the filtrate was evaporated by using a rotary evaporator which was concentrated to obtain the crude ethanol (16g) extract. Then the crude extract was divided into two portions. One portion (3g) was poured into glass vials to be tested as crude ethanol extract, whereas the second portion (13g) was dissolved in 200 ml ethanol and partitioned successively with chloroform, petroleum ether and water. By using the rotary evaporator, the fractions were then concentrated to find chloroform (yield weight 2.4g), petroleum ether (3.60g) and water (4.50g) fractions. The gummy extracts were then transferred to closed container and stored for further use.

Drugs and chemicals

Loperamide, atropine sulfate, castor oil and activated charcoal were purchased from Mark Germany. Ethanol, chloroform and petroleum ether were bought from Mark India. Normal saline solution was procured from Square Pharmaceuticals Ltd., Bangladesh. All the chemicals employed in this study were of highest analytical reagent grade.

Animals and insects

Swiss albino mice of both (male and female) sex, weighing 25-30 g were used for this experiment which were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). After purchase, the mice were kept in standard environmental conditions (24.0 ± 0 °C &55-65% relative humidity and 12 hour light/dark cycle) for two weeks to be acclimatized to laboratory environment prior to the experiment and ICDDR,B formulated rodent food and water *ad libitum* were feed. The experimental procedures for the use and maintenance of experimental animals were conducted in accordance with the recommendations of Comilla University, Bangladesh. The set of guidelines to be followed for this animal experiment were approved by the institutional animal ethical committee [14]. The insects, *Sitophilus oryzae* (L.), used in the experiment were taken from the stock cultures of the Pharmacy Laboratory, Comilla University, Bangladesh.

Acute toxicity test

A well-known method of Lorke [15] which was described previously was followed to investigate acute toxicity. One hundred mice of both sexes were randomly divided into twenty groups of five mice in each group and were fed orally with graded doses (100, 500, 1000, 1500 and 2000 mg/kg) of ALFS, ELFS, CLFS and PLFS by gastric gavage. The animals were allowed free access to feed and water. They were noticed over a 48 hours period for acutely toxic signs and death.

Castor oil-induced diarrhoea

The technique described by Shoba & Thomas [16] was used in this study. Mice were divided into fourteen groups having six animals in each group, and diarrhoea was induced by using of0.5 ml of castor oil which was administered orally in each mouse. Before one hour of castor oil administration, standard drug loperamide and the extracts were also supplied orally where control group took 1% CMC (10 ml/kg, b.w.) standard group received loperamide (10 mg/kg,b.w.) and the remaining groups received ALFS, ELFS, CLFS and PLFS at a dose of 50, 100 and 200 mg/kg, b.w.Number of both wet and dry diarrhoeal droppings were counted every hour and continued up to 4 hours, and mean value of the wet stools of treatment groups were compared with that of mean value of control group. Inhibition of faeces was calculated by the following formula.

Inhibition offaeces (%) = $(T_0\text{-}Te)/T_0 \times 100$

Where, T_0= Total number of faeces in control group, T_e = Total number of faeces in experimental group.

Effect on castor oil-induced enteropooling

For this experiment, a technique described by Robert [17] was followed for determination of intraluminal fluid accumulation. Grouping and treatment of mice were as same as castor oil induced diarrhoea protocol. The animals were sacrificed after two hours of treatment, and then the intestine was removed after ligation both at the pyrolic sphincter and at the ileocaecal junctions. The whole small intestinal contents were expelled into a graduated measuring cylinder. After that volume as well as weight of contents were recorded and percent inhibition of fluid accumulation was calculated by comparing with control group.

Inhibition of fluid accumulation (%) = $\{(V_c\text{-}V_t)/V_c\} \times 100$

Here, V_c =Volume (intestinal content) of control group, V_t= Volume of treatment/standard groups.

Effect on small intestinal transit

Here the experiment was carried out using previously described method given by Pazhani [18]. Mice were fasted for 18h and assigned into fourteen groups (n=6). Control group, standard group and extracts treatment groups were orally received 1% CMC, atropine sulfate (10 mg/kg b.w) and extracts (ALFS, ELFS, CLFS and PLFS at a dose of 50, 100 and 200 mg/kg, b.w). After one hour,1 ml 10% charcoal suspension in 5% CMC was administered orally to each mouse. After 1 hourall mice were sacrificed and distance

travelled by the charcoal in intestine was measured. Finally, percent inhibition of charcoal movement (Peristaltic Index) was measured by the following formula:

$PI = \{L_C/L_I\} \times 100$
Where, PI= Peristaltic index, L_C = Length of intestine travelled by charcoal; L_I = Total length of intestine.

$IT (\%) = \{(Pc-Pt)/Pc\} \times 100$
Where, IT= Intestinal transit, P_c= Peristaltic Index of control group, P_t = Peristaltic Index of treatment group.

Insecticidal activity
For insecticidal study, Film residue technique, described by Busvine [19] was appliedon *Sitophilus oryzae*. Firstly, 60mm petridishes were taken for control and every treatment group. Then 1 ml ALFS, ELFS, CLFS and PLFS solution (50,100, 200µg/ml concentration) was poured into the lower portion of eachpetridish of respective group. After completely drying by fan air, 30 adults of *S. oryzae* were released in each petridish. Similarly, 1ml ethanol was poured into the lower portion of a petridish served as control group. Mortality was assessed at 0.5, 12, 36, 48 and 72hours of the experiment. A simple microscope was used to check each beetle by observing natural movement of its organs. In some cases, hot needle was taken closer to the bodies (without movement) to verify death. The mortality records of the *Sitophilus oryzae* were corrected by the Abbott's formula [20].

$CM (\%) = \{(M_t-M_c)/100-M_c\} \times 100$
Where, CM=Corrected mortality, M_t = Observed mortality/death rate in treated group, M_c = Mortality rate of control group.

Statistical analysis
The data are expressed as mean ± SEM (n=6 mice per group). Statistical significance (p) calculated by ANOVA done in SPSS, Version 15.0, followed by Dunnett´s Test. $P^b<0.01$ and $P^a<0.001$ were considered as the statistically significant.

Results
Acute toxicity study
Behavior and the faeces condition of each mouse were normal during the study period. Moreover, no signs of weakness or mortality was reported up to 2000 mg/kg body weight dose for oral administration of the extracts.

***In vivo* anti-diarrhoeal effect**
All the extracts (ALFS, ELFS, CLFS and PLFS) showed significant ($P^b<0.01$, $P^a<0.001$)reduction of defecation frequency as dose dependently. Castor oil, after 30 minutes of oral administration has induced diarrhea in control (vehicle control) group. On the other hand, standard drug loperamide had inhibited the defecation (feaces) by 85.44%. CLFS had inhibited 79.67% defecation which was the highest among the extractsat 200 mg/kgdose. The order of feaces inhibition was CLFS> PLFS>ELFS> ALFS (Table1)

Table 1: Antidiarrhoeal activity of *L. falcatus* stem extracts on castor-oil induced diarrhoea.

Treatment	Dose (mg/kg, b.w.)	Total weight of stool (hard+wet) (g)	Weight of wet stool (g)	Total no. of stool (hard+wet)	No. of wet stool	Protection %
Control	--	1.22±0.20	0.85±0.03	14.76±2.05	10.58±1.10	00
Loperamide	10	0.13±0.02	0.018±0.01[a]	3.12±0.02[a]	1.54±0.02[a]	85.44
EDFS	50	0.83±0.12	0.66±0.02[a]	8.95±1.30[a]	7.05±0.47[a]	33.36
	100	0.58±0.08	0.49±0.01[a]	6.55±0.56[a]	5.90±0.35[a]	44.23
	200	0.41±0.16	0.37±0.02[a]	5.0±0.25[a]	4.15±0.20[a]	60.77
CDFS	50	0.68±0.02	0.53±0.04[a]	7.30±1.10[a]	5.42±0.02[a]	48.77
	100	0.43±0.02	0.21±0.01[a]	5.10±0.20[a]	3.73±0.05[a]	64.74
	200	0.20±0.11	0.13±0.01[a]	3.25±0.04[a]	2.15±0.10[a]	79.67
PDFS	50	0.72±0.16	0.60±0.13[a]	8.18±1.12[a]	6.25±0.10[a]	42.34
	100	0.52±0.14	0.35±0.02[a]	6.32±0.35[a]	4.77±0.04[a]	54.91
	200	0.35±0.02	0.20±0.01[a]	5.23±0.53[a]	3.12±0.03[a]	70.51
ADFS	50	1.14±0.02	0.73±0.03[b]	10.15±1.15[b]	7.75±0.45[a]	26.74
	100	0.73±0.20	0.62±0.07[b]	8.25±1.20[a]	6.12±0.67[a]	42.15
	200	0.60±0.02	0.49±0.10[a]	7.30±1.02[a]	5.65±0.13[a]	46.59

Data are Mean ± SEM, $P^b<0.01$, $P^a<0.001$ are considered as significance level compared with control group. ANOVA done in SPSS, version 15.0, followed by Dunnett ´s Test.

Effect on enteropooling
All the extracts significantly ($P^b<0.01$, $P^a<0.001$) inhibited intestinal fluid accumulation (enteropooling) as dose dependent manner which results reduction of intestinal content. Loperamide, standard drug, inhibited 85.83% and85.18% of weight and volume of intestinal content RESPECTIVELY. Among the four extracts, CLFS showed the highest inhibition (80.73% weight and 69.75% volume). The order of enter pooling among the extracts was CLFS> PLFS>ELFS> ALFS (Table2).

Table 2: Effect of *L. falcatus* stem extracts on castor oil induced enter pooling in mice.

Treatment	Dose (mg/kg, b.w)	Weight of intestinal content (g)	% Inhibition of weight	Volume of intestinal content (ml)	% Inhibition of volume
Control	--	3.53±0.05	00	3.24±0.05	00
Loperamide	10	0.50±0.03[a]	85.83	0.48±.01[a]	85.18
ELFS	50	1.85±0.07[a]	47.59	2.47±0.06[b]	23.76
	100	1.53±0.06[a]	56.65	1.98±0.05[a]	38.88
	200	1.18±0.04[a]	66.56	1.57±0.03[a]	51.54
CLFS	50	1.36±0.05[a]	61.47	1.83±0.05[a]	43.51
	100	0.93±0.04[a]	73.65	1.32±0.04[a]	59.25
	200	0.68±0.02[a]	80.73	0.98±0.02[a]	69.75

PLFS	50	1.64±0.04[a]	53.54	2.14±0.05[a]	33.95
	100	1.15±0.06[a]	67.42	1.66±0.05[a]	48.76
	200	0.86±0.03[a]	75.63	1.22±0.03[a]	62.34
ALFS	50	2.56±0.08[b]	27.47	2.63±0.07[b]	18.82
	100	2.05±0.06[a]	41.92	2.23±0.12[a]	31.17
	200	1.64±0.04[a]	53.54	1.76±0.04[a]	45.67

Data are Mean ± SEM, $P^b<0.01$, $P^a<0.001$ are considered as significance level compared with control group. ANOVA done in SPSS, version 15.0, followed by Dunnett ´s Test.

Gastrointestinal transit

All the extracts significantly ($P^b<0.01$, $P^a<0.001$) reduced gastrointestinal transit as dose dependant manner. Among the four extracts, CLFS exhibited the highest inhibition (74.33%) while ALFS exhibited the lowest (40.42%) inhibition of peristaltic index at 200mg/kg dose. The standard drug atropine sulfate showed 83.30% inhibition of peristaltic index at 10mg/kg dose. The order of gastrointestinal transit was atropine sulfate> CFFS> PFFS> EFFS> AFFS (Table3)

Table 3: Effects of *L. falcatus* stem extracts on small intestinal transit.

Treatment	Dose (mg/kg,b.w)	Length of intestine	Distance travelled by charcoal	Peristaltic Index (%)	Inhibition (%)
Control	--	64.20±5.16	52.02±4.20	81.02±7.32	00
Atropine sulfate	10	60.21±6.10	8.15±1.20a	13.53±1.45a	83.30
ELFS	50	62.05±4.19	38.16±3.22[a]	61.49±5.29[a]	24.10
	100	59.83±6.10	30.20±5.20[a]	50.47±4.35[a]	37.70
	200	63.12±5.10	26.10±2.12[a]	41.34±4.20[a]	48.97
CLFS	50	64.23±4.16	24.15±3.23[a]	37.59±3.25[a]	53.60
	100	60.43±3.20	17.40±3.13[a]	28.79±3.21[a]	64.46
	200	63.10±5.11	13.12±2.12[a]	20.79±2.05[a]	74.33
PLFS	50	64.03±4.10	33.63±4.50[a]	52.52±5.80[a]	35.17
	100	60.12±3.85	23.30±2.63[a]	38.75±4.25[a]	52.17
	200	62.27±6.13	16.42±2.52[a]	26.36±2.11[a]	67.46
ALFS	50	64.94±5.67	45.15±5.57[b]	76.60±6.20[b]	14.19
	100	62.24±3.75	37.73±3.18[a]	60.62±5.32[a]	25.17
	200	60.43±4.46	29.17±2.10[a]	48.27±4.23[a]	40.42

Data are Mean ± SEM, $P^b<0.01$, $P^a<0.001$ are considered as significance level compared with control group. ANOVA done in SPSS, version 15.0, followed by Dunnett ´s Test.

Insecticidal activity

All the extracts showed insect (*S. oryzae*) mortality at 50, 100, 200µg/ml concentration. This effect was recorded after 0.5hours of the experiment. However, effective time for death of *S. oryzae* was between 12 hours to 72 hours. Among the extracts, PLFS had maximum effect than other extracts while ALFS showed minimum effect. The order of insecticidal effect was PLFS> CLFS>ELFS>ALFS (Table 4**).**

Table 4: Insecticidal effect of *L. falcatus* stem extracts on *Sitophilus oryzae.*

Treatment	Concentration (µg/ml)	Number of insects used	Number of dead insects					Total no. of dead insects after 72H	% Corrected mortality after 72H
			0.5H	12H	36H	48H	72H		
Control	--	30	0	0	0	0	0	0	0
EDFS	50	30	0	5	6	5	7	23	76.66
	100	30	0	6	5	3	10	24	80
	200	30	0	8	6	7	6	27	90
CDFS	50	30	0	8	7	5	5	25	83.33
	100	30	0	7	6	7	6	26	86.66
	200	30	0	9	7	8	5	29	96.66
PDFS	50	30	0	7	6	8	7	28	93.33
	100	30	0	8	7	6	8	29	96.66
	200	30	0	10	8	3	9	30	100
ADFS	50	30	0	2	5	6	5	18	60.00
	100	30	0	5	3	8	6	22	73.33
	200	30	0	6	6	4	8	24	80.00

Data are Mean ± SEM

Discussion

This experiment was performed to evaluate the antidiarrheal activity of the plant extract of *L. falcatus* stem in experimental mice. Results of the study revealed that all the extracts are significantly ($P^b<0.01$, $P^a<0.001$)effective to control castor oil-induced diarrhea in mice where they were inhibited onset of diarrhea, weight of wet stools, number of wet stools, and total number of stools as compared to the control group. This result is in accordance with previous claims in respect of antidiarrheal herbs. Antidiarrheal plants are known to reduce number of wet stools, consistency of fecal droppings as well as delay in the onset of diarrhea as reported for *Pterocarpus erinaceus* [21].

Castor oil after ingestion converts toricinoleic acid in intestine by the action of lipases enzyme. The acid promotes local irritation and inflammation to the intestinal mucosa leading to potentiation inflammatory mediators release like prostaglandins, histamine and nitric oxide [22]. These

mediators are responsible for increasing epithelial permeability, vasodilatation, mucus secretions and edema formation in the intestinal mucosa. Consequently, it stimulates gastrointestinal motility and prevents reabsorption of Na^+, K^+as well as water by forming ricinoleate salts with Na^+ and K^+ in the lumen of the intestine [23]. Prostaglandins (PG) of the E series are diarrheogenic factors in animals. Therefore, inhibitors of prostaglandin biosynthesis are considered to delay castor oil–induced diarrhea [24]. Based on these facts, it is reasonable to suggest that the extract may reduce PG induced secretion of water and electrolytes due to the inhibition of prostaglandin synthesis [25]. Furthermore, the efflux of Cl^- ions from the cell occurs due to activation of Cl^- channels which is associated with the secretory diarrhoea. As a result, massive secretion of water into intestinal lumen is produced leading to potentiate the production of profuse watery diarrhoea. The extract may hinder the secretion of the water into the intestinal lumen by inactivation of the Cl^- channel [26].

It has been evidenced that several phytochemicals like, tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoidsetc. are effective to control castor oil induced diarrhoeal disorders [27]. Tannins denature proteins in the intestinal mucosa forming protein-tannates complex causing the intestinal mucosa more resistant, and hence inhibits the secretions. Flavonoids are active to suppress intestinal motility and to constrain excess electrolytes secretion [28]. In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism, thus, reducing prostaglandin induced fluid secretion [29]. Polyphenols reveals their antidiarrhoeal activity by the inhibition of cytochrome P450 systems [30]. Therefore, the antidiarrhoeal activity of ELFS, CLFS, PLFS and ALFS could be due to the existence of tannins, flavonoids or polyphenolic substances.

Some secondary metabolites of plant can efficiently be treated as insecticides which are very toxic to insects. These secondary metabolites exhibit their insecticidal activity through binding with a wide range of molecular targets like (a) proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), (b) nucleic acids, (c) bio membranes, and (d) other cellular components [31-32]. The physiology of insect modifies in many ways at various receptor sites by the interaction of secondary metabolites with these targets [33]. Terpenes, steroids, sterols, and cardiac glycosides, terpenoids, flavonoid glycosides have been proven to expose their insecticidal activities through inhibition and regulation of growth, neurotoxicity, and acting as endogenous hormone agonist or antagonist [34]. Some alkaloids such as ryanodine, physostigmine, dictamine, harmalineetc. are potent photosensitizing compounds which cause toxicity to insect larvae in sun light. Proteins like lectins and hemolysins are mostly responsible for showing the insecticidal activity [35]. The leading cause for mortality of insects are the alteration of the digestive enzyme machinery, reduction of feeding and growth which are induced by lectins [36].

Some active biochemicals including alkaloids, tannins, flavonoids, saponinsetc have reported in *L. falcatus* stem extracts [37] which might be responsible for the *in vivo* anti-diarrhoeal and insecticidal activities of the plant. In this studythe experimented plant extracts demonstrated potential to reduce castor oil-induced diarrhea and to destroy the *Sitophilus oryzae* effectively. These natural products might be treated as an antidiarrhoeal agent or an important component for integrated pest management system. Further research will be needed for the separation of the active chemical constituent(s) responsible for the antidiarrhoeal and insecticidal activities and visualization of the proper mechanism of action.

Conclusion

Based on the findings of the present study it can be concluded that the *L. falcatus* stem has significant antidiarrhoeal and insecticidal effects. The outcomes of the study give the scientific basis to support the traditional use of the plant. Finally, this study suggested the isolation of single compound to evaluate the antidiarrheal and insecticidal effects on biological model and to establish the mechanisms of action of the compounds.

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Conflict of interest

Authors have no conflict of interest.

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