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# EVALUATION OF ANTIDIARRHOEAL AND INSECTICIDAL ACTIVITIES OF ETHANOL EXTRACT AND ITS FRACTIONS OF *DENDROPHTHOE FALCATA* (L.) LEAVES

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**Keywords:**

*Dendrophthoe falcate*, Antidiarrhoeal activity, Insecticidal activity, *Sitophilus oryzae*

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**ABSTRACT:** *Dendrophthoe falcata* L. is an important folk medicinal plant for its medicinal uses against different types of diseases. Therefore the present study was designed to investigate the antidiarrhoeal and insecticidal activities of aqueous, ethanol, chloroform and petroleum ether fractions of *D. falcata* leaves. Antidiarrhoeal activities of these extracts were evaluated in Swiss albino mice by three different approaches: (i) castor oil–induced diarrhea, (ii) castor oil–induced enteropooling and (iii) activated charcoal induced small intestinal transit. Three doses of the leaf extracts (50, 100 and 200 mg/kg.p.o.) and standard drug loperamide (5mg/kg.p.o.) were used to conduct the research which showed a significant ($P<0.001$) reduction in the severity and frequency of diarrhea (total number and weight of stools), the volume and weight of intestinal content, as well as intestinal transit compared to control group at dose-dependent manner. Ethanol extract exhibited maximum inhibition (83.47%) among the four fractions, which are slightly less than loperamide (89.23%) whereas aqueous fraction produced the lowest inhibition (50.95%). The insecticidal activity of these extracts was investigated by the film residue method against *Sitophilus oryzae* L. In the insecticidal activity, the result showed that four fractions of *D. falcata* leave exhibited strong to moderate toxicity in concentration-dependent fashion on the insect. Among the extracts, ethanol fraction showed maximum mortalityt.

**INTRODUCTION:** Diarrhoea is characterized by an increase in the fluidity, volume, frequency of bowel movements, increased frequency of bowel sound, wet stools, and abdominal pain, accompanied by increased secretion and decreased absorption of fluid, and thus loss of water and electrolytes [1, 2, 3, 4]. It is one of the leading causes of mortality and morbidity in developing countries accounting for more than 5-8 million deaths per year in infants and small children fewer than 5 years old [5, 6, 7, 8].

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It is estimated that during the next 20-30 years, diarrhea, along with other infectious diseases will remain a cause of global health concern [9]. The major cause of this disease is malnutrition, and it may be brought about by viruses, bacteria, fungi, protozoa, drugs, and bacterial endotoxins [10, 11].

Generally, the treatment of diarrhea is non-specific and is usually aimed at reducing the discomfort, inconvenience of frequent bowel movements [12] and the frequency of feces [1, 13]. Oral rehydration therapy has been a key strategy for effective case management. However, it often fails in high stool output state [14]. Moreover, symptomatic therapy with anti-motility agents is contraindicated in infectious diarrhea, and there is an increasing threat of drug resistance to antibiotics [15]. In the recent past, there have been advances towards the treatment of infectious diarrhea with supportive

therapy such as the use of probiotics; but these are still under development [14, 16].

Consumption of medicinal herbs is tremendously increased over the past decades as an alternative approach to improve the quality of life and maintain good health. Medicinal plants have been used for centuries as remedies for human diseases. Recently, there has been growing interest in exploiting biological activities of flora and fauna owing to their natural origin, cost-effectiveness and lesser side effects [17, 18]. In developing countries, the majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea. Therefore, medicinal plants represent a promising source for the discovery of new antidiarrhoeal agents [19]. The World Health Organization (WHO) has encouraged studies for treatment and prevention of diarrhoeal disease based on traditional medicinal practices [20, 21]. Hence, medicinal plants may aid in developing cost-effective alternative approaches for the treatment of diarrhea. It would be interesting to search for plants with antidiarrhoeal activities that could be used against any type of diarrhoeal disease [2].

*Sitophilus oryzae* L. commonly known as rice weevil is a primary and major pest of agricultural stored products such as maize, sorghum, wheat, barley and rice [22, 23, 24]. It can feed on clean grains which reduces not only germination efficiency, but also quantitative, qualitative, nutritional and commercial values of that grain [25, 26, 27]. The annual losses of grains due to weevils are estimated to an average of 25% to 40% after 6 months of storage [22, 26]. High populations of this species can easily build up as it has a relatively short developmental period [28]. Additionally, the kernel damage caused by *S. oryzae* larvae enables other species to attack quickly, which are incapable of infesting sound grain. Thus increase the damage rapidly. Both white and brown rice are susceptible to the damage by the pest [29]. So, unless control measures are taken, heavy infestations may take place.

Control of these insects generally requires the use of chemical insecticides, although these insecticides are toxic to humans and domestic animals, and negatively impact the environment [30]. Therefore, there is a need to search for environmentally safe, degradable, and target specific insecticides [31]. Plants are a sound source of natural product in a most efficient way and with precise selectivity [32, 33, 34].

*Dendrophthoe falcata* belongs to the family Loranthaceae, commonly known as 'Porgassa in Bangla [35], and 'Banda' in Hindi [36]. It is also familiar as "Bandaaka, Vrkshaadani, Vrkshruuhaa" in the Indian Ayurvedic System of Medicine [37]. *D. falcata* is an evergreen perennial climbing [38] woody hemiparasitic plant with smooth bark grey, leaves opposite unequal, thick 1.6 - 25.4 cm long, flowers single, large, bisexual, orange-red or scarlet softly pubescent, berries soft ovoid-oblong, 1.3cm diameter [35, 39, 40]. It is found in Bangladesh and also widely distributed in Australia, India, China, Malaysia, Myanmar [35, 40, 41], Srilanka, and Thailand [38, 42, 43].

The entire plant is used extensively in traditional system of medicine as cooling, bitter, aphrodisiac, astringent, narcotic, diuretic, and is useful in pulmonary tuberculosis, asthma, menstrual disorders, swellings, wounds, ulcers, renal and vesical calculi, [37, 38, 39, 42, 44, 45, 46, 47]. Leaf paste is used in skin diseases where it is applied on boils, setting dislocated bones and extracting pus [43, 36]. The decoction of the whole plant is used to treat joint pains [48,] and leaf juice is used for relief from chest pain [49]. *D. falcata* is reported to have cytotoxic, immunomodulatory activities [50, 51] and wound healing, antimicrobial, antioxidant activities [39] as well as hepatoprotective activity [52]. In the traditional system of medicine, *D. falcata* is recommended for the treatment of epilepsy [44, 53].

Preliminary phytochemical screening mainly revealed the presence of carbohydrates, alkaloids, phytosterols, fixed oils, and phenolic compounds [54, 55]. Several enzymes are separated from the leaves of *D. falcata* such as L-Threonine dehydratase, hexokinase, glucan phosphatase[35]. It has also been reported by the isolation and identification of several possible active chemical constituents such as β-amyrin acetate, β-sitostirol, stigmasterol, oleanolic acid [49, 56], kaempferol, quercetin [41, 47] quercetin–3-O-rhamnoside, rutin, myricetin, and their glycosides: leucocyanidin, kaempferol-3-O-α-L-rhamnopyranoside and quercetin-3-O-α-L-rhamnopyranoside, *etc*. [44, 52, 57]

It also contains tannins comprising of gallic acid, chebulinic acid [43] ellagic acid [57] and (+) – catechin [43, 44, 56, 58]. Three cardiac glycosides such as strospeside, odoroside F, and neritaloside were isolated from the leaves of *D. Falcate* [44]. Pentacyclic triterpenes: 3β-acetoxy-1β-(2-hydroxy-2-propoxy)-11α-hydroxy-olean-12-ene [59-65].

The study was undertaken to evaluate antidiarrhoeal, and insecticidal activity of aqueous, ethanol, chloroform and petroleum ether extract of *D. falcata* leaves in albino mice and *Sitophilus oryzae* which will unveil the rationality of use of the plant as traditional medicines.

## MATERIALS AND METHODS:

**Plant Materials:** For the investigation, *Dendrophthoe falcata* L. leaves, mistletoe of *Swietenia fabrilis* tree were collected from Joypurhat, Bangladesh in September 2012 and identified by experts of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. 39432. The collected plant parts were cleaned, dried for one week and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

**Extract Preparation:** Approximately 800 g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol and similarly 400g of the powder was soaked in distilled water. Both the containers with its contents were sealed and kept for 5 days. Then extraction was carried out using an ultrasonic sound bath accompanied by sonication (40 min). The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and was concentrated to obtain the ethanol (12g) and aqueous (4g) crude extracts.

The ethanol extract was divided into two portions. One portion (2 g) was poured into glass vials to be tested as crude ethanol extract, whereas the second portion (10 g) was dissolved in 100 mL ethanol and partitioned successively with chloroform and petroleum ether. The fractions were then concentrated using a rotary evaporator to obtain chloroform fraction (yield weight 1.5 g), and petroleum ether fraction (yield weight 2.60 g). This process rendered a gummy concentrated reddish black color. The gummy extracts were transferred to a closed container for further use and storage.

**Drugs and Chemicals:** The active drugs loperamide and atropine sulfate; active chemical castor oil and activated charcoal were purchased from Mark Germany. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Ethanol, chloroform, and petroleum ether were purchased from Mark Germany. All the chemicals used in this study were of analytical reagent grade.

**Animals and Insects:** Swiss Albino mice of either sex weighing approximately 25-30 g were used for this experiment. The mice were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). After their purchase, the mice were kept in standard environmental conditions (24.0 ± 0°C & 55-65% relative humidity and 12 h light/dark cycle) for one week to acclimate and fed ICDDR, B formulated rodent food and water *ad libitum*. The experimental procedures involving animals were conducted by the guidelines of Southeast University, Dhaka, Bangladesh. The study protocol was approved by Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee of the University. The set of rules followed for the animal experiment were approved by the institutional animal ethical committee [66]. Insect's *Sitophilus oryzae* (L) used in the present experiment were taken from the stock cultures of the pharmacy Laboratory, Southeast University, Dhaka, Bangladesh.

**Acute Toxicity Testing:** One hundred and two mice were divided in seventeen groups of six animals. Four extracts (aqueous, ethanol, chloroform and petroleum ether) of *D. falcata* leaves were administrated orally at doses of 200, 400, 800, 1600 and 3200 mg/kg body weight to the animal groups (one dose per group). The control group received normal saline (mg/kg). General signs of weakness and symptoms of toxicity, food and water intake and mortality were recorded for a period of 48 h and then for 14 days.

***In-vivo* Anti-Diarrheal Activity:** The experiment was performed according to the method described by Shoba & Thomas [67]. Briefly, mice fasted for 24 hours were randomly allocated to fourteen groups of six animals in each group. All the animals were screened initially by giving 0.5 ml of castor oil. Only those showing diarrhea were selected for the final experiment. Group I received 1% CMC (10 ml/kg, *p.o*), group II received loperamide (10 mg/ kg, *p.o.*), groups III, IV and V received orally the aqueous extract (50, 100 and 200 mg/kg); group VI, VII and VIII received orally the ethanol extract (50, 100 and 200 mg/kg); group IX, X, and XI received orally the chloroform extract (50, 100 and 200 mg/kg), and group XII, XIII and XIV received orally the petroleum ether extract (50, 100 and 200 mg/kg) respectively. After 1 h of oral ingestion, 0.5 ml of castor oil was administered orally in each animal.

Each animal of respective group was placed in an individual digestive cage, the floor of which was lined with blotting paper. This paper was changed after 1hour and observed up to 4 hour. The total number and weight of stools (both diarrhoeal and non-diarrhoeal) counted in each group were compared with the control group, and the results were expressed as a percentage of inhibition of diarrhea (stool output).

$$\text{Inhibition of stools (\%)} = (T_0 - T_1) / T_0 \times 100$$

Where, $T_0$ = Total number of stools in the control group, $T_1$ = Total number of stools in the test group.

**Castor Oil Induced Enteropooling:** This study was done as described by Robert *et al.*, [69] with slight modification. Eighty-four mice were divided into fourteen groups of six animals in each group and were fasted for 24 h with free access to water. The first group (control group) received 1% CMC (10 ml/kg, p.o); the second group received the standard drug, loperamide (10 mg/kg body weight). Group III-XIV received aqueous, ethanol, chloroform and petroleum ether extracts of three doses (50, 100 and 200 mg/kg) orally in the respective group which is described in castor oil induced diarrhea model. After 1 h, 1 ml of castor oil was administered orally in each animal. The mice were anesthetized 1h later by inhalation of chloroform. Then the animals were sacrificed, and the small intestine was removed after ligation both at the pyrolic sphincter and at the ileocaecal junctions. The entire small intestine contents were expelled into a graduated measuring cylinder, and volume, as well as the weight of contents, was recorded and expressed as percent inhibition of fluid accumulation.

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

Here, Vc = Volume of intestinal content of control group, Vt = Volume of intestinal content of the treatment group.

**Gastrointestinal Transit:** Eighty-four mice were divided into fourteen groups of six animals in each group and were fasted for 18 h with free access to water. The first group (control group) received 1% CMC (10 ml/kg, p.o); the second group received the standard drug, atropine sulfate (10 mg/kg body weight). Group III-XIV received aqueous, ethanol, chloroform and petroleum ether extracts of three doses (50, 100 and 200 mg/kg) orally in the respective group which is described in castor oil induced diarrhea model. After 60 minutes, 1 ml of the activated charcoal meal (10% charcoal suspension in 5% CMC) was administered orally in each animal. All the animals were anesthetized with chloroform after 1 hour of charcoal administration. Then the animals were sacrificed and distance traveled by the charcoal in the intestine was measured and expressed as percent inhibition of charcoal movement [68] (Peristaltic Index).

$$PI = Lc \times 100 / LI$$

Where, PI= Peristaltic Index, Lc = Length of Charcoal Meal; LI = Length of Intestine.

$$IT (\%) = (Pc - Pt) \times 100 / Pc$$

Where, IT = Inhibition of transit, Pc = Peristaltic Index of the control group, Pt = Peristaltic Index of the treatment group.

**Insecticidal Activity:** To conduct the study, film residue method [70] was used to test the mortality of the adults of *Sitophilus oryzae* L. To perform the test 60mm Petri dishes were taken for control and each extract group. 1 ml of aqueous, ethanol, chloroform and petroleum ether extract solution (50, 100, 200 µg/ml) was poured into the lower part

of each Petri dish of respective group and allowed them to dry out firstly by fanning and finally by heating in an electric oven at 40 ºC temperature. 15 adults of *S. oryzae* were released in each Petri dish, and the whole experiment was done three times for each group. A control experiment by applying the only solvent into the Petri dish was also set at the same time under the same condition [71]. After completing all the arrangements, treated Petri dishes were placed in a secured place at room temperature. Mortality was assessed after 0.5, 12, 36, 48, and 72 h of the treatment.

A simple microscope was used to check every beetle by tracing the natural movement of its organs. In some cases, hot needle was taken closer to the bodies (without movement) to confirm the death. The mortality records of the *Sitophilus oryzae* L. adults were corrected by the Abbott's formula [72].

$$CM\ (\%) = (Mt - Mc) \times 100 / 100 - Mc$$

Where, CM = Corrected mortality, Mt = Observed mortality rate in treated group, Mc = Mortality rate of the control group.

**Statistical Analysis:** The data are expressed as mean ± S.E.M. (n=6 mice per group). Statistical significance (p) calculated by ANOVA done in SPSS, Version 15.0, followed by Dunnett ´s Test. P**<0.01 and P***<0.001 were considered to be statistically significant.

## RESULTS:

**Acute Toxicity Study:** The behavior and the feces of the animals were normal. We did not observe others signs of weakness or mortality in mice receiving up to a dose 3200 mg/kg body weight by oral administration of the aqueous, ethanol, chloroform and petroleum ether extracts of *D. falcata* leaves. This finding suggests that the extracts of *D. falcate* leaves are safe or non-toxic to mice up to 3200mg/kg p.o.

***In-vivo* Castor Oil-Induced Anti-Diarrhoeal Test:** In the castor oil induced diarrhoeal mice, the aqueous, ethanol, chloroform and petroleum ether extracts of *D. falcata* leaves at the dose of 50, 100 and 200 mg /kg b. wt. significantly lessen the total number of stools, total number of wet stools, the weight of total stools and weight of wet stools in a dose-dependent manner. After a 30-min administration of castor oil, diarrhea was clinically apparent for the next 4 h in the control group. This condition was markedly reduced by 89.23% by loperamide at a dose of 10 mg/kg.

All of our extracts also demonstrated statistically significant (P<0.001) inhibition of castor oil-induced diarrhea in a dose-dependent manner. Amongst four extracts, the ethanol fraction had better activity against diarrhea and produced 83.47% inhibition at 200 mg/kg, while aqueous fraction showed lowest inhibition (50.95%) at the same dose **Table 1**.

**TABLE 1: EFFECT OF AQUEOUS, ETHANOL, CHLOROFORM AND PETROLEUM ETHER EXTRACTS OF *D. FALCATA* LEAVES ON CASTOR OIL INDUCED DIARRHOEA IN MICE**

| Treatment | Group | Dose (mg/kg p.o.) | Total weight of stool (hard + wet) (g) | Weight of wet stool (g) | Total no. of stool (hard + wet) | No. of wet stool | Protection % |
|---|---|---|---|---|---|---|---|
| Control | I | -- | 1.07±0.012 | 0.78±0.01 | 13.53±1.27 | 9.38±1.17 | 00 |
| Loperamide | II | 10 | 0.11±0.02*** | 0.013±0.01*** | 4.23±0.03*** | 1.01±0.01*** | 89.23 |
| Aqueous | III | 50 | 1.02±0.11** | 0.64±0.11** | 11.26±1.42** | 8.23±0.26** | 12.26 |
| fraction | IV | 100 | 0.69±0.10*** | 0.53±0.17*** | 10.21±1.45** | 6.13±0.29*** | 34.64 |
| | V | 200 | 0.55±0.15*** | 0.39±0.15*** | 8.31±1.20*** | 4.60±0.20*** | 50.95 |
| Ethanol | VI | 50 | 0.62±0.17*** | 0.36±0.05*** | 7.75±0.35*** | 6.05±0.18*** | 35.50 |
| fraction | VII | 100 | 0.36±0.06*** | 0.29±0.02*** | 4.35±0.32*** | 2.98±0.10*** | 68.23 |
| | VIII | 200 | 0.22±0.10*** | 0.18±0.03*** | 3.23±0.10*** | 1.55±0.27*** | 83.47 |
| Chloroform | IX | 50 | 0.89±0.05*** | 0.52±0.06*** | 8.60±0.40*** | 7.06±0.05*** | 24.73 |
| fraction | X | 100 | 0.52±0.03*** | 0.45±0.06*** | 6.60±0.47*** | 4.23±0.25*** | 54.90 |
| | XI | 200 | 0.25±0.10*** | 0.22±0.07*** | 3.85±0.25*** | 2.00±0.20*** | 78.67 |
| Petrolium | XII | 50 | 1.03±0.19** | 0.61±0.11** | 11.10±0.18** | 8.19±0.21** | 12.68 |
| ether | XIII | 100 | 0.64±0.16*** | 0.31±0.01*** | 8.10±0.14*** | 6.10±0.14*** | 34.96 |
| fraction | XIV | 200 | 0.45±0.03*** | 0.24±0.02*** | 5.30±0.30*** | 4.30±0.30*** | 54.15 |

Data are Mean ± SEM, P**<0.01, P***<0.001 are considered as significance level compared with the control group. ANOVA did in SPSS, version 15.0, followed by Dunnett ´s Test

**Castor Oil Induced Enteropooling:** All the extracts of *D. falcata* leaves were effective to inhibit fluid accumulation in the intestine (enteropooling) and consecutively weight gain. They significantly (P<0.01, P<0.001) inhibited the enteropooling by the strength of the extracts. Shortly, Loperamide inhibited 83.89% and 81.67% of volume and weight of intestinal content. Among the extracts, the highest inhibition was found in ethanol fraction 74.22% of volume and 70.49% of weight at 200 mg/kg dose. Aqueous, chloroform and petroleum ether extracts showed 65.5255, 65.21% and 55.275% of weight, and 68.01%, 71.42% and 64.59% of volume inhibition at 200mg/kg dose of the extracts **Table 2**.

**TABLE 2: EFFECT OF AQUEOUS, ETHANOL, CHLOROFORM AND PETROLEUM ETHER EXTRACTS OF *D. FALCATA* LEAVES ON CASTOR OIL INDUCED ENTEROPOOLING IN MICE**

| Treatment | Group | Dose (mg/kg, *p.o*) | Weight of intestinal content(g) | % Inhibition of weight | The volume of intestinal content(ml) | % Inhibition of volume |
|---|---|---|---|---|---|---|
| Control | I | -- | 3.22±0.03 | 00 | 2.98±0.07 | 00 |
| Loperamide | II | 10 | 0.59±0.02*** | 81.67 | 0.48±.01*** | 83.89 |
| | III | 50 | 2.36±0.01** | 36.02 | 2.38±0.05** | 20.13 |
| Aqueous | IV | 100 | 1.66±0.03*** | 48.44 | 1.58±0.03*** | 50.93 |
| fraction | V | 200 | 1.11±0.02*** | 65.52 | 1.03±0.02*** | 68.01 |
| | VI | 50 | 2.06±0.02** | 26.70 | 2.07±0.04** | 30.53 |
| Ethanol | VII | 100 | 1.16±0.01*** | 63.97 | 1.01±0.02*** | 68.63 |
| fraction | VIII | 200 | 0.95±0.04*** | 70.49 | 0.83±0.03*** | 74.22 |
| | IX | 50 | 2.13±0.02** | 33.85 | 2.24±0.05** | 24.83 |
| Chloroform | X | 100 | 1.50±0.04*** | 53.41 | 1.52±0.04*** | 52.79 |
| fraction | XI | 200 | 1.12±0.03*** | 65.21 | 0.92±0.02*** | 71.42 |
| | XII | 50 | 2.52±0.01** | 21.73 | 2.35±0.07** | 21.14 |
| Petroleum | XIII | 100 | 1.55±0.02*** | 51.86 | 1.43±0.06** | 55.59 |
| ether fraction | XIV | 200 | 1.44±0.03*** | 55.27 | 1.14±0.03*** | 64.59 |

Data are Mean ± SEM, P**<0.01, P***<0.001 are considered as significance level compared with the control group. ANOVA did in SPSS, version 15.0, followed by Dunnett´s Test.

**Gastrointestinal Transit:** All the extracts of *D. falcata* leaves have shown their effects on intestinal transit in mice. The extracts (aqueous, ethanol, chloroform and petroleum ether fractions) cause a significant (P<0.001) dose-dependent (50, 100, 200 mg/kg p.o.) reduction of the distance traveled by charcoal meal (Intestinal transit) compared to the control mice. Among the extracts highest inhibition of the transit (57.28%) was produced by the ethanol fraction, and the lowest inhibition (39.44) was produced by an aqueous fraction at 200 mg/kg dose. The standard drug atropine sulfate showed 69.16% inhibition of the gastrointestinal transit at 10 mg/kg dose **Table 3**.

**TABLE 3: EFFECT OF AQUEOUS, ETHANOL, CHLOROFORM AND PETROLEUM ETHER EXTRACTS OF *D. FALCATA* LEAVES ON CHARCOAL INDUCED SMALL INTESTINAL TRANSIT IN MICE**

| Treatment | Group | Dose (mg/kg,p.o) | Length of intestine | Distance traveled by charcoal | Peristaltic Index (%) | Inhibition (%) |
|---|---|---|---|---|---|---|
| Control | I | -- | 60.33±4.36 | 48.16±3.27 | 79.82761±2.36 | 00 |
| Atropine sulfate | II | 10 | 62.37±5.30 | 15.35±2.45*** | 24.61±3.01*** | 69.16 |
| | III | 50 | 61.25±3.39 | 39.56±4.20*** | 64.58±3.89*** | 19.08 |
| Aqueous | IV | 100 | 58.73±5.06 | 35.10±2.20*** | 59.76±3.27*** | 25.12 |
| fraction | V | 200 | 61.78±4.01 | 29.86±3.78*** | 48.33±3.30*** | 39.44 |
| | VI | 50 | 60.03±2.26 | 29.45±2.33*** | 49.05±3.09*** | 38.53 |
| Ethanol fraction | VII | 100 | 62.41±5.12 | 24.61±4.10*** | 39.43±2.31*** | 50.59 |
| | VIII | 200 | 59.60±4.51 | 20.32±3.18*** | 34.09±2.25*** | 57.28 |
| | IX | 50 | 60.93±3.89 | 34.48±3.20*** | 56.58±3.81*** | 29.10 |
| Chloroform | X | 100 | 63.45±5.01 | 31.36±4.23*** | 49.42±3.15*** | 38.07 |
| fraction | XI | 200 | 60.80±4.22 | 25.45±2.02*** | 41.85±3.10*** | 47.55 |
| | XII | 50 | 57.44±3.77 | 34.55±4.57*** | 60.14±3.49*** | 24.64 |
| Petroleum ether | XIII | 100 | 60.56±2.02 | 29.23±2.78*** | 48.26±2.22*** | 39.53 |
| fraction | XIV | 200 | 63.13±5.17 | 25.87±2.16*** | 40.97±2.55*** | 48.66 |

Data are Mean ± SEM, P**<0.01, P***<0.001 are considered as significance level compared with the control group. ANOVA did in SPSS, version 15.0, followed by Dunnett´s Test.

**Insecticidal Activity:** The toxic effects of aqueous, ethanol, chloroform, and petroleum ether extracts of *D. falcata* leaves were evaluated against *S.oryzae* by using surface film method. All the extracts at different concentrations (50, 100, 200 µg/ml) revealed toxicity at a concentration gradient manner. The numbers of dead *Sitophilus oryzae* L. were counted after 0.5, 12, 36, 48 and 72 h at the three concentrations respectively. Then the percentages of corrected mortality were calculated by using Abbott's formula, and the results are shown in **Table 4**. Briefly, both ethanol and chloroform fractions produced highest mortality (100%) while Petroleum ether and aqueous fractions showed 92% and 80% respectively at 200µg/ml concentration **Table 4**.

**TABLE 4: INSECTICIDAL PROFILE OF *D. FALCATE* LEAF EXTRACTS ON *SITOPHILUS ORYZAE* L. BY FILM RESIDUE METHOD**

| Treatment | Group | Conc. (µg/ml) | Number of an insect used | Number of dead insects | | | | | Total no. of dead insects after 72 h | % Corrected mortality after 72 h |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.5 h | 12 h | 36 h | 48 h | 72 h | | |
| Control | I | -- | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | II | 50 | 25 | 0 | 0 | 2 | 3 | 5 | 11 | 44 |
| Aqueous | III | 100 | 25 | 0 | 2 | 1 | 5 | 7 | 15 | 56 |
| fraction | IV | 200 | 25 | 0 | 3 | 4 | 6 | 7 | 20 | 80 |
| | V | 50 | 25 | 0 | 4 | 2 | 6 | 5 | 17 | 68 |
| Ethanol | VI | 100 | 25 | 0 | 5 | 4 | 3 | 6 | 20 | 80 |
| fraction | VII | 200 | 25 | 0 | 8 | 7 | 4 | 6 | 25 | 100 |
| | VIII | 50 | 25 | 0 | 2 | 5 | 3 | 8 | 18 | 72 |
| Chloroform | IX | 100 | 25 | 0 | 4 | 6 | 7 | 5 | 22 | 88 |
| fraction | X | 200 | 25 | 0 | 6 | 5 | 6 | 8 | 25 | 100 |
| | XI | 50 | 25 | 0 | 2 | 6 | 4 | 6 | 16 | 64 |
| Petroleum | XII | 100 | 25 | 0 | 5 | 3 | 8 | 3 | 19 | 76 |
| ether fraction | XIII | 200 | 25 | 0 | 5 | 5 | 6 | 7 | 23 | 92 |

**DISCUSSION:** Most of the people throughout the world normally using plant(s) or plant-derived preparations (such as Ayurvedic and Herbal products), and consider them to be efficacious against diarrhoeal disorders without any scientific basis to explain the action of such plants. *D. falcata*, mistletoe of *Swietenia fabrilis*, is regarded to use against diarrhoeal disease in Bangladeshi traditional medicine [18]. Diarrhea may be characterized as the abnormally frequent defecation of stools of low consistency, which may be a consequence of a disturbance in the transport of water and electrolytes in the intestines.

There are four major mechanisms responsible for the pathophysiology in water and electrolytes transport in diarrhea such as (a) increased luminal osmolarity (osmotic diarrhea), (b) increased electrolytes secretion (secretory diarrhea), (c) decreased electrolytes absorption, and (d) deranged intestinal motility causing a decreased transit time [18, 73]. It is unveiled that castor oil after oral ingestion is metabolized into ricinoleic acid [18] in the intestinal lumen by the action of lipases [74]. This acid causes irritation and inflammation to the intestinal mucosa resulting in the release of inflammatory mediators, such as prostaglandins, histamine, and nitric oxide which in turn stimulates gastrointestinal motility, mucus secretions, epithelial permeability, vasodilatation, smooth muscle contraction and edema of the intestinal mucosa, thereby preventing the reabsorption of $Na^+$, $K^+$ and water [74]. Prostaglandins of the E series are considered to have good diarrheogenic effects in experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil–induced diarrhoea[75].

The secretory diarrhea is associated with activation of $Cl^-$ channels, causing $Cl^-$ efflux from the cell. The efflux of $Cl^-$ results in the massive secretion of water into the intestinal lumen and profuse watery diarrhea. The extract may inhibit the secretion of the water into the lumen by inactivation of the $Cl^-$ channel [76]. Polyphenols, by their antidiarrhoeal property, interact with and inhibit cytochrome P450 systems. This can impact the pharmacokinetics of any co-administered drugs metabolized by these systems [77].

The antidiarrhoeal activity of the aqueous, ethanol, chloroform and petroleum ether extracts of the leaf of *D. falcata*, therefore, could be due to the presence of tannins, flavonoids and polyphenols [19]. Plants possessing tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids are responsible for anti-diarrhoeal activity [2, 18, 74, 75]. Tannins present in anti-diarrhea plants denature proteins in the intestinal mucosa by forming protein tannates complex. Protein tannates make the intestinal mucosa more resistance and hence, reduce secretion [18, 78]. Studies on the functional role of tannins also unveil that they could also bring similar functions by reducing the intracellular $Ca^{2+}$ inward current or by activation of the calcium pumping system (which induces the muscle relaxation) [79]. Anti-diarrhoeal activities of flavonoids have been attributed to their ability to restrain intestinal motility and hydro electrolytic secretions which are known to be altered in diarrhoeic conditions [3, 18].

Secondary metabolites of the plant are the active toxic ingredients to insects that are evolved to protect them from herbivores [80]. These secondary metabolites such as terpenes, terpenoids, flavonoid, saponins, alkaloids, and some proteins, phenolic compounds as well as tannins exert insecticidal activity through a wide range of molecular targets [31]. These targets are: (a) proteins (enzymes, receptors, signaling molecules, ion-channels, and structural proteins), (b) nucleic acids, (c) biomembranes, and (d) other cellular components. When secondary metabolites interact with these targets, insect physiology alters in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage, release, binding, and re-uptake, receptor activation and function, enzymes involved in signal transduction pathway) [80]. Terpenes, steroids, sterols, and cardiac glycosides, pyrethroids [81, 82], terpenoids, (azadirachtin), flavonoid glycosides [83] have been demonstrated to have insecticidal or insect-inhibiting activities through growth inhibition, neurotoxicity, growth regulation, endogenous hormone agonist or antagonist [84]. Some alkaloids like Ryanodine, Physostigmine, dictamine, harmaline, etc are potent photosensitizing compounds that are highly toxic to insect larvae in sun light. Proteins such as lectins and hemolysins are responsible for most of the insecticidal activity [85]. lectins provoke a wide range of detrimental effects, including alteration of the digestive enzyme machinery, reduction of feeding, growth and development inhibition that is the main reason of mortality of insects [86].

*D. falcata* leaf extracts possess active biochemicals such as alkaloids, tannins, flavonoids, saponins, *etc.* which may be responsible for the *in-vivo* anti-diarrhoeal and *in-vitro* insecticidal effects of the plant.

From the results, it is clear that the use of *D. falcate* leaves as traditional medicine is rational and also it will be more or less effective for controlling insect, specially *Sitophilus oryzae* L. It is available throughout the country, and the farmers may use this plant in their storehouses for the management of stored grain pests. Further investigation for the identification of active chemical compounds responsible for the antidiarrhoeal and insecticidal effect of the extracts is almost needed.

**CONCLUSION:** In conclusion, the results presented in this study revealed that crude ethanol extract and aqueous, chloroform as well as petroleum ether fractions of *D. falcate* L. leaves possess significant antidiarrhoeal and insecticidal activities. These results further support the traditional use of this plant as medicine. The potential of the extracts as antidiarrhoeal and insecticidal activities may be due to the presence of phytoconstituents like flavonoids, tannins, phenolics, *etc*. However, more detail phytochemical analysis will be necessary to isolate and characterize the active compounds which are responsible for these activities, and that will give a way to draw the proper mechanisms of action of these activities.

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