Bioactivity studies on *Musa seminifera* Lour

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Submitted: 05-09-2012 Revised: 04-11-2012 Published: 07-09-2013

ABSTRACT

Background: *Musa seminifera* Lour is a tree-like perennial herb that has been used in folk medicine in Bangladesh to heal a number of ailments. **Objective:** To evaluate the antioxidant, analgesic, antidiarrheal, anthelmintic activities, and general toxicity of the ethanol extract of the roots. **Materials and Methods:** The extract was assessed for free-radical-scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, total phenolic content (TPC) by the Folin Ciocalteu reagent, antioxidant activity by the ferric reducing power assay, analgesic activity by the acetic acid-induced writhing and hot-plate tests, antidiarrheal activity by the castor oil-induced diarrhea model in mice, anthelmintic activity on Paramphistomum cervi and Haemonchus contortus, and general toxicity by the brine shrimp lethality assay. **Results:** The extract showed free-radical-scavenging activity with an IC_{50} value of 44.86 µg/mL. TPC was 537.89 mg gallic acid equivalent/100 g of dried plant material. It showed concentration-dependent reducing power, and displayed 42.11 and 69.32% writhing inhibition at doses of 250 and 500 mg/kg body weight, respectively. The extract also significantly raised the pain threshold at the above-mentioned dose levels. In vivo antidiarrheal property was substantiated by significant prolongation of latent period and decrease in total number of stools compared with the control. The LC_{50} against brine shrimp nauplii was 36.21 µg/mL. The extract exhibited dose-dependent decrease in paralysis and death time of the helminths. **Conclusion:** The above results demonstrated that the plant possesses notable bioactivities and somewhat supports its use in folk medicine.

Key words: Analgesic activity, anthelmintic activity, brine shrimp lethality, 2,2-diphenyl-1-picrylhydrazyl, Folin Ciocalteu's reagent, reducing power

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.117827

Quick Response Code:

INTRODUCTION

Musa seminifera Lour (Synonym: *M. sylvestris*, fam: Musaceae) is native to Asia, grows in 5-9 m in height with tuberous rhizome, hard pseudo-stem, and a crown of large extended green leaves. The inflorescence can be consumed as vegetable. The ripe fruits are sweet, juicy, and full of seeds. The peel is thicker than other species of the genus *Musa*. In Bangladesh, this plant is commonly known as Bichi kola, Aitta kola, or Doia kola and distributed throughout the country. The fruit is used in the treatment of diarrhea, dysentery, and excess menstruation.[1] The flower is used to treat diarrhea, diabetes, and menorrhagia. Stem juice is used to treat cholera, hemoptysis, otalgia, and dysentery.[2]

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There are no reports available on any bioactivity studies on the roots of this species. However, other species of this genus, e.g., *Musa acuminata*, *Musa paradisiaca,* and *Musa sapientum*, were studied before. The antidiarrheal, antioxidant, and antimicrobial activities of the seeds[3] and hypoglycemic activity of the flowers[4] of *M. sapientum* were studied. The fruits of *M. sapientum* revealed antibacterial, wound-healing, and antiallergic properties.[5-7] Phytochemical analysis on *M. sapientum* indicated the presence of triterpenes (β-amyrin, cyclomusalenone, cyclomusalenol, 24-methylenecycloartanol stigmast-7-en-3-ol, stigmast-7-methylenecycloartanol), flavonoids (quercetin and its 3-*O*-glucoside, 3-*O*-galactoside and 3-*O*-rhamnosyl glucoside),[8] 4-epicyclomusalenone, and 4-epicycloeucalenone.[9] Antihyperglycemic effect of the roots of *M. paradisiaca* was investigated in streptozotocin-induced diabetic male Albino rats.[10] Inhibition of dehydrogenase activity in pathogenic bacteria was studied for aqueous fruit peel and leaf extracts of *M. paradisiaca*.[11] Chemical investigation on *M. acuminata* led to the isolation

of 4,4'-dihydroxy-anigorootin, 4'-hydroxy-anigorootin, 3,3'-bishydroxyanigorufone,[12] (*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid,[13] and oxabenzochrysenones.[14]

As part of our ongoing studies on medicinal plants form the Bangladeshi flora,[15-30] we now, for the very first time, report on the antioxidant, analgesic, antidiarrheal, anthelmintic activity, and general toxicity of the ethanol extract of the roots of *M. seminifera*.

MATERIALS AND METHODS

Plant material

Roots of *M. seminifera* were collected from Khulna University area, Khulna, Bangladesh, in November 2011. A voucher specimen (DACB 37523) was lodged at the Bangladesh National Herbarium, Dhaka.

Extraction

The roots were washed with water, shed dried, ground, and soaked in ethanol for 3 days with occasional shaking. The extract was filtered through a cotton plug and dried using a rotary evaporator at 50°C to obtain crude extract (yield 1.83%).

Test animals

Young Swiss Albino mice aged 4-5 weeks, average weight 20-25 g, procured from the International Centre for Diarrheal Disease and Research, Bangladesh (ICCDR, B) were used. The mice were provided with ICCDR, B formulated rodent food, and water *ad libitum*. The experiments were conducted strictly in accordance with the animal ethics guidelines[31] and the ethical guidelines and permission provided by the ICDDR, B.

Test pathogens

Live parasites *Paramphistomum cervi* (Trematoda) and *Haemonchus contortus* (Nematode) were collected from freshly slaughtered cattle at local abattoirs and identified by Dr. Md. Royhan Gofur (Rajshahi University, Rajshahi). After cleaning, parasites were stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with 8.01 g NaCl, 0.20 g KCl, 1.78 g Na_2HPO_4, and 0.27 g KH_2PO_4 in 1 L of distilled water at 37 ± 1°C.

Chemicals and reagents

Folin Chiocalteu's reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, USA. Gallic acid, ascorbic acid, trichloroacetic acid, potassium ferricyanide, sodium carbonate, acetic acid, dimethyl sulfoxide (DMSO), and ferric chloride were purchased from Merck, Germany. Castor oil and tween-80 were procured from Loba Chemie Pvt., Ltd, India. Solvents and all other chemicals used in the study were of analytical grade. Standard vincristine sulphate was obtained from Cipla Pharmaceuticals, India. Standard diclofenac sodium and albendazole were from Beximco Pharmaceuticals Ltd, Bangladesh. Standard morphine was collected from Popular Pharmaceuticals Ltd, Bangladesh.

Determination of DPPH radical scavenging activity

In vitro antioxidant activity was determined by the DPPH assay.[32] Stock solution (512 μg/mL) of the root extract was prepared in ethanol, and was serially diluted with ethanol to obtain concentrations of 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL. Serially diluted solutions (1 mL) were mixed with 3 mL DPPH solution (0.004% in ethanol) and kept for 30 min to complete any reaction that occurs. The absorbance was recorded at 517 nm. Ascorbic acid was used as the positive standard. Percent inhibition was calculated using the formula $(1–A_1/A_0) \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance of sample or standard. IC_{50} was determined from % inhibition versus concentration (μg/ml) plot.

Determination of total phenolic content (TPC)

Ground roots (0.5 g) were mixed with 80% aq. MeOH (50 mL), sonicated for 20 min, and a portion (2 mL) was centrifuged for 15 min at 14,000 rpm. TPC was determined by the Folin Ciocalteu reagent.[33] Gallic acid solution in MeOH was prepared at 500, 250, 125, 62.5, 31.25, and 15.62 mg/L concentrations. From each of the concentrations, and from the extract, a volume of 1 mL was transferred to a 25 mL volumetric flask and 9 mL distilled water was added. Diluted (1 mL reagent in 9 mL distilled water) Folin Ciocalteu's reagent (1 mL) was added and mixed by continuous shaking. After 5 min, 7% Na_2CO_3 (10 mL) was added to each flask and distilled water was added to make the final volume of 25 mL. Keeping for 30 min at room temperature, absorbance was measured at 750 nm against blank, which was prepared by following all the above-mentioned steps except for the addition of extract or gallic acid. Standard curve was prepared by plotting absorbance versus respective concentration of gallic acid. TPC of the extract was determined from the standard curve and expressed as mg gallic acid equivalent (GAE)/100 g dried plant material.

Reducing power assay

Reducing power was determined by the method described by Oyaizu (1986).[34] The root extract (1 mL) at different concentrations (15.62-500 μg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added, and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.1%, w/v, 0.5 mL). Reducing power was determined by measuring the formation of

Pearl's Prussian blue at 700 nm. Ascorbic acid was used as the positive standard.

Evaluation of analgesic activity

Randomly screened animals were divided into four groups – Groups I, II, III, and IV – consisting of six mice in each. Groups I and II were considered as control and positive control, respectively, whereas groups III and IV were test groups.

Acetic acid-induced writhing test

Mice ($n = 6$) were administered orally with diclofenac sodium 25 mg/kg body weight as standard drug, root extract at the doses of 250 and 500 mg/kg and control as 1% Tween-80 in distilled water. Thirty minutes after the treatment, the mice were subjected to intraperitoneal injection of 0.7% acetic acid at the dose of 10 mL/kg to induce writhing. After 5 min of acetic acid administration, the writhes were counted for 10 min. The numbers of writhes of standard and extract were compared to controls.[35]

Hot-plate test

Animals were screened based on their reaction time at 3-5 s when subjected to pain stimulus. Mice were placed on a hot plate at 55 ± 0.5°C. Reaction time was recorded when mice licked their fore and hind paws and jumped at before (0 min) and 30, 60, 90, and 120 min after oral administration of extract at the doses of 250 and 500 mg/kg, standard drug morphine, at the dose of 5 mg/kg (i.p.) and control as 1% Tween-80 in distilled water (10 mL/kg, p.o.). Cutoff point was considered 15 s to avoid accidental damage of the paws of mice.[36]

Evaluation of in vivo antidiarrheal activity

In vivo antidiarrheal activity was investigated by castor oil-induced diarrheal episode in mice.[37] Test animals were divided into control, positive control, and test groups containing six mice in each group. The control group received 1% Tween-80 in distilled water (10 mL/kg) whereas the positive control group received standard loperamide (2 mg/kg). The test groups received root extract at doses of 250 and 500 mg/kg. All the treatments were administered orally, 1 h prior to oral administration of castor oil at the dose of 0.5 mL per mouse. Each mouse was placed in an individual cage and the floor was lined with blotting paper. The blotting paper was changed every hour. The observation period was 4 h. The latent period and total stool count after 4 h were recorded. Antidiarrheal activity was expressed as the prolongation of latent period and percentage of inhibition of defecation.

Brine shrimp lethality assay for general toxicity

General toxicity of the extract was tested by the brine shrimp lethality assay.[38] Artificial sea water was prepared by dissolving 20 g of NaCl and 18 g of table salt in 1 L of distilled water and was filtered off to obtain a clear solution. A rectangular tank was divided into two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. Eggs of *Artemia salina* were hatched at 25-30°C for 24-48 h. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. They were then collected by a pipette. Extract was dissolved in distilled water with DMSO and transferred to test tubes to obtain concentrations of 320,160, 80, 40, 20, 10, and 5 μg/mL in 5 mL artificial sea water with ten nauplii in each test tube. Anticancer drug vincristine sulphate was used as positive control at concentrations of 5, 2.5, 1.25, 0.625, and 0.312 μg/mL. The concentration of DMSO did not exceed 0.01% in any of the test tubes. Control test tubes were subjected to DMSO in artificial seawater at the same concentration as in test tubes for samples. After 24 h incubation at 25-30°C, the number of viable nauplii was counted using a magnifying glass.

Evaluation of anthelmintic activity

Anthelmintic activity of the root extract was investigated on live parasites *P. cervi* and *H. contortus* of cattle.[39,40] The parasites were divided into different groups consisting of six parasites in each group. Extracts at concentrations of 25, 50, 100, and 200 mg/mL and reference standard albendazole at the concentration of 15 mg/mL of 10 mL in PBS were prepared and transferred to Petri dishes. The control group was treated with 0.1% Tween-80 in PBS. Six parasites were placed in each Petri dish and observed. The time of paralysis was recorded when no movement was observed unless shaken vigorously. The death time was recorded after ensuring that the parasites did not move when shaken vigorously, dipped in warm water (50°C), or subjected to external stimuli. Anthelmintic activity was expressed as the time required for paralysis and death of parasites as compared to control.

Statistical analysis

All the values were expressed as mean ± Standard error for mean (SEM). Student's *t*-test was used to estimate significant differences between the test and control groups. Statistical analysis was performed with Prism 5.0 (GraphPad software Inc., San Diego, CA). Results were considered as statistically significant when the *P* value was less than the alpha factor (0.05).

RESULTS

DPPH scavenging activity

Both the extract and ascorbic acid showed a linear increase in DPPH scavenging activity at lower concentrations until they reached a plateau at higher concentrations [Figure 1]. IC_{50} values for both test and standard were within the linear region. Using the values of the linear region, IC_{50} of the extract and ascorbic acid was determined as 44.86 and 13.25 μg/mL, respectively.

TPC determination

The TPC of *M. seminifera* root extract was 537.89 mg GAE/100 g of dried plant material [Figure 2].

Reducing power assay

The root extract of *M. seminifera* displayed concentration-dependent increase in the reducing power. At the concentrations of 15.62, 31.25, 62.5, 125, and 250 μg/mL, the extract showed absorbance of 0.482, 0.528, 0.657, 0.687, and 0.758, whereas at the same concentrations, standard ascorbic acid showed absorbance of 0.725, 0.855, 1.236, 1.688, and 2.523, respectively [Figure 3].

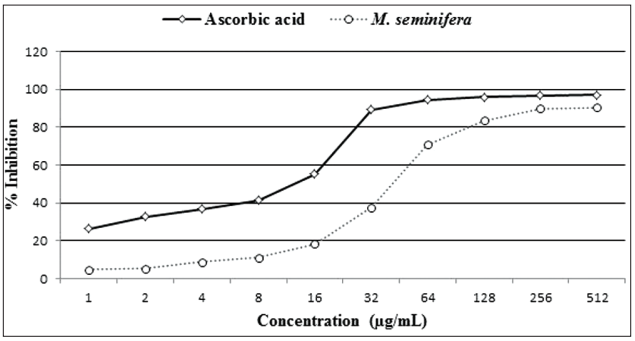

Figure 1: 2, 2-diphenyl-1-picrylhydrazyl scavenging activity of *Musa seminifera* root

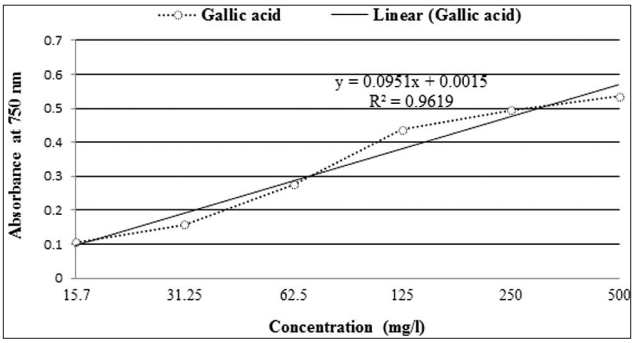

Figure 2: Standard calibration curve of gallic acid

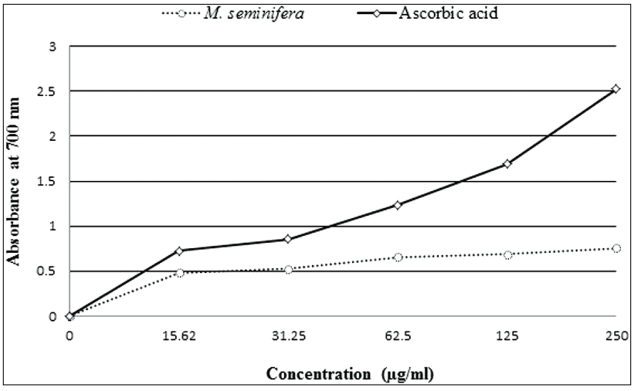

Figure 3: Reducing power of *Musa seminifera* root

The acetic acid-induced writhing test

The extract revealed significant and dose-dependent pain inhibition at both dose levels tested. The extract showed 42.11% and 69.32% writhing inhibition at doses of 250 and 500 mg/kg body weight, respectively, whereas standard diclofenac sodium (25 mg/kg) showed 78.95% writhing inhibition [Table 1].

The hot-plate test

The extract significantly and dose-dependently increased the pain threshold. The extract showed maximum reaction times of 5.77 and 6.48 s at doses of 250 and 500 mg/kg body weight, respectively, whereas morphine (5 mg/kg) showed a reaction time of 9.30 s [Table 2].

In vivo antidiarrheal activity

The extract showed significant and dose-dependent prolongation of latent period as well as decrease in number of stools throughout the whole observation period compared to control. The extract demonstrated 57.89% and 77.19% inhibition of defecation at doses of 250 and 500 mg/kg body weight, respectively, whereas loperamide (3 mg/kg) showed 88.58% inhibition of defecation [Table 3].

The brine shrimp lethality assay

LC_{50} was calculated using LdP line Probit analysis software, USA. The LC_{50} for *M. seminifera* root extract was found to be 36.21 μg/mL and that of vincristine sulphate was 0.43 μg/mL.

Anthelmintic activity

The root extract revealed significant anthelmintic activity in a concentration-dependent manner. The extract, at concentrations of 25, 50, 100, and 200 mg/mL exhibited paralysis at 24.17, 11.17, 6.16, and 2.25 min and death at 33.33, 21.67, 9.50, and 5.16 min, respectively, for *P. cervi,* and paralysis at 39.33, 23.00, 13.50, and 7.16 min and death at 51.33, 32.00, 22.83, and 13.00 min, respectively, for *H. contortus*. Standard drug albendazole at the concentration of 15 mg/mL showed paralysis at 12.50 min and death at 20.00 min for *P. cervi* and paralysis at 24.17 min and death at 35.50 min for *H. contortus* [Table 4].

Table 1: Effect of *Musa seminifera* root on acetic acid induced writhing in mice

Treatment (n=6)	Dose (mg/kg)	No. of writhes	% Inhibition
Control	1% tween-80 in water	19±0.73	-
Diclofenac sodium	25	4±0.51*	78.95
Extract 250	250	11±0.77**	42.11
Extract 500	500	5.83±0.65*	69.32

Values are expressed as mean±SEM, SEM: Standard error for mean, *n*: Number of mice (6), *P<0.001 and **P<0.01 versus control, Student's *t*-test

Table 2: Effect of *Musa seminifera* root in hot-plate test in mice

Treatment (n=6)	Dose (mg/kg)	Reaction time(s)				
		0 min	30 min	60 min	90 min	120 min
Morphine	5	3.52±0.12*	6.24±0.13**	7.32±0.13**	9.30±0.12**	8.20±0.08**
Extract 250	250	3.50±0.10*	4.37±0.07**	5.77±0.08**	5.05±0.04**	4.02±0.07**
Control	1% tween-80 in water	3.15±0.06	3.26±0.04	3.27±0.08	3.23±0.07	3.23±0.06
Extract 500	500	3.47±0.11*	5.15±0.09**	6.48±0.11**	5.90±0.09**	4.90±0.08**

Values are expressed as mean±SEM, SEM: Standard error for mean, *n*: Number of mice (6), **P*<0.05 and ***P*<0.001 versus control, Student's *t*-test

Table 3: Effect of *Musa seminifera* root on castor oil induced diarrhea in mice

Treatment (n=6)	Dose (mg/kg)	Onset of diarrhea (min)	No. of stools after 4 h	% Inhibition of defecation
Control	1% tween-80 in water	33±3.59*	22.80±1.63*	-
Loperamide	3	197.40±0.77*	2.60±0.50*	88.59
Extract 250	250	101±3.64*	9.60±0.74*	57.89
Extract 500	500	178.80±5.00*	5.20±0.58*	77.19

Values are expressed as mean±SEM, SEM: Standard error for mean, n: Number of mice (6), **P*<0.001 versus control, Student's *t*-test

Table 4: Anthelmintic activity of *Musa seminifera* root

Treatment (n=6)	Conc.(mg/ml)	Time (min) taken for paralysis (P) and death (D)			
		Paramphistomum cervi		Haemonchus contortus	
		P	D	P	D
Control	0.1% Tween-80 in PBS	-	-	-	-
Albendazole	15	12.50±0.42*	20.00±0.57*	24.17±0.47*	35.50±0.42*
Extract 25	25	24.17±0.65*	33.33±0.80*	39.33±0.66*	51.33±0.49*
Extract 50	50	11.17±0.47*	21.67±0.71*	23.00±0.63*	32.00±0.57*
Extract 100	100	6.16±0.40*	9.50±0.42*	13.50±0.56*	22.83±0.70*
Extract 200	200	2.25±0.25*	5.16±0.40*	7.16±0.30*	13.00±0.51*

Values are expressed as mean±SEM, SEM: Standard error for mean, n: Number of parasites (6), * *P*<0.001 versus control, Student's *t*-test, PBS: Phosphate-buffered saline

DISCUSSION

Free-radicals are responsible for many disease conditions including rheumatoid arthritis, cardiovascular disorders, cysticfibrosis, inflammation, neurodegenerative diseases (e.g., Parkinsonism, Alzheimer's disease), AIDS, and cancer.[41] Antioxidants can prevent these disease conditions by scavenging harmful free-radicals. *M. seminifera* showed potential antioxidant activity in the DPPH radical-scavenging assay. The antioxidant activity of plant extracts is generally due to phenolic components such as flavonoids, chalcones, and polyhydroxy benzoic acid derivatives.[42] The TPC of the extract was much higher than some of the phenol-rich fruits such as strawberry and plum (244.1 and 303 mg GAE/100 g, respectively);this supports the observed strong antioxidant activity.[33] Besides neutralizing free radicals, reducing power may serve as a significant indicator to assess the antioxidant activity of natural extracts.[43] The reducing power assay is based on the fact that the substances having reaction potential react with potassium ferricyanide (Fe_3^+) to reduce into potassium ferrocyanide (Fe_2^+), which reacts with ferric chloride to form ferric ferrous complex (Prussian's blue) that has an absorption maximum at 700 nm. Reducing ability was increased with the increase in concentration of the extract, which indicated that some compounds in the extract are able to react with free radicals to convert them into stable forms as well as to terminate chain reactions.

The extract showed analgesic activity in both of the methods. The abdominal constriction response, i.e., 'writhing' induced by acetic acid, is a model to asses s peripherally acting analgesic activity.[44,45] The extract significantly and dose dependently reduced writhing count compared to control, which indicated potential peripheral analgesic activity through prostaglandin

inhibition. The hot-plate test is a model to assess centrally acting analgesic activity, which focuses on changes only in the spinal cord level.[46-48] The extract significantly increased the pain threshold, probably through central mechanisms involving these receptor systems.

Despite the use of conventional antidiarrheal therapies, many plants are used in Bangladesh to treat diarrhea. Castor oil liberates ricinolic acid, which results in irritation and inflammation of intestinal mucosa; subsequently, intestinal motility and secretion are stimulated due to the release of prostaglandins.[49] The *M. seminifera* root extract significantly and dose dependently reduced the quantity of feces as well as prolonged the onset of diarrhea. The result suggested that the extract might have potential antidiarrheal activity via the antisecretory mechanism.

The brine shrimp lethality bioassay is an easy and sensitive bench-top assay for predicting important pharmacological activities such as enzyme inhibition, ion channel interference, and cytotoxic activity.[37,50,51] The correlation between the brine shrimp lethality bioassay and *in vitro* growth inhibition of rapidly growing human tumor cell lines was established by National Cancer Institute (NCI, USA).[52] In the present study, both the extract and vincristine sulphate showed a gradual increase in mortality rate with the increase in concentration. The LC_{50} value for the crude extract was found to be very low, signifying that the extract may contain potent pharmacologically active compound(s).

The anthelmintic activity of plant materials is usually judged on the basis of loss of movement or paralysis and complete destruction or death of live parasites in *in vitro* studies.[53-55] The extract showed significant and concentration-dependent decrease in both paralysis and death time compared with standard albendazole. Although the exact mechanism involved with the observed anthelmintic activity of the extract is not known, polyphenolics present in the extract may play a role in it. Polyphenolic compounds such as tannins have been found to be responsible for the strong anthelmintic activity by interfering in energy generation in helminths by uncoupling oxidative phosphorylation.[56]

CONCLUSION

The results of the present pharmacological investigation support the uses of this plant in folk medicine. The extract showed significant and dose-dependent antioxidant, analgesic, antidiarrheal and anthelmintic activities, and general toxicity.

ACKNOWLEDGMENT

We thank the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICCDR, B) for providing experimental mice, and Beximco Pharmaceuticals Ltd., Bangladesh, for the standard diclofenac sodium and albendazole.

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Cite this article as: Saha S, Shilpi JA, Mondal H, Gofur R, Billah M, Nahar L, *et al*. Bioactivity studies on *Musa seminifera* Lour. Phcog Mag 2013;9:315-22.
Source of Support: Nil. **Conflict of Interest:** None declared.